

Respiration

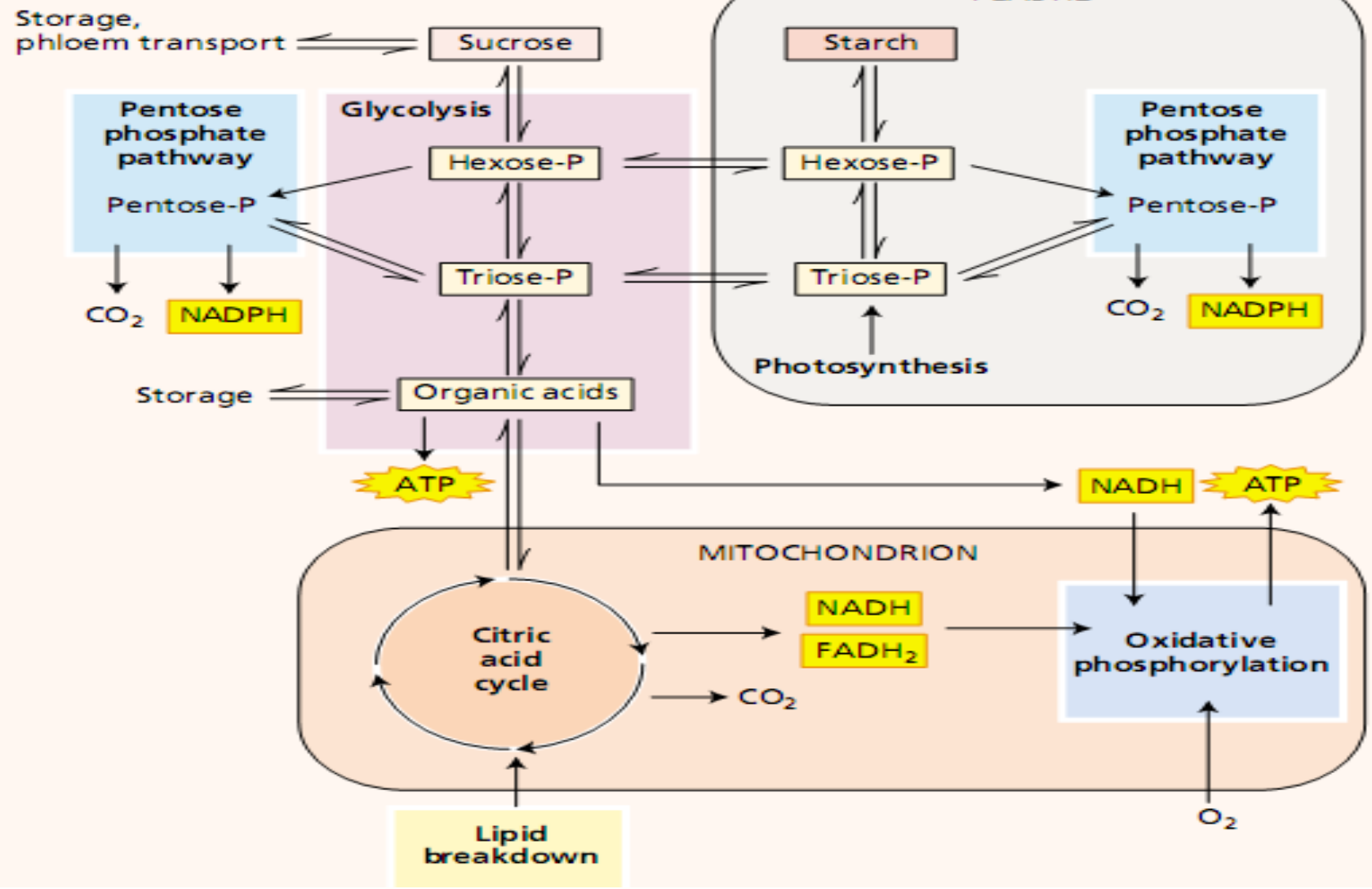
- **Glucose 1 mole (180g)=2880kJ**
- **Starch, sucrose, fructan, other sugars, lipids, organic acids, proteins**
- **To prevent damage, energy released in a multistep process**
- **Metabolites derived from various intermediates- aminoacids, pentoses in cell walls and nucleotide biosynthesis, glycerol needed to synthesize phospholipids**

Respiration

- **Glycolysis** and the pentose phosphate pathways in the cytosol and plastid convert sugars to organic acids, via hexose phosphates and triose phosphates, generating NADH or NADPH and ATP.
- The organic acids are oxidized in the mitochondrial **citric acid cycle**, and the NADH and FADH produced provide the energy for ATP synthesis by the **electron transport chain** and ATP synthase in **oxidative phosphorylation**.
- In **gluconeogenesis**, carbon from lipid breakdown is broken down in the glyoxysomes, metabolized in the citric acid cycle, and then used to synthesize sugars in the cytosol by reverse glycolysis.

CYTOSOL

PLASTID

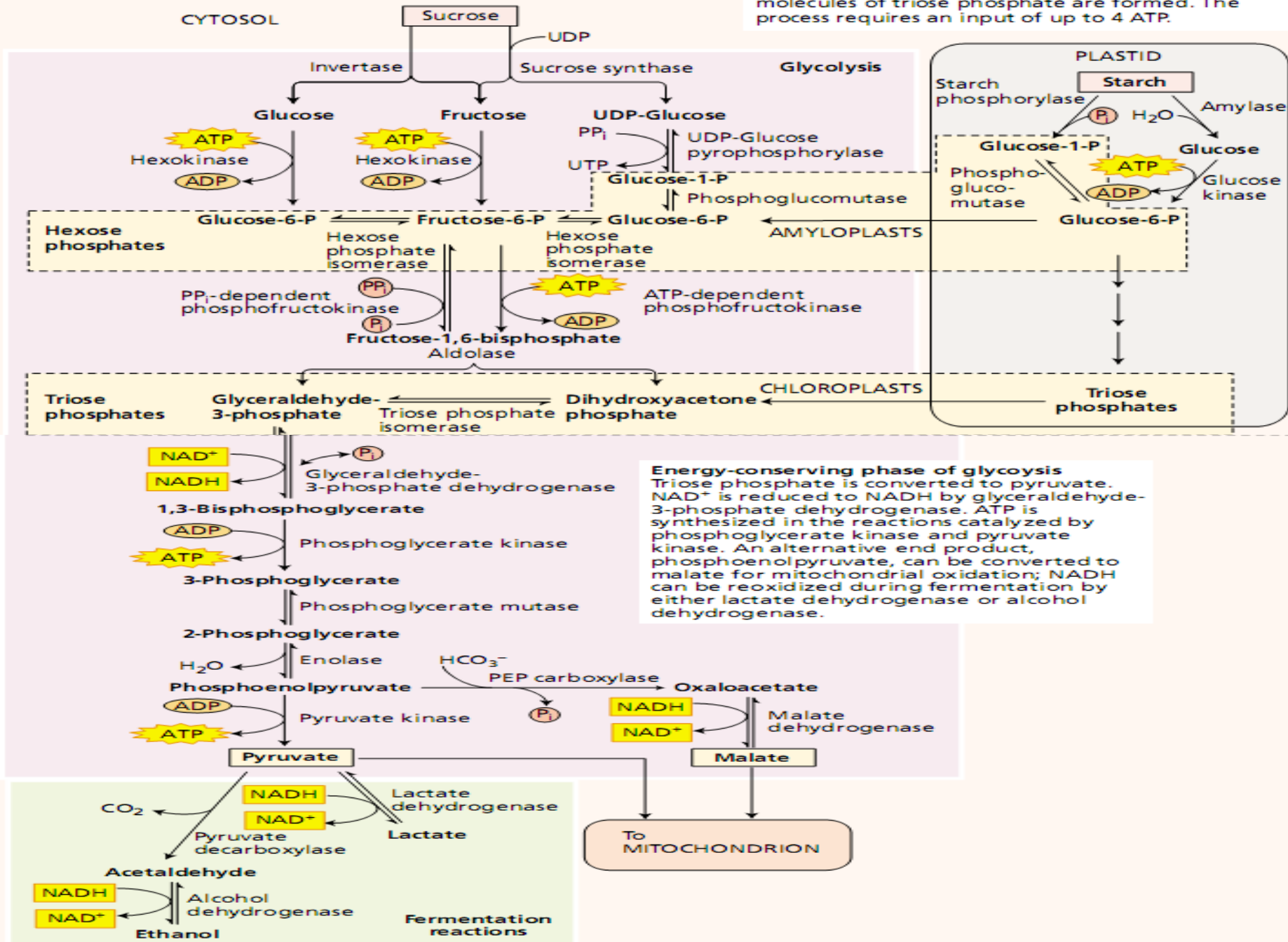


Glycolysis, (Sucrolysis) glykos=sugar, lysis=splitting

- Glycolysis is main source of energy via fermentation
- Source of energy, when oxygen levels are low. Eg. Roots in water flooded soil
- In animals, glycolysis takes place in supramolecular complex attached to outer mitochondrial membrane

