Respiration

7

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Energy requirement for growth and functions in all living beings is met through ATP generated during respiration. Additionally in plants, light energy is conserved as ATP and NADPH, which are subsequently utilized for CO_2 assimilation. Besides, carbohydrates thus synthesized are consumed by animals as the primary source of energy. Though in animals lipids may also be consumed, in plants carbohydrates remain the main energy source. Plants store carbohydrates mainly as starch since it is osmotically inactive. All carbon accumulation in plants is the result of photosynthesis, which remains the source of energy as well as for biosynthesis of various other biomolecules. In cells hexose monophosphate pool constitutes an important component of carbohydrate metabolism. **Hexose monophosphate pool** maintained in cells consists of glucose 6-phosphate, glucose 1-phosphate, and fructose 6-phosphate. At the sinks, sucrose translocated from source is consumed during respiration. Respiration is an exergonic redox reaction with $\Delta G^{0'}$ value -5760 kJ.mol^{-1} of sucrose. Sucrose is oxidized to CO_2 coupled with the reduction of O_2 to water as follows:

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12C + 48H^+ + 48e^-$$

$$12O_2 + 48H^+ + 48e^- \rightarrow 24H_2O$$

Overall reaction can be written as:

$$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O$$

The reaction is apparently reverse of photosynthesis which also is a redox reaction. In photosynthesis, oxidation of H_2O, resulting in release of O_2, is coupled with reduction of CO_2 to carbohydrates. It is an endergonic reaction for which light energy is used. Since glucose is primarily utilized as energy source, starch is converted to simple sugars in storage organs of plants. The translocated form of carbohydrates, i.e., sucrose, is also hydrolyzed to produce monosaccharides. As a

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result, it is mainly glucose which is catabolized. Respiration may take place either in presence or in absence of oxygen depending upon the availability of oxygen. However, generally oxygen is not limiting for plants except when they grow under certain conditions including waterlogged situations and roots do not get any O_2 or enough O_2. Under aerobic conditions, complete oxidation of glucose results in free energy change of -2840 kJ.mol^{-1}. Respiration is a multistep process in which energy released during oxidation of the substrate is conserved as ATP. **Combustion** is also an energy-releasing process, but it differs from respiration, the latter being a multistep process catalyzed by enzymes, while combustion is burning of fuel with release of energy in a single step. In this chapter mainly the catabolism of glucose has been taken up since it is the major molecule which is produced either by hydrolysis of sucrose or starch. However, carbohydrates in other forms, such as triose phosphates or hexose phosphates, may also enter at relevant steps in the pathway. The major steps in respiration include catabolism of glucose in the cytosol, which involves glycolysis (synthesis of pyruvate from glucose), metabolic fate of pyruvate in the absence of oxygen (fermentation), and oxidative pentose phosphate pathway. Studying pyruvate metabolism in the presence of O_2, which occurs in mitochondrial matrix, will involve conversion of pyruvate to acetyl-CoA and tricarboxylic acid (TCA) cycle. Reduction of NAD^+ to NADH and FAD to $FADH_2$ occurs during TCA cycle. These reduced cofactors are oxidized through electron transport chain which is located in the inner mitochondrial electron transport. Proton gradient is created across the inner membrane of mitochondria coupled with electron transport which is responsible for ATP synthesis (Fig. 7.1). Mechanism of ATP synthesis is dealt in Chap. 8.