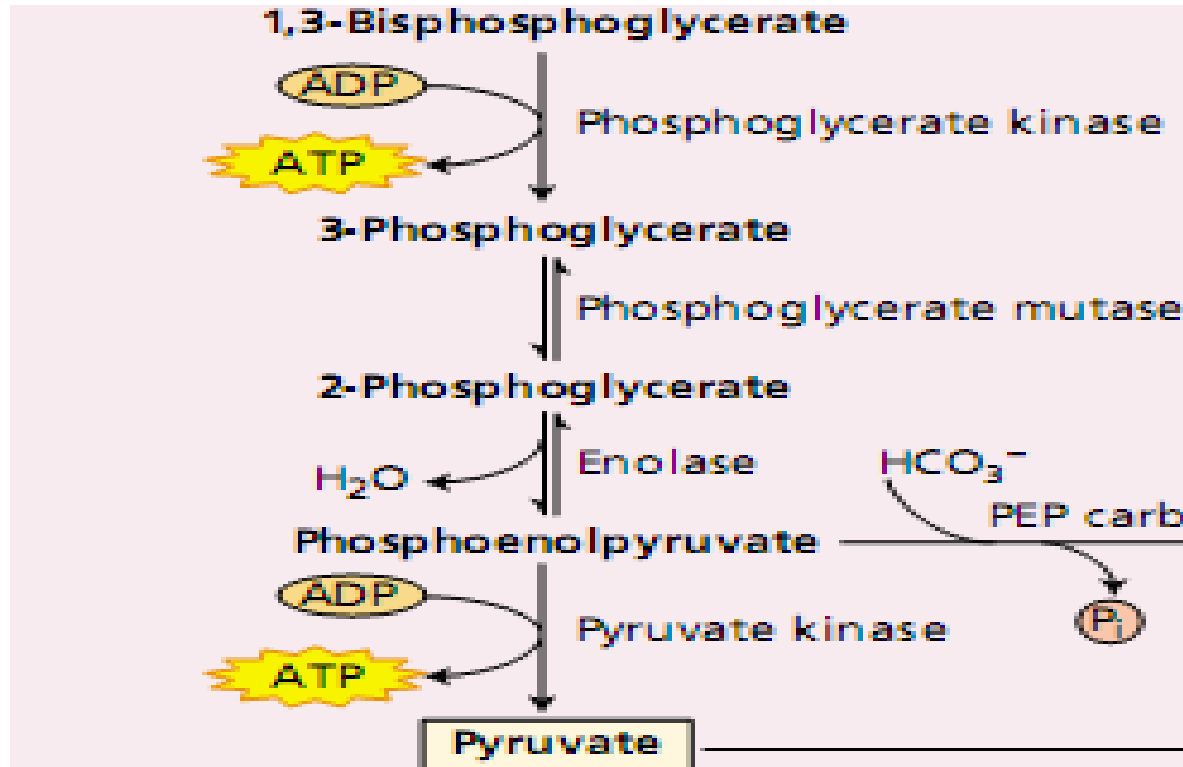
(A)

Initial phase of glycolysis Substrates from different sources are channeled into triose phosphate. For each molecule of sucrose that is metabolized, four molecules of triose phosphate are formed. The process requires an input of up to 4 ATP.



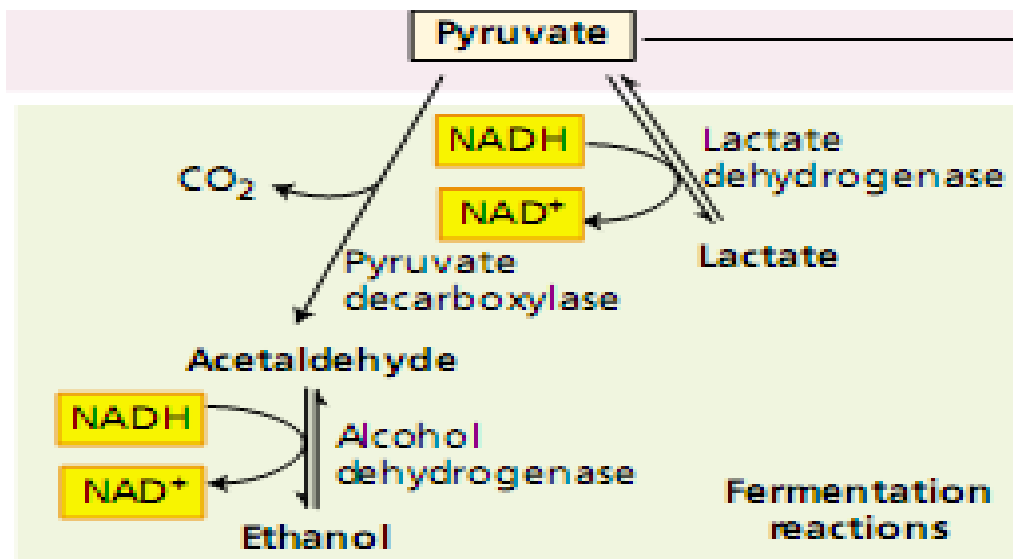
Substrate level phosphorylation:

- Direct transfer of P moiety from a substrate molecule to ADP



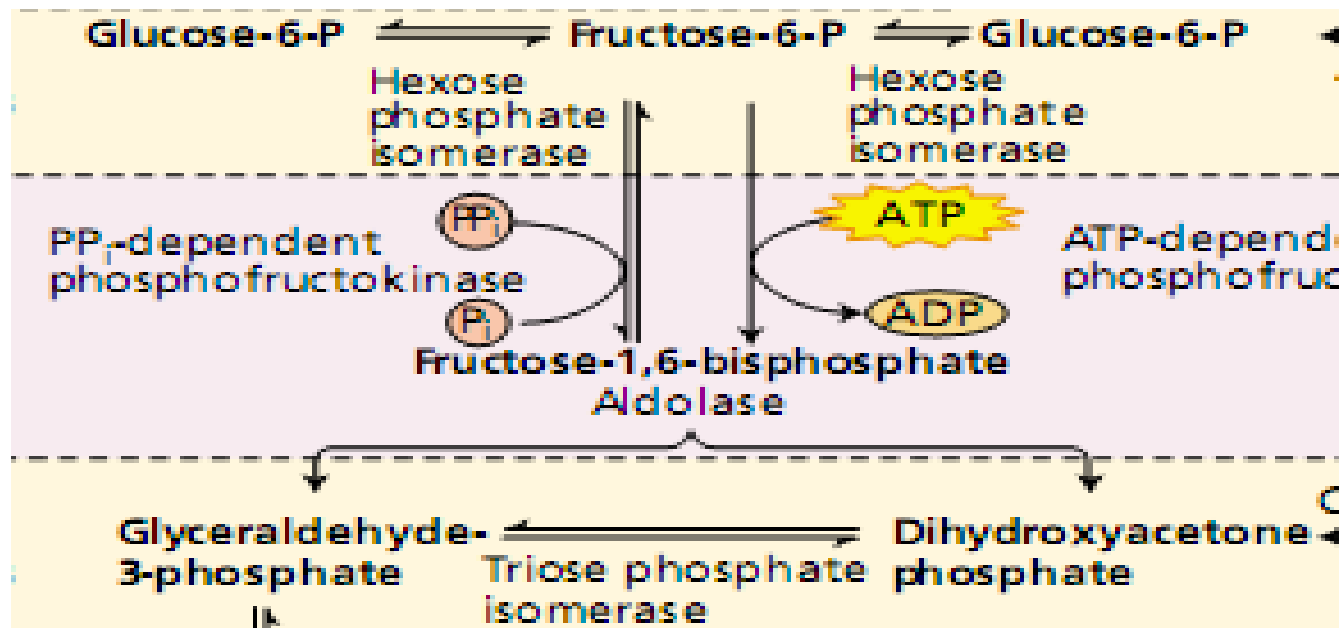
Fermentation

- No oxygen-No TCA & ETC
- When NADH/NAD ratio is high, to overcome the above situation fermentative metabolism occurs
- Ethanol less deleterious
- Lactate acidifies the cytosol