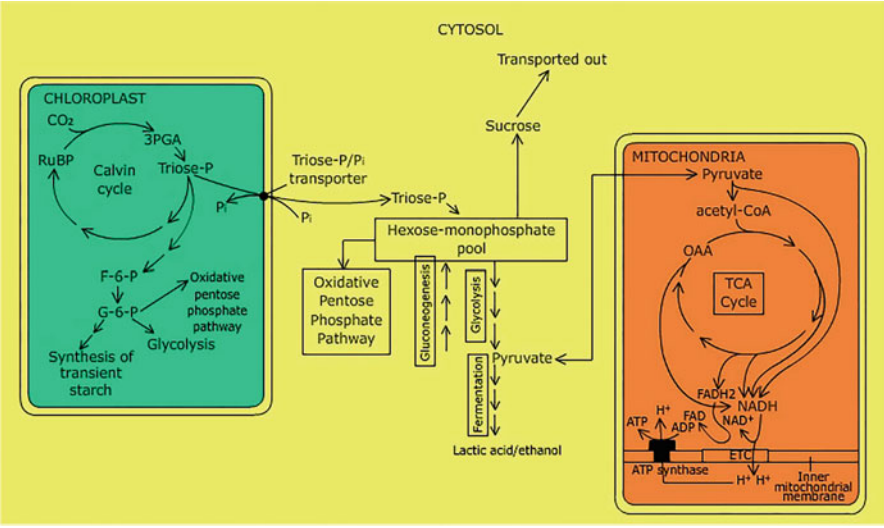

Fig. 7.1 Overview of respiration in a plant cell

7.1 Glycolysis

Glycolysis (Gr. glykos, sweet, and lysis, splitting) is a ten-step catabolic pathway during which a glucose molecule is converted to two molecules of three-carbon compound pyruvate. Two molecules of ATP are produced, and 2 NAD^+ molecules are reduced to NADH simultaneously. Many scientists, working with various strategies, have helped in elucidating the path of glucose to pyruvate, a pathway occurring in all organisms. Glycolytic pathway was understood mainly while working on fermentation process, the oldest biochemical process known. In 1860 Louis Pasteur had postulated that fermentation required intact living yeast cells, i.e., some kind of vital force was required for the fermentation to occur. However, this observation was found to be incorrect when in 1897 Hans Buchner and Eduard Buchner, German biologist brothers, demonstrated that fermentation could be carried out by cell-free extract of yeast as well. This leads to conclusion that since no vital force is required, fermentation could have been a chemical process. When the fermentation declined after sometime, it could be restored on adding inorganic phosphate to the medium. They demonstrated the presence of fructose 1,6-bisphosphate in the medium. After observing glucose 6-phosphate in the medium along with fructose 1,6-bisphosphate in a ratio of 3:1, Robinson concluded that phosphorylated intermediates are produced during fermentation. In one experiment with fermentation, Harden and Young used cell-free extract of yeast. The extract lost its fermentation capacity when heated or dialyzed, the catalytic activity could be restored after mixing both the fractions, i.e., dialyzable components in the heated fraction and functional proteins in dialyzed fractions. Loss in the catalytic activity of the heated fraction was due to denaturation of proteins, while the dialyzable components, which included smaller molecules such as NAD^+, ATP, etc., were lost due to dialysis of the second fraction. The two components were named as zymase (heat-labile) and co-zymase (dialyzable). Another approach used by Embden for elucidating the pathway was adding inhibitors of certain enzymes to the medium. He used iodoacetate, an inhibitor of enzymes containing –SH groups, which lead to the accumulation of fructose 1,6-bisphosphate. Otto Warburg identified the enzyme aldolase and the reaction catalyzed by it. Embden also observed that adding fluoride (another inhibitor which inhibits activity of enolase) to the fermentation medium leads to the accumulation of 3-phosphoglycerate and 2-phosphoglycerate, thus demonstrating the role of both aldolase and enolase. Complete pathway of fermentation was worked out. In RBCs or in some bacteria, glycolytic pathway is the sole source of energy. Even in plants growing under waterlogged conditions, since their roots are exposed to hypoxia or anoxia, this pathway is the only source of energy. Besides providing energy, various intermediates of the glycolytic pathway serve as precursors for many biosynthetic reactions. Glycolytic pathway is universally present in all organisms and is similar in fermentation as well as aerobic respiration. Unlike animal cells, glycolysis occurs both in cytosol and plastids of the plant cells, and the pathway is almost complete in both the locations, except that one or two enzymes may be missing in the plastidial pathway. These pathways do not occur in isolation, and exchange of various intermediates between the cytosol and plastids is

facilitated by the transporters localized on the inner membrane of plastids. Universality of this pathway indicates its significance. The ten reactions of the glycolysis can be considered into two phases: the *preparatory* phase is an energy investment phase in which two ATP molecules are consumed for each glucose molecule, which is converted to two molecules of triose phosphates. This phase is also known as mobilization phase since it correlates with mobilization of carbohydrates other than glucose, which include storage forms or the translocating form (sucrose) of carbohydrates. The second phase of glycolysis represents *payoff* phase which is an energy generation phase. During this phase triose phosphates are converted to pyruvate coupled with synthesis of four molecules of ATP and two molecules of NADH. This is also known as Embden-Meyerhof-Parnas (EMP) pathway after the name of the scientists who facilitated the elucidation of this pathway. Figure 7.2 represents the abbreviated scheme of the conversion of glucose to pyruvate. In case glucose enters glycolysis, first five reactions of the *investment phase* involve two phosphorylation reactions, two isomerization reactions, and one hydrolytic reaction, catalyzed by kinases, isomerases, and aldolase, respectively, thereby yielding two molecules of triose phosphate, i.e., glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Both triose phosphates are interconvertible and maintain equilibrium, but glyceraldehyde 3-phosphate is on the main pathway. During the latter *payoff* phase of glycolysis, glyceraldehyde 3-phosphate undergoes further activation and oxidation to yield compounds containing energy-rich phosphate bonds, i.e., 1,3-bisphosphoglycerate and phosphoenolpyruvate which are considered as super high-energy compounds since these have higher energy of hydrolysis than required for ATP synthesis. ATP is synthesized by the transfer of high-energy phosphate group of these compounds to ADP. ATP synthesis by this process without involvement of any electron transport is called **substrate-level phosphorylation**, i.e., transfer of phosphoryl group from a high-energy compound to ADP, yielding ATP. Substrate-level phosphorylation is different both from oxidative phosphorylation and photophosphorylation, since ATP synthesis is not coupled to any electron transport. Net reaction of glycolysis can be summarized as follows:

$$C_6H_{12}O_6 + 2ADP + 2P_i + 2NAD^+ \rightarrow \quad 2CH_3COCOOH + 2ATP + 2NADH + 2H^+ + 2H_2O$$

Initially produced phosphorylated sugars in glycolysis are low-energy compounds. In enzyme-catalyzed reactions, these are converted into compounds with high phosphoryl group transfer potential which subsequently generate ATP. Glycolytic equation can be resolved into two processes. The first one is exergonic reaction involving synthesis of pyruvate from glucose.

$$\text{Glucose} + 2NAD^+ \rightarrow \quad 2\text{Pyruvate} + 2NADH + 2H^+$$

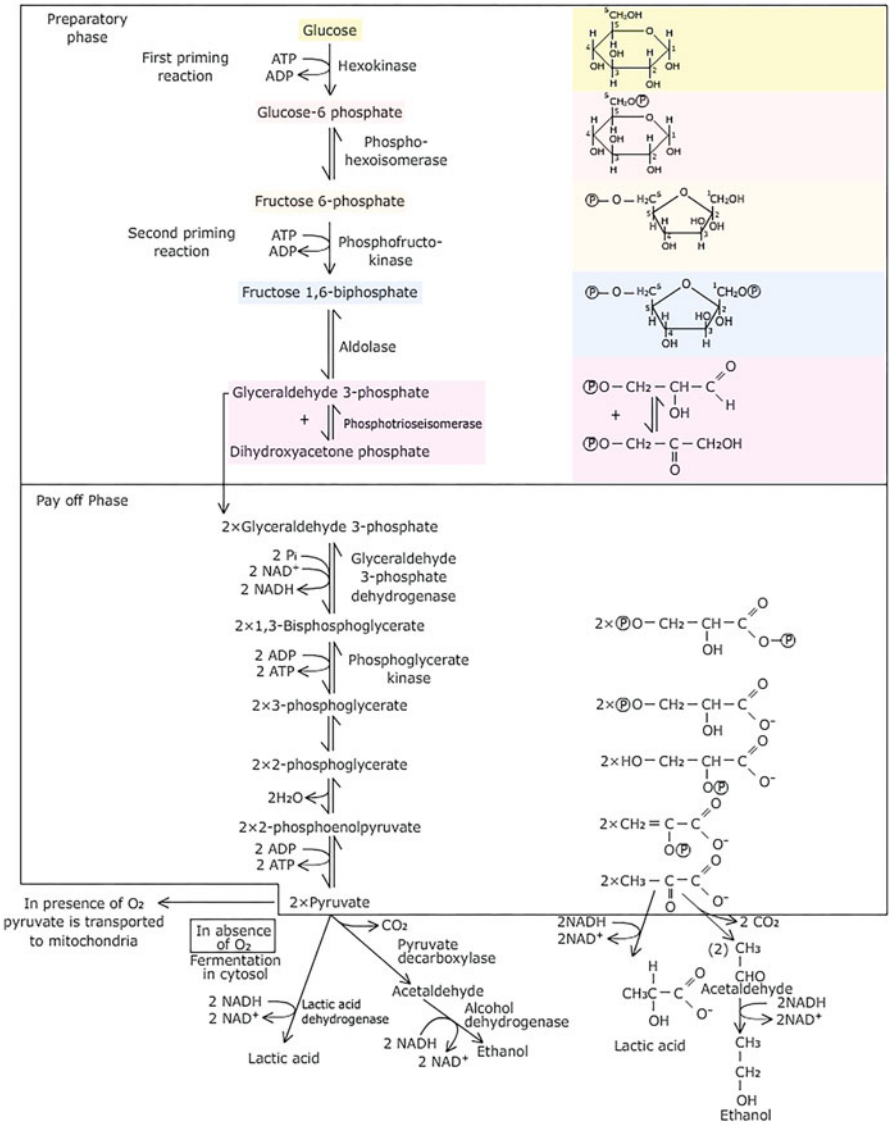

Fig. 7.2 Glycolysis and fermentation pathway. Fate of pyruvate is determined by the availability of O_2

$\Delta G^{0'}$ of the reaction is -146 kJ.mol^{-1}. The second process involves synthesis of ATP from ADP and is endergonic.

$$2ADP + 2P_i \rightarrow 2ATP$$

$\Delta G^{0'}$ of the reaction is 61 kJ.mol^{-1} (2×30.5 kJ.mol^{-1}).

Overall free energy change ($\Delta G^{0'}$) during glycolysis is -85 kJ.mol^{-1} (-146 kJ.mol^{-1} + 61 kJ.mol^{-1}). Let us consider the details of ten reactions occurring during conversion of glucose to pyruvate.

7.1.1 Preparatory Steps

Reaction 1: Phosphorylation of glucose: Though glucose is the source of energy, its energy status needs to be stepped up before it can enter glycolysis. Phosphorylation of glucose results in stepping up its energy state. The first reaction of glycolytic pathway, which results in phosphorylation of glucose at the sixth carbon (glucose 6-phosphate), is catalyzed by hexokinase (kinases are enzymes which transfer γ phosphate from ATP to the substrate or vice versa, but ATP is involved). Since phosphorylation of glucose is thermodynamically unfavorable, coupling this with an exergonic reaction is required which involves ATP hydrolysis. 30.5 kJ.mol^{-1} of energy is released when a molecule ATP is hydrolyzed, while phosphorylation of glucose requires 13.8 kJ.mol^{-1} (Table 7.1).

$$\alpha D - \text{Glucose} + \text{P}_\text{i} \rightarrow \quad \text{Glucose 6} - \text{phosphate} \qquad \Delta G^{0'} = 13.8 \text{ kJ.mol}^{-1}$$

$$\text{ATP} + \text{H}_2\text{O} \quad \rightarrow \quad \text{ADP} + \text{P}_\text{i} \qquad \Delta G^{0'} = -30.5 \text{ kJ.mol}^{-1}$$

Thus, the net free energy change during the reaction is -16.7 kJ.mol^{-1} of glucose under the standard conditions making the reaction irreversible.