Alternative glycolytic reactions

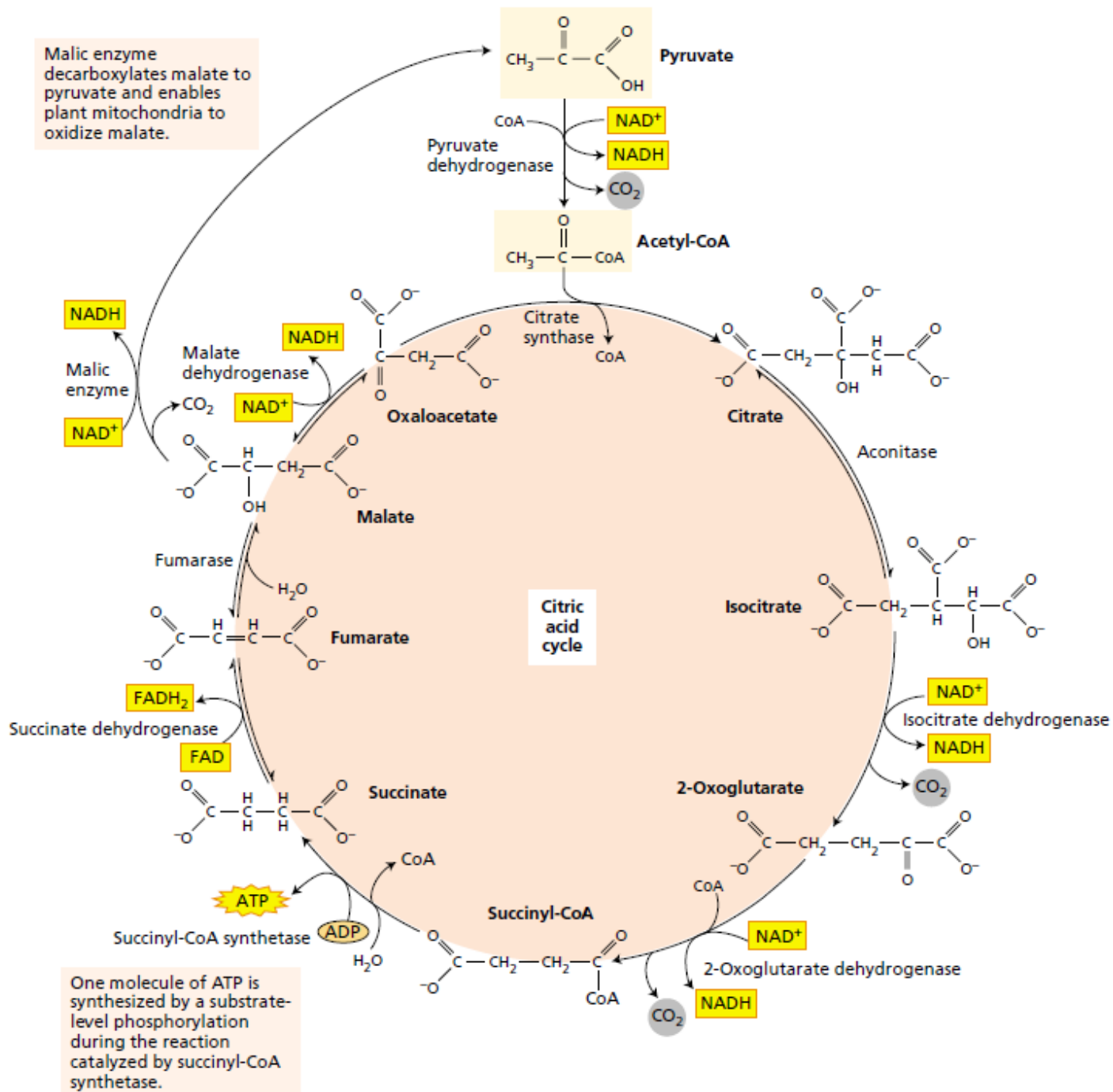
- Gluconeogenesis: Synthesis of glucose through reversal of glycolytic pathway, operates in seeds (castor bean, sunflower)



TCA Cycle-citric acid cycle

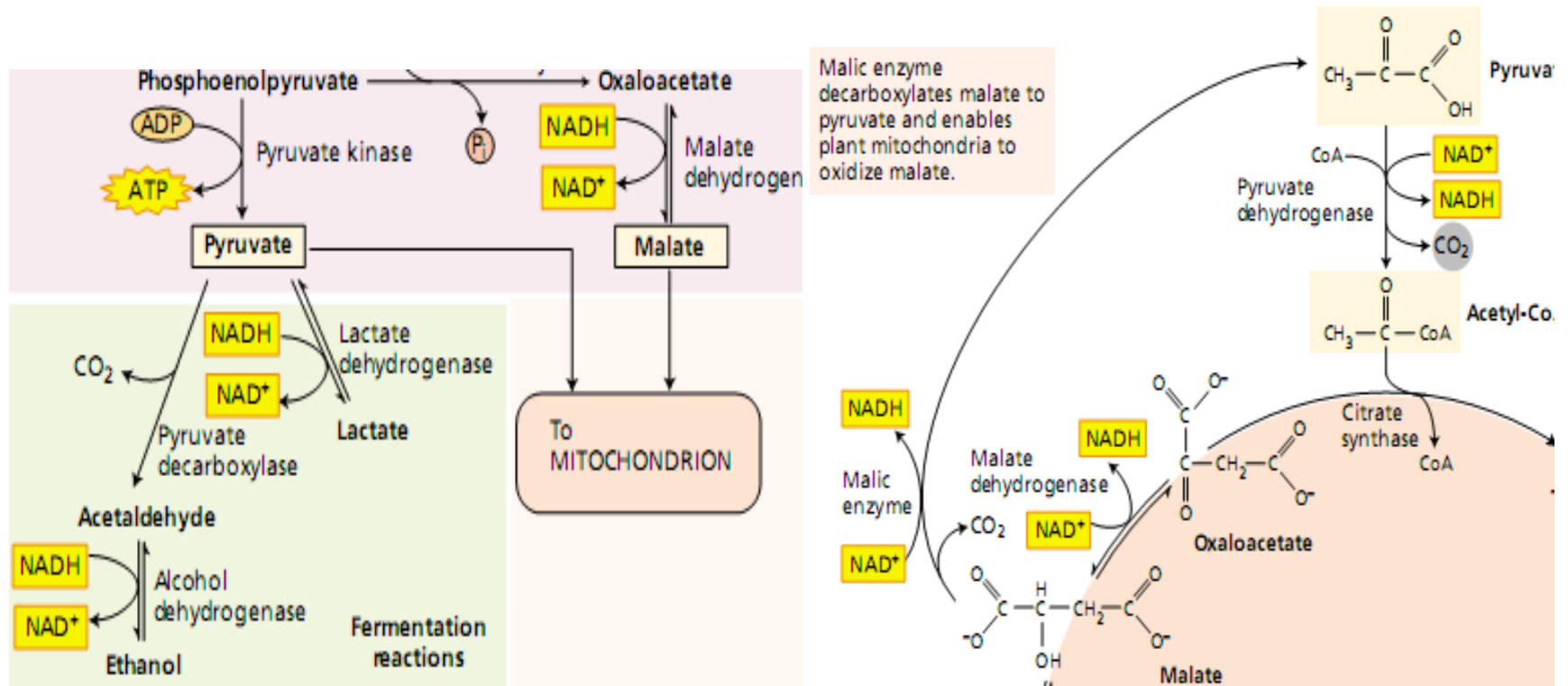
- Hans A Krebs-1937
- In mt matrix
- Pyruvate transported into mt matrix through pyruvate translocater (Pyruvate-OH)
- Pyruvate oxidatively decarboxylated into CO_2 , NADH and Acetic acid
- Acetic acid formed linked to cofactor (CoA) via a thioester bond to form acetyl CoA