$$\alpha D - \text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase;Mg}^{2+}} \alpha D - \text{Glucose 6} - \text{phosphate} + \text{ADP}$$
$$\Delta G^{0'} = -16.7 \text{ kJ.mol}^{-1}$$

Carbohydrates enter into metabolism in phosphorylated form. Phosphorylation of glucose is important, as glucose being neutral molecule could diffuse into and out of the cell across membrane. However, phosphorylation gives it a negative change which causes plasma membrane to be impermeable to glucose 6-phosphate. Glucose cannot be metabolized without getting phosphorylated. In most animals, plants and microbial cells, the enzyme that phosphorylates glucose is hexokinase. Magnesium ion (Mg^{2+}) is required for this reaction since substrate for the hexokinase reaction is Mg. ATP $^{2-}$ not ATP^{4-}. Mg^{2+} shields two negative charges of ATP making the terminal phosphorus atom an easier target for nucleophilic attack by an –OH group of glucose. In plants, glucose cannot be phosphorylated by any other enzyme, except hexokinases. The other way by which phosphorylated form of glucose can be produced is due to phosphorylatic breakdown of starch. No hexose phosphate phosphatase has been identified in plants. Hexokinases are generally characterized by their broad specificity for sugars. Thus they catalyze phosphorylation of various hexose sugars, which include fructose and mannose besides glucose, leading to their

Table 7.1 Reactions of glucose catabolism during glycolysis

	Reactions	Enzyme	$\Delta G^{0'}$ of the reaction in kJ. mol^{-1}
1.	Glucose + ATP → Glucose 6 − phosphate + ADP	Hexokinase	−16.7
2.	Glucose 6 − phosphate ↔ Fructose − 6 phosphate	Glucose-6-phosphate isomerase	1.7
3.	Fructose 6 − phosphate + ATP → Fructose 1, 6 − biphosphate + ADP	Phosphofructokinase	−14.2
	(In plants) Fructose 6 − phosphate + PP_i ↔ Fructose 1, 6 bisphosphate + P_i	PP_i-dependent phosphofructokinase	−2.9
4.	Fructose 1, 6 − bisphosphate ↔ 3 − Phosphoglyceraldehyde + dihydroxyacetone phosphate	Aldolase	23.8
5.	3 − Phosphoglyceraldehyde ↔ Dihydroxyacetone phosphate	Triose phosphate isomerase	7.5
6.	3 − Phosphoglyceraldehyde + P_i + NAD^+ ↔ 1, 3 − bisphosphoglycerate + NADH + H^+	G3P dehydrogenase	6.3
7.	1, 3 − bisphosphoglycerate + ADP ↔ 3 − Phosphoglycerate + ATP	Phosphoglycerate kinase	−18.5
8.	3 − Phosphoglycerate ↔ 2 − Phosphoglycerate	Phosphoglyceromutase	4.4
9.	2 − Phosphoglycerate + ADP ↔ Phosphoenolpyruvate + ATP	Enolase	7.5
10.	Phosphoenolpyruvate + ADP → Pyruvate + ATP	Pyruvate kinase	−31.4

utilization via glycolysis. Hexokinases are inhibited by the product of the reaction glucose 6-phosphate, which regulates the influx of hexose sugars into the glycolytic pathway. Glucose 6-phosphate can also be metabolized through oxidative pentose phosphate pathway. Structure of hexokinase provides striking evidence for induced fit model of enzyme catalysis. Hexokinase occurs in single form and consists of two lobed 50 kDa subunits. The enzyme is characterized by the presence of ATP-binding site. Daniel Koshland had proposed "induced fit" model and predicted many years back that hexokinase undergoes conformational change on binding with substrates, $Mg.ATP^{2-}$ and glucose. On binding with $Mg.ATP^{2-}$, there is a conformational change in the enzyme protein which brings it closer to the bound glucose. As a result, access to the water molecules is stopped, and phosphate group of the ATP is transferred to glucose molecule rather than being attacked by water. These predictions were later on confirmed in the enzyme obtained from yeast, both in presence or in absence of glucose. Unlike animals, hexokinase in plants is the only enzyme that phosphorylates glucose. However, fructose can be phosphorylated either by hexokinase or fructokinase. Almost three to ten genes have been identified which encode hexokinase. While different isoforms have been identified from the cytoplasm, only one hexokinase isoform is present in the plastids. Four isoforms of the enzyme have been identified in wheat germ. One isoform, called glucokinase, is found in the human liver, which is responsible for lowering blood sugar level.

Reaction 2: Isomerization of glucose 6-phosphate to fructose 6-phosphate: The second step in glycolysis is the isomerization of glucose 6-phosphate. The carbonyl oxygen of glucose 6-phosphate is shifted from C-1 to C-2. This results in isomerization of an aldose, i.e., glucose 6-phosphate, to a ketose—fructose 6-phosphate. Enzyme which catalyzes this reaction is called either phosphohexoisomerase or phosphoglucoisomerase or glucose phosphate isomerase. The enzyme facilitates conversion of pyranose ring of glucose to furanose ring of fructose. Isomerization has two relevances: First in the subsequent step of glycolysis when phosphorylation occurs at C1, the hemiacetal group –OH of glucose 6-phosphate would be more difficult to phosphorylate than a simple primary hydroxyl –OH. Second, for C-C bond cleavage, which occurs in the next step, isomerization to fructose with carbonyl group coming to C-2 activates C-3, making cleavage of the –C-C bond easy. In humans, this enzyme requires Mg^{2+} for the activity, and it is highly specific for glucose 6-phosphate. The $\Delta G^{0'}$ is 1.67 $kJ.mol^{-1}$. This small value means that the reaction operates near equilibrium in the cell and is easily reversible.

Reaction 3: ATP-driven second priming reaction: investment of another ATP: In the previous reaction, carbonyl group of glucose 6-phosphate moves from C-1 to C-2 portion, and the hydroxyl group at C-1 becomes available. Phosphorylation of this group occurs which is catalyzed by the enzyme **phosphofructokinase (PFK)**. Similar to reaction 1, phosphoryl group is provided by ATP and requires Mg^{2+}. Phosphorylation of fructose 6-phosphate is endergonic and is another priming reaction.

$$\text{Fructose 6} - \text{phosphate} + \text{P}_\text{i} \rightarrow \text{Fructose 1,6} - \text{bisphosphate}$$

$$\Delta G^{0'} = 16.4 \text{ kJ.mol}^{-1}$$

When coupled with the hydrolysis of ATP, the overall reaction becomes exergonic.

$$\text{Fructose 6} - \text{phosphate} + \text{ATP} \rightarrow \text{Fructose 1,6} - \text{bisphosphate}$$

$$\Delta G^{0'} = -14.2 \text{ kJ.mol}^{-1}$$

Reaction catalyzed by ATP-dependent PFK is essentially irreversible. At pH 7 and 37 °C, reaction catalyzed by phosphofructokinase lies mostly toward right, similar to the reaction catalyzed by the enzyme hexokinase, and is irreversible. Conversion of fructose 6-phosphate to fructose 1,6-bisphosphate, catalyzed by phosphofructokinase, is a committed step for glucose to be used for energy-releasing process rather than converting it to another sugar or just storing it. Thus, reaction catalyzed by PFK is an important regulatory step in glycolysis. ATP acts both as a substrate as well as allosteric inhibitor of the enzyme. Therefore, phosphofructokinase has two distinct binding sites for ATP, a high-affinity substrate site and a low-affinity regulatory site. In the presence of high ATP concentration, phosphofructokinase subunits behave cooperatively, i.e., the activity of one subunit influences the activity of all other subunits. When enzyme activity is plotted against fructose 6-phosphate, a sigmoidal curve is obtained which is typical of an allosterically regulated enzyme. Inhibition is reversed by AMP. Rise in the levels of AMP within the cell is coupled with decrease in ATP, and it is the ratio of ATP/AMP which regulates the activity of phosphofructokinase (PFK). Thus, the activity of phosphofructokinase depends on the levels of both ATP and AMP and is a function of cellular energy status. Glycolytic rate is decreased when ATP is in excess and increases when more ATP is required by the cell. In plants an additional cytosolic enzyme PP_i-dependent phosphofructokinase (pyrrophosphate: fructose 6-phosphate 1-phosphotransferase) is present which phosphorylates fructose 6-phosphate using pyrophosphate not ATP in a reversible reaction.

$$\text{Fructose 6} - \text{phosphate} + \text{PP}_\text{i} \rightarrow \text{fructose 1,6} - \text{biphosphate} + \text{P}_\text{i}$$

$$\Delta G^{0'} = -2.9 \text{ kJ.mol}^{-1}$$

The PP_i-dependent PFK (PP-PFK-1) is present in quite an appreciable amount in the cytosol of plant cells, but ATP-dependent PFK can take over the function of PP-PFK-1 in case of its absence or is present with reduced activity. This shows that quite a flexible system present in plants to provide more alternative reactions. Other molecules which are potent allosteric activators of PFK are fructose 2,6-bisphosphate and ribulose 5-phosphate. Besides, in plants the activity of PFK

is also inhibited by PEP. Thus, there are multple layers of regulation for the activity of PFK, which indicates reaction catalyzed by this enzyme to be the primary regulatory step of glycolysis.

Reaction 4: Hydrolytic cleavage of fructose 1,6-bisphosphate to two molecules of triose phosphate: Fructose 1,6-bisphosphate aldolase cleaves fructose 1,6-bisphosphate between the C-3 and C-4 carbons to yield two molecules of triose phosphates. The products are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P). The reaction is reversible but is in favor of fructose 1,6-bisphosphate. The reaction has $\Delta G^{0'}$ of +23.9 kJ.mol^{-1}. Equilibrium of the reaction involving the formation of two molecules of triose phosphates from one molecule fructose 1,6-bisphosphate depends on the concentration of all the three (the substrate and the products). The enzyme is called aldolase as the reaction is similar to the reverse of an aldol condensation reaction and is strongly endergonic. Aldolase is a tetrameric protein and an example of covalent catalysis. The enzyme activates the reaction by condensing the keto carbon at position two with ε-amino group of lysine residue present at the active site of the enzyme.