- Pyruvate dehydrogenase (complex of several enz), catalyze the reaction in a 3 step process: decarboxylation, oxidation, conjugation to CoA
- Citrate synthase combines acetyl CoA with OAA to form citrate
- 3 carbon of pyruvate entering the cycle is released as 3 CO₂ molecules
- Energy in the thioester bond of succinyl CoA conserved through synthesis of ATP (Substrate level phosphorylation)
- Succinate dehydrogenase is a membrane associated protein-the cofactor FAD is converted into FADH₂

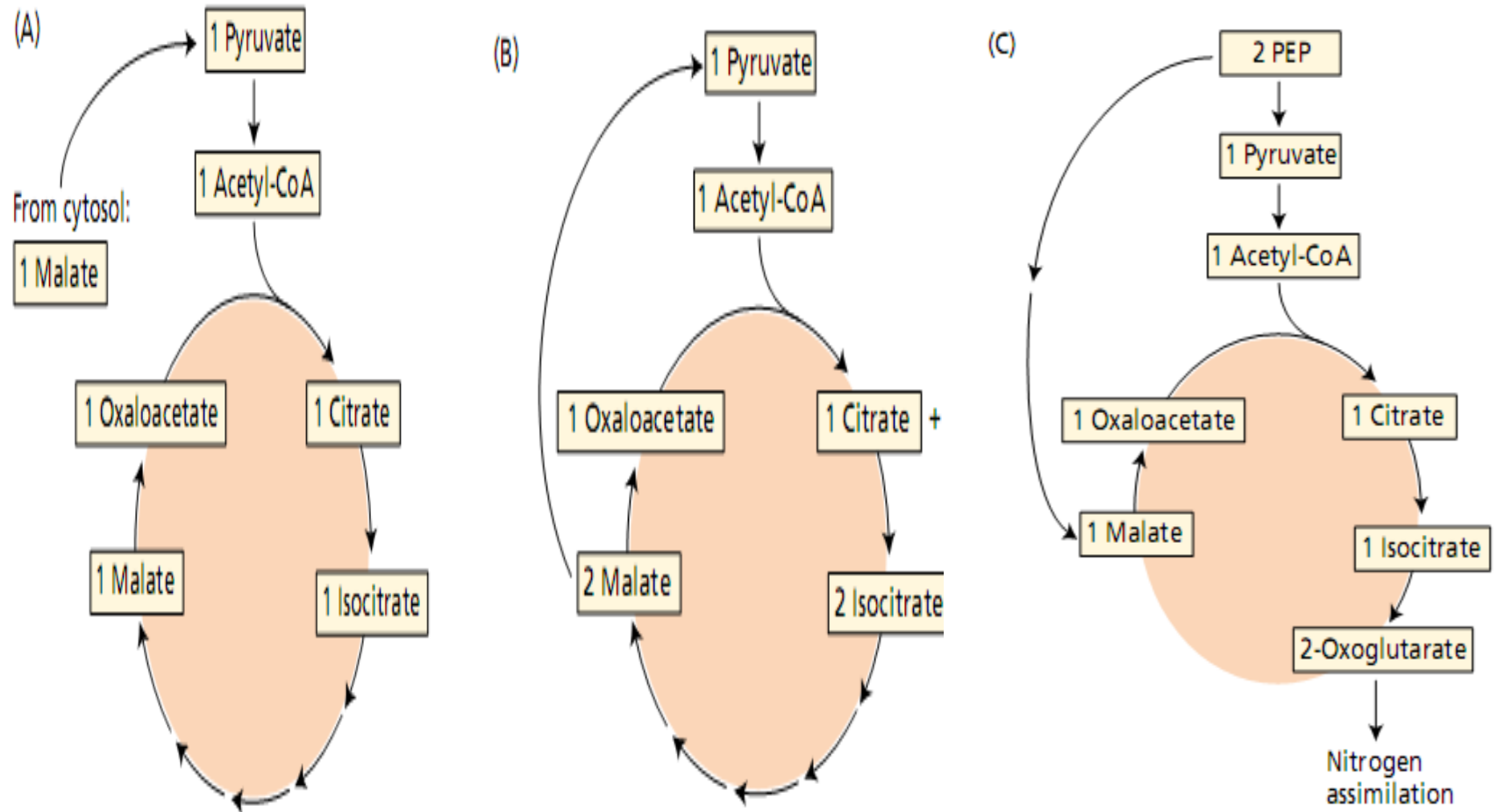


TCA cycle unique features

- ATP in plants, GTP in animals
- NAD⁺ Malic enzyme

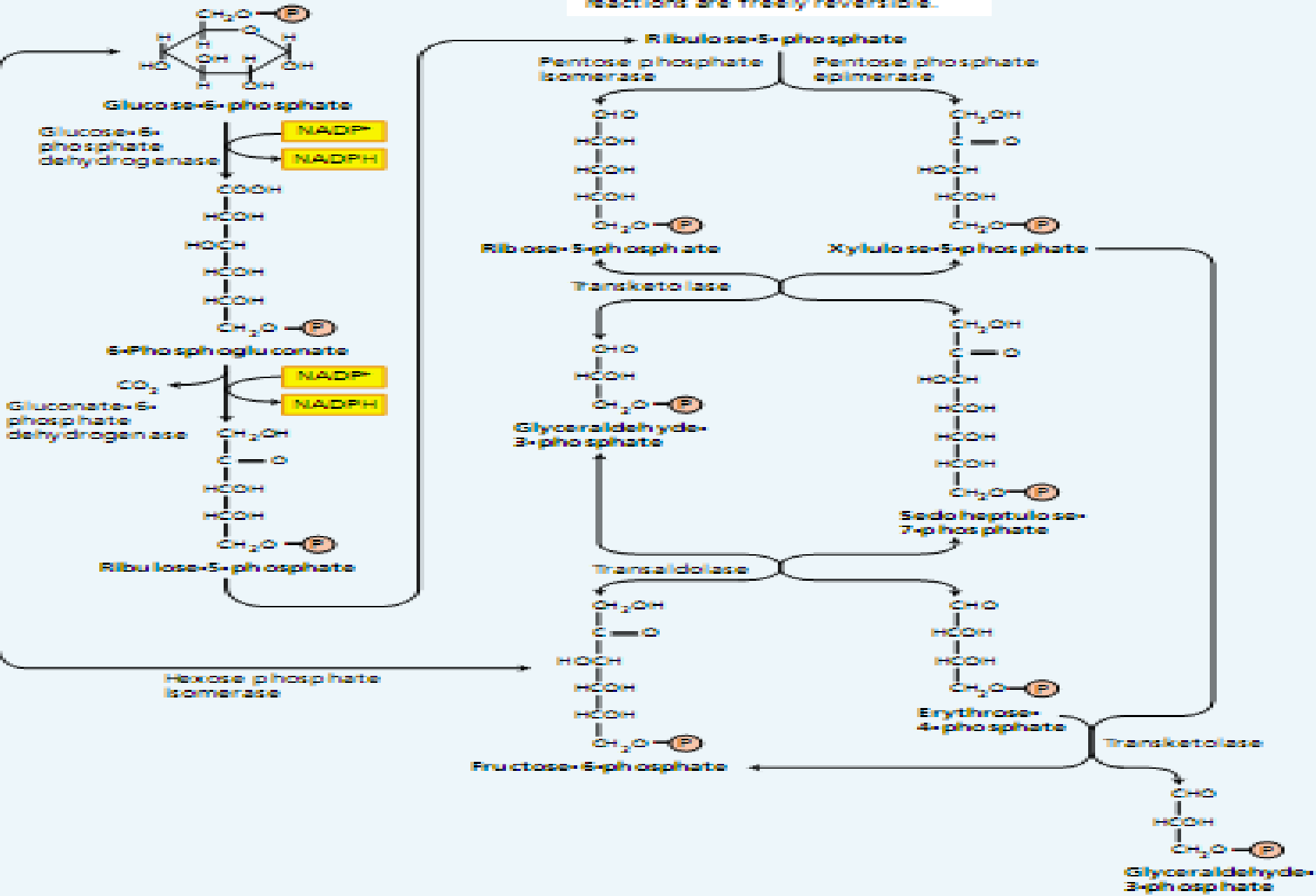


Malic enzyme and PEPcarboxylase provide plants with metabolic flexibility for the metabolism of Phosphoenolpyruvate.