Reaction 5: Interconversion of Triose Phosphates: The two triose phosphates, 3-phosphoglyceraldehyde (G3P) and dihydroxyacetone phosphate (DHAP), produced from the previous reaction are interconvertible with the help of enzyme triose phosphate isomerase (TPI). The two triose phosphates occur in equilibrium favoring DHAP over G3P in a ratio of 22:1. Since G3P is on the main pathway of glycolysis and is substrate in the next glycolytic reaction, more of dihydroxyacetone phosphate (DHAP) is converted to G3P to maintain the equilibrium. The reaction is endergonic under standard conditions, but low intracellular concentration of glyceraldehyde 3-phosphate makes the reaction shift to right. Thus both triose sugars produced from glucose molecules are permitted to proceed further in glycolytic pathway. C-1, C-2, and C-3 of the initial molecules become equivalent to C-6, C-5, and C-4, respectively. Triose phosphate isomerization reaction completes the first phase of glycolysis. Each glucose molecule that passes through glycolysis is converted to two molecules of glyceraldehyde 3-phosphate. Till this point, two ATP molecules are consumed. In plants sucrose is the main carbohydrate to be metabolized. In case of sucrose catabolism, the number of ATP consumed, during this phase, will depend upon the mechanism of sucrose hydrolysis. If sucrose is hydrolyzed by invertase, 4 ATP will be consumed per molecule of sucrose, since two molecules of hexoses, i.e., glucose and fructose, will be produced as a result of hydrolysis of sucrose. In case hydrolysis of sucrose occurs by sucrose synthase activity, 2 ATP per molecule of sucrose will be consumed. Hydrolysis by this enzyme will result in the formation of one molecule of fructose, and another hexose will be phosphorylated, i.e., glucose 6-phosphate. As a result of this, 2 ATP will be saved. G3P is metabolized further to generate high-energy compounds which are responsible for synthesis of ATP. The next phase of glycolysis involves the *payoff* phase of glycolysis.

7.1.2 Entry of Molecules in Glycolysis Other than Glucose

Since in plants generally sucrose is translocated, this is the principal form of carbohydrates, which enters into the metabolism. Plant metabolism is characterized by **metabolic redundancy** because of the presence of alternate pathways. Starch is hydrolyzed either by hydrolytic or phosphorolytic enzymes in chloroplasts or in plastids of storage tissue. In chloroplasts, **transistory starch** is hydrolyzed during night, while storage starch hydrolysis occurs in the storage organs either in daytime or nighttime. Since glucose 1-phosphate is the product of starch hydrolysis, phosphorolytic enzymes have the advantage over hydrolytic enzymes as one ATP, required for the first priming reaction, is saved. Triose phosphates produced in light during CO_2 assimilation may enter glycolysis in chloroplasts directly during daytime. Glycerol is an important simple substance which can also be metabolized through glycolytic pathway. This metabolite, which is produced in substantial amount in cytosol as a result of hydrolysis of triglycerides especially in germinating oil seeds, is converted to glycerol 3-phosphate by the action of glycerol kinase. Glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate by cytosolic glycerol phosphate dehydrogenase, with NAD^+ as coenzyme. The dihydroxyacetone phosphate thus produced enters the glycolytic pathway as a substrate of triose phosphate isomerase.

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Glycerol kinase;}Mg^{2+}]{} \text{Glycerol phosphate} + \text{ADP}$$

$$\text{Glycerol phosphate} + NAD^+ \longrightarrow \text{Dihydroxyacetone phosphate} + \text{NADH} + H^+$$

7.1.3 *Payoff* Phase

The second half of the glycolytic pathway involves partial oxidation of triose phosphates as well as the reactions that convert terminal phosphate groups of the phosphorylated sugars into ATP.

Reaction 6: Generation of first energy-rich compound in glycolysis: It is the first oxidation reaction of glycolysis in which glyceraldehyde 3-phosphate is oxidized to 1,3-bisphosphoglycerate by the action of glyceraldehyde 3-phosphate dehydrogenase. Oxidation of glyceraldehyde to the acid is coupled to reduction of NAD^+ to NADH. Energy released during oxidation is conserved as carboxylic acid anhydride bond with phosphate group, known as acyl phosphate. The compound formed is 1,3-bisphosphoglycerate (BPG) which has very high $\Delta G^{0'}$ of hydrolysis, i.e., -49.4 kJ.mol^{-1}. Hydrolysis of 1,3-bisphosphoglycerate drives synthesis of ATP from ADP, as acyl phosphate group of BPG is much more energy rich than the phosphate anhydride of ATP. Glyceraldehyde 3-phosphate dehydrogenase activity

is inhibited by iodoacetate, which blocks cysteine -SH group present at the active site of the enzyme, necessary for carrying out reaction.

Reaction 7: First ATP generation reaction in glycolysis: Because of its high-energy transfer potential, 1,3-bisphosphoglycerate has a strong tendency to transfer its acyl phosphate group to ADP, resulting in the formation of ATP and 3-phosphoglycerate. This **substrate-level phosphorylation** reaction is catalyzed by phosphoglycerate kinase. Since each molecule of glucose produces two triose phosphates, two ATPs will be produced as a result of this reaction. The phosphoglycerate kinase reaction pays off the debt created by the priming reactions. This phosphoryl transfer is facilitated by Mg^{2+} since true nucleotide substrate for the reaction is $Mg.ADP^{2-}$. It is appropriate to consider the sixth and seventh reactions of glycolysis as coupled reactions since 1,3-bisphosphoglycerate is as an intermediate produced during the sixth reaction. The sum of both reactions can be written as:

$$\begin{aligned}&\text{Glyceraldehyde 3} - \text{phosphate} + \text{NAD}^+ + \text{ADP} + \text{P}_\text{i}\\ &\rightarrow 3 - \text{phosphoglycerate} + \text{NADH} + \text{H}^+ + \text{ATP}\end{aligned}$$

The reaction is exergonic and net change in standard free energy ($\Delta G^{0'}$) is -12.2 kJ.mol^{-1}. Under cellular conditions, the reaction is reversible.