NADPH is generated in the first two reactions of the pathway, where glucose-6-phosphate is oxidized to ribulose-5-phosphate. These reactions are essentially irreversible.

The ribulose-5-phosphate is converted to the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate through a series of metabolic interconversions. These reactions are freely reversible.



The oxidative pentose phosphate pathway (in plastids) plays several roles in plant metabolism:

- In nongreen plastids, such as amyloplasts, and in chloroplasts functioning in the dark, the pathway may also supply NADPH for biosynthetic reactions such as lipid biosynthesis and nitrogen assimilation.
- Because plant mitochondria are able to oxidize cytosolic NADPH via an NADPH dehydrogenase localized on the external surface of the inner membrane, some of the reducing power generated by this pathway may contribute to cellular energy metabolism; that is, electrons from NADPH may end up reducing O and generating ATP.
- The pathway produces ribose-5-phosphate, a precursor of the ribose and deoxyribose needed in the synthesis of RNA and DNA, respectively.
- Another intermediate in this pathway, the four-carbon erythrose-4-phosphate, combines with PEP in the initial reaction that produces plant phenolic compounds, including the aromatic amino acids and the precursors of lignin, flavonoids, and phytoalexins
- During the early stages of greening, before leaf tissues become fully photoautotrophic, the oxidative pentose phosphate pathway is thought to be involved in generating Calvin cycle intermediates.

ETC-ATP synthesis

Complex I (NADH dehydrogenase).

- Electrons from NADH generated in the mitochondrial matrix during the citric acid cycle are oxidized by complex I (an NADH dehydrogenase).
- The electron carriers in complex I include a tightly bound cofactor (flavin mononucleotide [FMN], which is chemically similar to FAD) and several iron sulfur centers.
- Complex I then transfers these electrons to ubiquinone.
- Four protons are pumped from the matrix to the intermembrane space for every electron pair passing through the complex.

Ubiquinone,

- A small lipid-soluble electron and proton carrier, is located within the inner membrane. It is not tightly associated with any protein, and it can diffuse within the hydrophobic core of the membrane bilayer.

Complex II (succinate dehydrogenase)

- Oxidation of succinate in the citric acid cycle is catalyzed by this complex, and the reducing equivalents are transferred via the FADH₂ and a group of iron-sulfur proteins into the ubiquinone pool.
- This complex does not pump protons.

Complex III (cytochrome bc complex)

Complex III (cytochrome bc_1 complex). This complex oxidizes reduced ubiquinone (ubiquinol) and transfers the electrons via an iron–sulfur center, two *b*-type cytochromes (b_{565} and b_{560}), and a membrane-bound cytochrome c_1 to cytochrome *c*. Four protons per electron pair are pumped by complex III.

Cytochrome *c* is a small protein loosely attached to the outer surface of the inner membrane and serves as a mobile carrier to transfer electrons between complexes III and IV.

Complex IV (cytochrome c oxidase). This complex contains two copper centers (Cu_A and Cu_B) and cytochromes a and a_3 . Complex IV is the terminal oxidase and brings about the four-electron reduction of O_2 to two molecules of H_2O . Two protons are pumped per electron pair (see Figure 11.8).

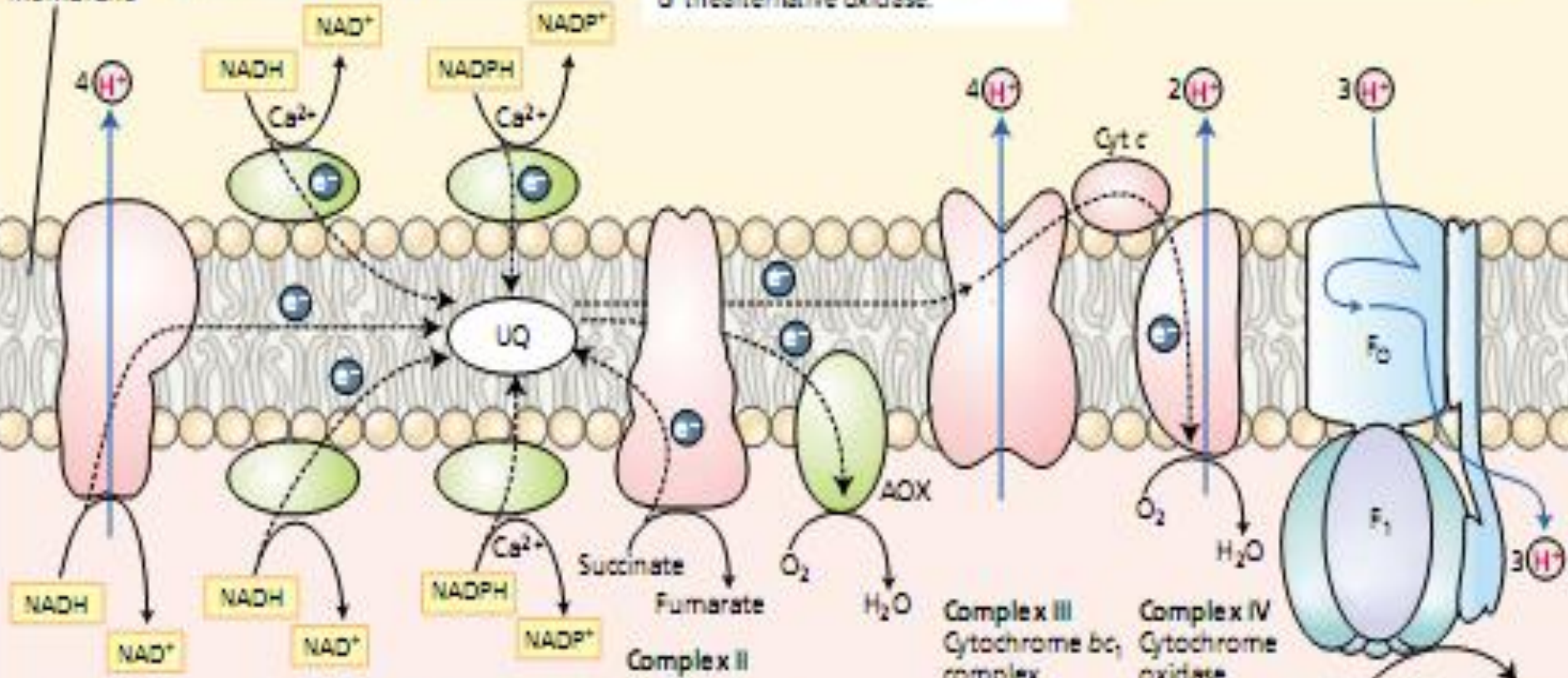
INTERMEMBRANE SPACE

External (rotenone-insensitive) NAD(P)H dehydrogenases can accept electrons directly from NAD(P)H produced in the cytosol.

The ubiquinone (UQ) pool diffuses freely within the inner membrane and serves to transfer electrons from the dehydrogenases to either complex III or the alternative oxidase.

Cytochrome c is a peripheral protein that transfers electrons from complex III to complex IV.

Inner membrane



Complex I
NADH dehydrogenase

Rotenone-insensitive NAD(P)H dehydrogenases exist on the matrix side of the membrane.

Complex II
Succinate dehydrogenase

An alternative oxidase (AOX) accepts electrons directly from ubiquinone.

Complex III
Cytochrome bc₁ complex

Complex IV
Cytochrome oxidase

Complex V
ATP synthase

MATRIX

ATP synthesis is coupled to electron transport

Substrate	Theoretical ^a	Experimental
Malate	2.5	2.4–2.7
Succinate	1.5	1.6–1.8
NADH (external)	1.5	1.6–1.8
Ascorbate	1.0 ^b	0.8–0.9

^aIt is assumed that complexes I, III, and IV pump 4, 4, and 2 H⁺ per 2 electrons, respectively; that the cost of synthesizing one ATP and exporting it to the cytosol is 4 H⁺ (Brand 1994); and that the non-phosphorylating pathways are not active.

^bCytochrome c oxidase pumps only two protons when it is measured with ascorbate as electron donor. However, two electrons move from the outer surface of the inner membrane (where the electrons are donated) across the inner membrane to the inner, matrix side. As a result, 2 H⁺ are consumed on the matrix side. This means that the net movement of H⁺ and charges is equivalent to the movement of a total of 4 H⁺, giving an ADP:O ratio of 1.0.

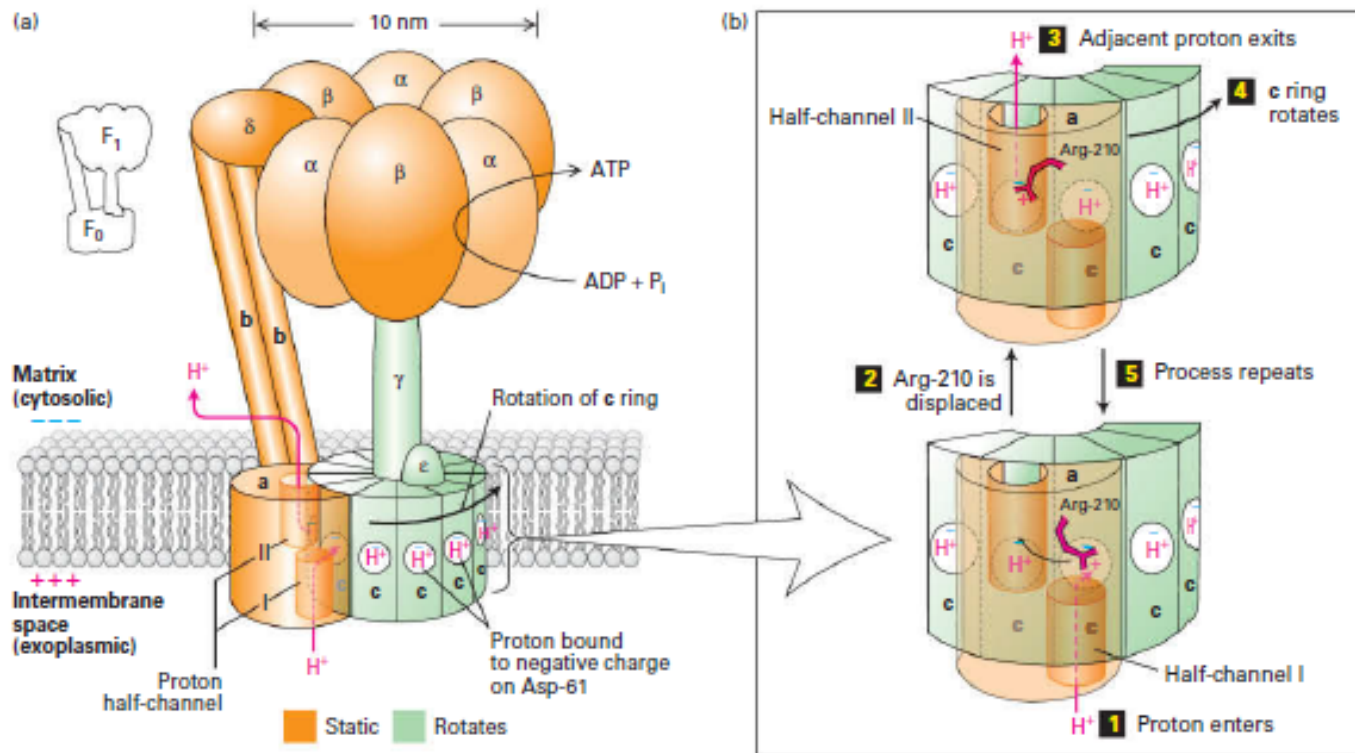


FIGURE 12-31 Structure of ATP synthase (the F_0F_1 complex) in the bacterial plasma membrane and mechanism of proton translocation across the membrane. (a) The F_0 membrane-embedded subcomplex of ATP synthase is built of three integral membrane proteins: one copy of **a**, two copies of **b**, and an average of ten copies of **c** arranged in a ring in the plane of the membrane. Two proton half-channels near the interfaces of subunit **a** with the **c** subunits mediate proton movement across the membrane (proton path is indicated by red arrows). Half-channel I allows protons to move one at a time from the exoplasmic medium (equivalent to intermembrane space in mitochondria) to the negatively charged side chain of Asp-61 in the center of a **c** subunit near the middle of the membrane. The proton-binding site in each **c** subunit is represented as a white circle with a blue "−" representing the negative charge on the side chain of Asp-61. Half-channel II permits protons to move from the Asp-61 of an adjacent **c** subunit into the cytosolic medium. The detailed structure of the **c** ring and a portion of the adjacent **a** subunit is shown in Figure 12-34. The F_1 subcomplex of ATP synthase contains three copies each of subunits α and β , which form a hexamer resting atop the single rod-shaped γ subunit, which is inserted into the **c** ring of F_0 . The ϵ subunit is rigidly attached to the γ subunit and also to several of the **c** subunits. The δ subunit permanently links one of the α subunits

in the F_1 subcomplex to the **b** subunit of F_0 . Thus the F_0 **a** and **b** subunits and the F_1 δ subunit and $(\alpha\beta)_3$ hexamer form a rigid structure (orange) anchored in the membrane. During proton flow, the **c** ring and the attached F_1 ϵ and γ subunits rotate as a unit (green), causing conformational changes in the F_1 β subunits, leading to ATP synthesis. (b) Potential mechanism of proton translocation. Step **1**: A proton from the exoplasmic space enters half-channel I and moves toward the "empty" (unprotonated) Asp-61 proton-binding site. The negative charge (blue "−") on the unprotonated side chain Asp-61 is balanced, in part, by a positive charge on the side chain of Arg-210 (red "+"). Step **2**: The proton fills the empty proton-binding site and simultaneously displaces the positively charged Arg-210 side chain, which swings over to the filled proton-binding site on the adjacent **c** subunit (curved arrow). As a consequence, the proton bound at that adjacent site is displaced. Step **3**: The displaced adjacent proton moves through half-channel II and is released into the cytosolic space, leaving an empty proton-binding site on Asp-61. Step **4**: Counterclockwise rotation of the entire **c** ring moves the "empty" **c** subunit over half-channel I. Step **5**: The process is repeated. See M. J. Schnitzer, 2001, *Nature* **410**:878; P. D. Boyer, 1999, *Nature* **402**:247; and C. von Ballmoos, A. Wiedenmann, and P. Dimroth, 2009, *Annu. Rev. Biochem.* **78**:649.