Reaction 8: Phosphoryl Transfer Reaction: Phosphoglycerate mutase catalyzes the transfer of phosphate group from the third to second position in 3-phosphoglycerate to produce 2-phosphoglycerate. Mg^{2+} is required for this reversible endergonic reaction. $\Delta G^{0'}$ value of this reaction is 4.4 kJ.mol^{-1}. The intracellular level of 3-phosphoglycerate is high relative to that of 2-phosphoglycerate so that in vivo the reaction proceeds uninterrupted to the right. The enzyme phosphoglycerate mutase requires 2,3-bisphosphoglycerate as an activator initially to phosphorylate histidine, which is present at the active site of the enzyme, to phosphohistidine. During the reaction, firstly the phosphate group is transferred from phosphohistidine to the substrate 3-phosphoglycerate, resulting in the formation of an intermediate 2,3-bisphosphoglycerate. This is followed by the breakdown of the enzyme-bound intermediate regenerating the phosphorylated histidine of the enzyme. The product 2-phosphoglycerate is released. Mg^{2+} is required for the reaction. Enzyme for this particular reaction has been found to be absent in the chloroplasts, as 3-phosphoglycerate is also produced during initial reactions of Calvin cycle. The enzyme, phosphoglycerate mutase, if present would draw carbon from Calvin cycle to glycolysis.

Reaction 9: Synthesis of High-Energy Compound Phosphoenolpyruvate (PEP): Enolase catalyzes conversion of 3-phosphoglycerate, having very low potential as phosphoryl group donor, to phosphoenolpyruvate, which has a very high potential for phosphoryl group transfer. The $\Delta G^{0'}$ for this reaction is very low, i.e., 7.5 kJ.mol^{-1}. It may be difficult to understand how enolase reaction transforms a substrate with a relatively low free energy of hydrolysis into a product (PEP) with a

very high free energy of hydrolysis. 2-Phosphoglycerate and PEP contain about the same amount of potential metabolic energy with respect to their respective decomposition to P_i, CO_2, and H_2O. Enolase is responsible for rearrangement in the structure of the 2-phosphoglycerate by removing a water molecule into a form which is capable of releasing more of the potential energy upon hydrolysis. Enzyme is inhibited by fluoride ions in the presence of phosphate. Yeast enolase is a dimer of identical subunits.

Reaction 10: Generation of Second Molecule of ATP PEP produced in the previous reaction has a very high phosphoryl group transfer potential and $\Delta G^{0'}$ for hydrolysis of PEP is -61.9 kJ.mol^{-1}. This reaction is catalyzed by the enzyme pyruvate kinase. It is another example of substrate-level phosphorylation in which phosphoryl group of phosphoenolpyruvate is transferred to ADP. It is Mg^{2+} requiring reaction which is stimulated by K^+. Significant change in the free energy for the conversion of PEP to pyruvate is mainly because of highly favorable and spontaneous conversion of enol **tautomer** of pyruvate to the more stable keto form after the phosphoryl transfer step. About half of the energy, which is released on hydrolysis of PEP, is conserved as ATP phosphoanhydride bond ($\Delta G^{0'} = -30.5$ kJ.mol^{-1}), while the remaining half (-31.4 kJ.mol^{-1}) is responsible for pushing the reaction forward.

7.1.4 Stoichiometry of Glycolysis

For each molecule of glucose metabolized through glycolysis, 2 ATP molecules are consumed during initial five reactions which constitute the preparatory phase of glycolysis. During *payoff* phase, there is generation of 4 ATPs and 2 NADH (since one glucose molecule produces two triose phosphates, and each triose phosphate produces 2 ATP and 1 NADH). As a result, there is net gain of 2 ATP and 2 NADH. However, the number of ATP molecules may vary depending upon the kind of carbohydrates metabolized and also the process of hydrolysis involved in sucrose or starch.

Glycolysis is the sole source of energy under anaerobic conditions which plants rarely experience except in certain situations such as in germinating seeds or in roots exposed to hypoxia or anoxia under waterlogged conditions. However, since the amount of energy produced is much less because of partial oxidation under anaerobic conditions, more amount of glucose is consumed (**Pasteur effect**). Since oxidized form NAD^+ is required for continuation of glycolysis, under anaerobic conditions NADH is oxidized during fermentation. Many glycolytic intermediates serve as precursors (Fig. 7.3).