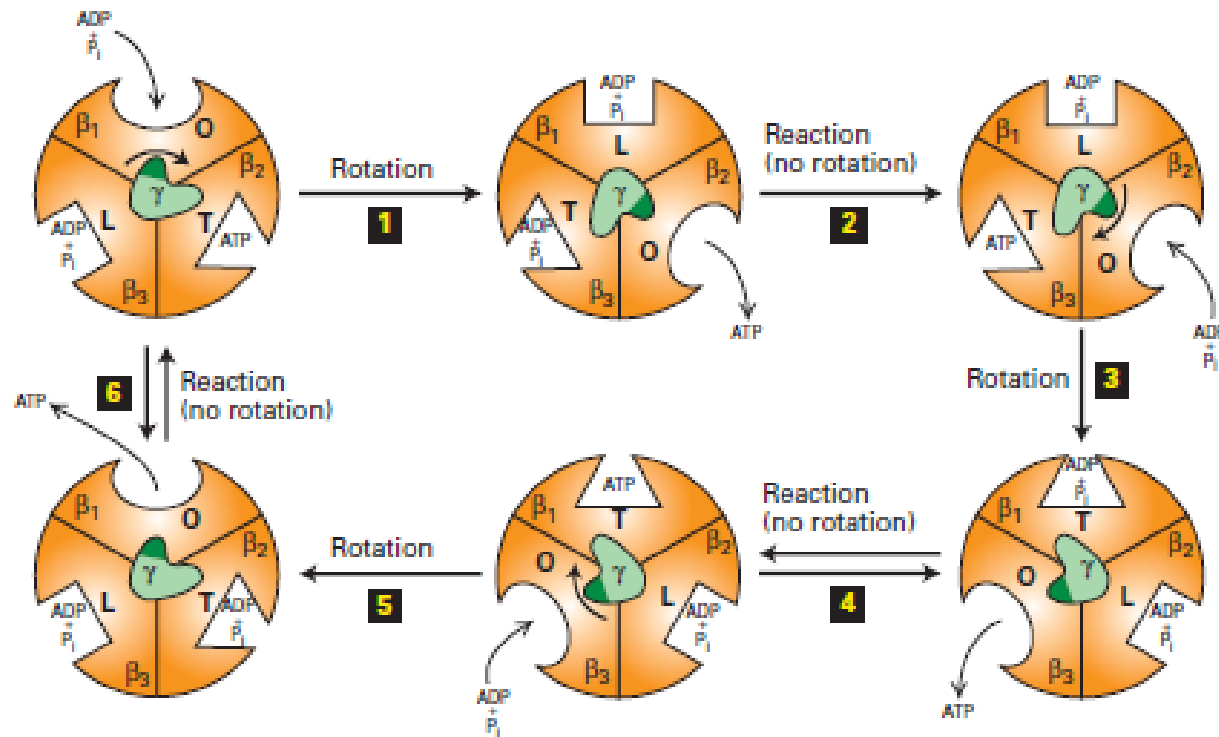


FIGURE 12-32 The binding-change mechanism of ATP synthesis from ADP and P_i . This view is looking up at F_1 from the membrane surface (see Figure 12-31). As the γ subunit rotates by 120° in the center, each of the otherwise identical F_1 β subunits alternates between three conformational states (O, open, with oval representation of the binding site; L, loose, with a rectangular binding site; T, tight, with a triangular site) that differ in their binding affinities for ATP, ADP, and P_i . The cycle begins (upper left) when ADP and P_i bind loosely to one of the three β subunits (here, arbitrarily designated β_1) whose nucleotide-binding site is in the O (open) conformation. Proton flux through the F_0 portion of the protein powers a 120° rotation of the γ subunit (relative to the fixed β subunits) (step **1**). This causes the rotating γ subunit, which is asymmetric, to push differentially against the β subunits, resulting in a conformational change and an increase in the binding affinity of the β_1 subunit for ADP and P_i (O \rightarrow L), an increase in the binding affinity of the β_3 subunit for ADP and P_i that were previously bound (L \rightarrow T),

and a decrease in the binding affinity of the β_2 subunit for a previously bound ATP (T \rightarrow O), causing release of the bound ATP. Step **2**: Without additional rotation, the ADP and P_i in the T site (here, in the β_3 subunit) form ATP, a reaction that does not require an input of additional energy due to the special environment in the active site of the T state. At the same time, a new ADP and P_i bind loosely to the unoccupied O site on β_2 . Step **3**: Proton flux powers another 120° rotation of the γ subunit, consequent conformational changes in the binding sites (L \rightarrow T, O \rightarrow L, T \rightarrow O), and release of ATP from β_3 . Step **4**: Without additional rotation, the ADP and P_i in the T site of β_1 form ATP, and additional ADP and P_i bind to the unoccupied O site on β_3 . The process continues with rotation (step **5**) and ATP formation (step **6**) until the cycle is complete, with three ATPs having been produced for every 360° rotation of γ . See P. Boyer, 1989, *FASEB J.* 3:2164; Y. Zhou et al., 1997, *Proc. Natl. Acad. Sci. USA* 94:10583; and M. Yoshida, E. Muneyuki, and T. Hisabori, 2001, *Nat. Rev. Mol. Cell Biol.* 2:669.

Uncouplers stimulates the rate of e transport

- Dinitrophenol, many detergents
- can dramatically increase the proton permeability of the membrane and thus act as an uncoupler. As a result, less ATP and more heat is generated.
- Heat production appears to be one of the uncoupling protein's main functions in mammalian cells.

Plant uniqueness

- NAD(P)H dehydrogenase complex facing intermembrane space facilitate oxidation of NAD(P)H
- This is insensitive to rotenone (rotenone resistant bypass)
- Existence of alternate oxidase

Cyanide Resistant Respiration (Alternate pathway)

- Alternate oxidase (32KDa-Membrane protein)-inhibitor Salicyl Hydroxamic acid-SHAM)
- Araceae(arum family)-25oC-volatilizes amines & indoles
- When cell saturated of ATP
- During stress-prevents the overreduction of ubiquinone pool

The internal, rotenone-insensitive NADH dehydrogenase, ND_{in} (NADH).

- This is one of the multiple NAD(P)H dehydrogenases found in plant mitochondria. It has been suggested to work as a nonproton-pumping bypass when complex I is overloaded. Complex I has a higher affinity for NADH (ten times lower K_m), than ND_{in} (NADH). At lower NADH levels in the matrix, typically when ADP is available (state 3), complex I will dominate, whereas when ADP is rate limiting (state 4), NADH levels will increase and ND_{in} (NADH) will be more active. The physiological importance of this enzyme is, however, still unclear.

Aerobic Respiration Yields about 60 Molecules of ATP per Molecule of Sucrose

The complete oxidation of a sucrose molecule leads to the net formation of

- 8 molecules of ATP by substrate-level phosphorylation (4 during glycolysis and 4 in the citric acid cycle)
- 4 molecules of NADH in the cytosol
- 16 molecules of NADH plus 4 molecules of FADH_2 (via succinate dehydrogenase) in the mitochondrial matrix

Energy stored per ATP-50.2KJ

Total=3012KJ

1 Sucrose= 5760KJ

Efficiency=52-55%

Fermentation=5%

Respiration-Regulation

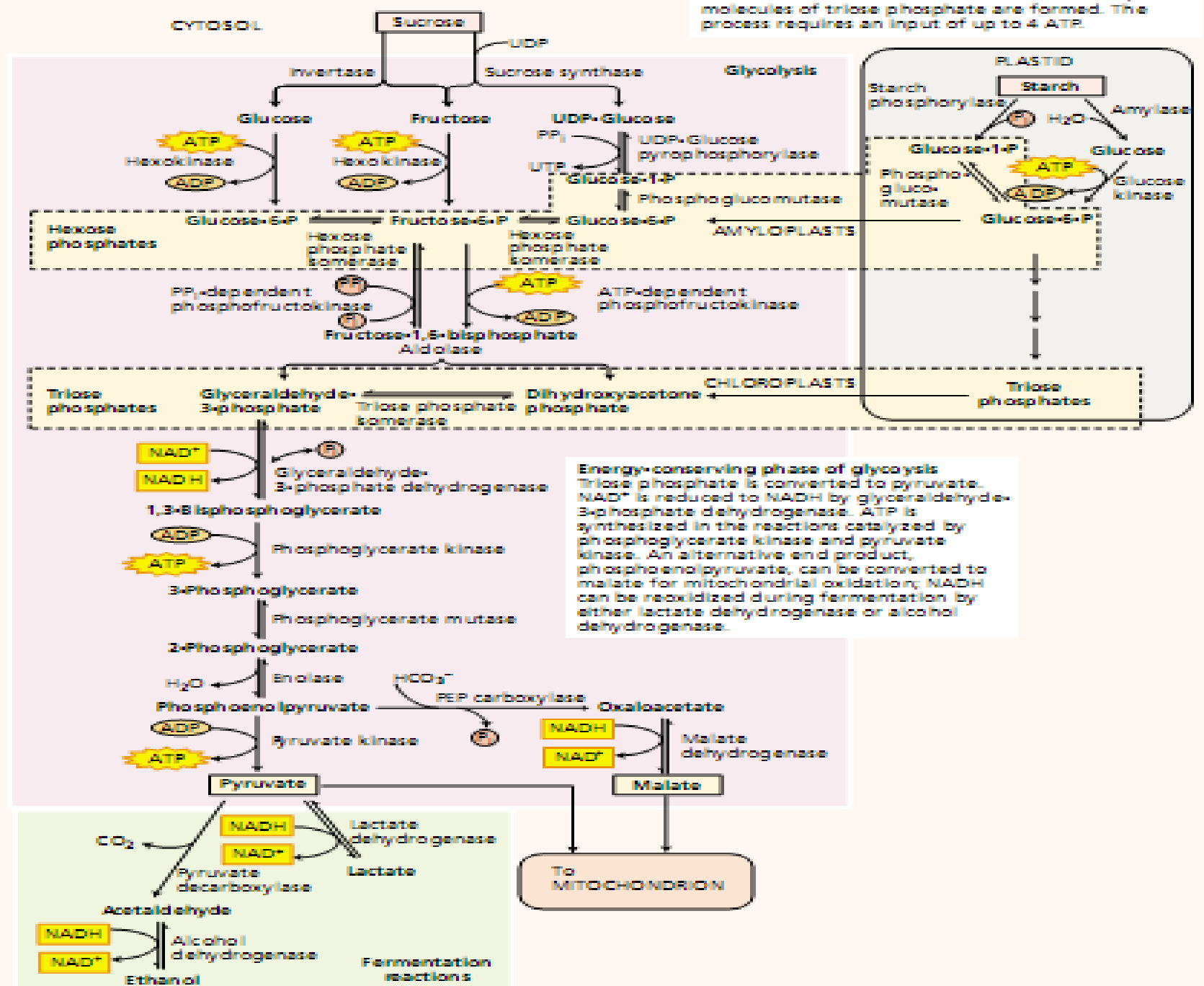
- General Factors
 - Rate at which photosynthate synthesized
 - Import into plant tissues
 - ATP utilization

Regulation of Glycolysis

- In plants, the control of glycolysis comes from the bottom up with primary regulation at the level of PEP metabolism by pyruvate kinase and PEP carboxylase and secondary regulation exerted by PEP at the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate
- In animals, the primary control operates at the phosphofructokinase, and secondary control at the pyruvate kinase.

(A)

Initial phase of glycolysis Substrates from different sources are channeled into triose phosphate. For each molecule of sucrose that is metabolized, four molecules of triose phosphate are formed. The process requires an input of up to 4 ATP.



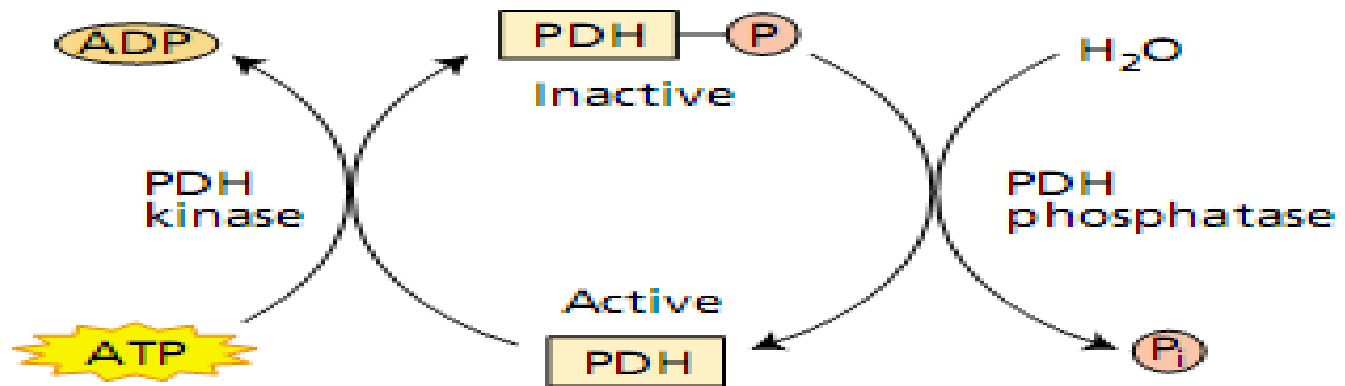
- **Phosphofructokinase ---PEP (inhibitor)—inhibition reduced by Pi (In animals, ATP inhibitor)**
- **Phosphofructokinase ---also feed back inhibited by 1)citrate 2)2-oxoglutarate 3)malate**
- **Reaction catalysed by Pyruvate kinase bypassed by PEP carboxylase**
- **Pyruvate kinase and PEPcarboxylase, the enzymes that metabolize PEP in the last steps of glycolysis are in turn sensitive to feedback inhibition by citric acid cycle intermediates and their derivatives, including malate, citrate, 2-oxoglutarate, and glutamate.**
- **Though the two enzymes are inhibited by similar metabolites, the PEPcarboxylase can under some conditions perform a bypass reaction around the pyruvate kinase. The resulting malate can then enter the mitochondrial citric acid cycle.**

The regulation of the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate is also complex.

- **Fructose-2,6-bisphosphate, another hexose bisphosphate, is present at varying levels in the cytosol.**
- **It markedly inhibits the activity of cytosolic fructose-1,6-bis-dependent phosphatase but stimulates the activity of P_{Pi} phosphofructokinase.**
- **These observations suggest that fructose-2,6-bisphosphate plays a central role in partitioning - dependent pathways flux between ATP-dependent and P_{Pi} of fructose phosphate metabolism at the crossing point between sucrose synthesis and glycolysis.**

Regulation-TCA Cycle

- Pyruvate dehydrogenase-phosphorylated-inactive
- Pyruvate dehydrogenase-non phosphorylated (phosphatase)-active
- PDH & citrate synthase-inhibited by NADH & ATP



Effect on PDH activity	Mechanism
Activating Pyruvate ADP Mg ²⁺ (or Mn ²⁺)	Inhibits kinase Inhibits kinase Stimulates phosphatase
Inactivating NADH Acetyl CoA NH ₄ ⁺	Inhibits PDH Stimulates kinase Inhibits PDH Stimulates kinase Inhibits PDH Stimulates kinase

Regulation of ETC

- Controlled by ATP/ADP ratio
- Less demand for ATP—less ADP—reduced oxidative phosphorylation—
increase in matrix NADH&ATP—inhibits pyruvate dehydrogenase, citrate sythataase—decreasae in TCA cycle activity and build up of intermediates (malate)—
inhibits pyruvate kinase—increasae in PEP—inhbits phosphofructokinase

60. All of the following are sources of energy for active transport except

(a) ATP

(b) proton gradients

(c) light

(d) All of the above

57. Actinomycin D is an inhibitor of
- (a) respiration
 - (b) photosynthesis
 - (c) protein synthesis
 - (d) transcription

64. The mitochondrial electron transport chain carriers are located

(a) in the inner mitochondrial membrane

(b) in the mitochondrial matrix

(c) in the inter-membrane space

(d) on the inner surface of the outer mitochondrial membrane

48. Which one of the following enzymes is tightly associated with the inner mitochondrial membrane?

- (a) Citrate synthase
- (b) Alpha-ketoglutarate dehydrogenase
- (c) Succinate dehydrogenase
- (d) Fumarase

53. During respiration in yeast the end product is-
- a. Water and CO_2
 - b. CO_2 , alcohol and energy
 - c. H_2S , $\text{C}_6\text{H}_{12}\text{O}_6$ and energy
 - d. Water and CO_2