7.1.5 Significance of Phosphorylated Intermediates

Nine out of ten intermediates of the glycolytic pathway are phosphorylated. This is significant because of the following reasons:

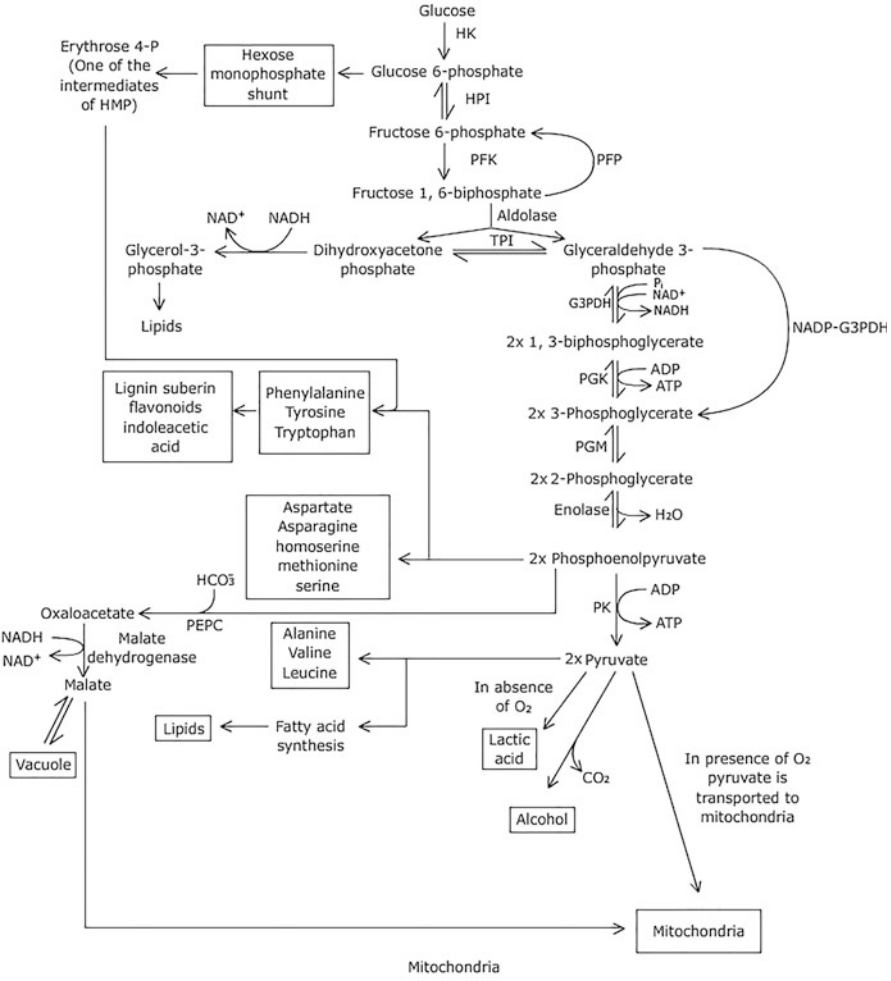

Fig. 7.3 Cytosolic glycolytic pathway and its role in metabolism of plants. HK, hexokinase; HPI, hexose phosphate isomerase; PFK, phosphofructokinase; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglyceromutase; PK, pyruvate kinase; PEPC, PEP carboxylase; TPI, triose phosphate isomerase; HMP, hexose monophosphate pathway

1. Plasma membrane is impermeable to phosphorylated sugars because of the absence of their transporters. As a result, the phosphorylated intermediates of glycolysis are retained inside the cell without any expenditure of energy involved.
2. There is a decrease in activation energy when phosphorylated substrates bind with the active sites of the enzymes. Active sites of many of the enzymes are specific for binding with Mg^{2+}complexes of phosphorylated sugars.

3. Energy consumed during priming of sugars at the expense of ATP is conserved as phosphorylated sugars, such as glucose 6-phosphate. High-energy compounds, formed due to rearrangement in the molecules in enzyme-catalyzed reactions, subsequently donate the phosphoryl group to ADP, resulting in the synthesis of ATP.

7.1.6 Regulation of Glycolysis in Plants

Regulation of any pathway is mainly determined by the requirement of the products. Irreversible reactions are the sites for regulation in glycolysis. Standard free energy changes ($\Delta G^{0'}$) vary from positive to negative during the ten reactions of glycolysis. Under cellular conditions, $\Delta G^{0'}$ of the reactions can be grouped into two distinct classes: (1) for reactions 2 and 4 through 9, free energy change is very close to zero, meaning these reactions operate essentially at equilibrium. Small changes in the concentration of reactants and products can push the reaction either forward or backward to maintain K_{eq}. (2) However, reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are exothermic, exhibiting large change in free energy under cellular conditions, and are not reversible. These reactions are the sites of glycolytic regulations. When these enzymes are active, gylcolysis proceeds in forward direction, and glucose is readily metabolized to pyruvate. Inhibition of these enzymes brings glycolysis to halt. The main point of regulation of glycolysis is the reaction which involves conversion of fructose 6-phosphate to fructose 1,6-bisphosphate. Both in plants and animals, there are two major enzymes which regulate this irreversible reaction, ATP-dependent PFK and fructose 1,6-biphosphate phosphatase, which regulate flux of carbon through glycolysis and **gluconeogenesis**, respectively. In plants, additional enzyme—PP_i-dependent phosphofructokinase—is present. The reaction catalyzed by this enzyme is readily reversible. The activity of the three enzymes (discussed above) is regulated to keep a balance between respiration and synthesis of sucrose or polysaccharides. A prominent difference between animal and plant glycolysis is the primary control point of regulation of glycolysis. In plants, primary control point is at the reaction catalyzed by pyruvate kinase. The enzyme is allosterically inhibited by pyruvate and ATP, resulting in the accumulation of PEP. PEP is an allosteric inhibitor of PFK. Reaction catalyzed by PFK is secondary regulatory point in which P_i acts as an activator of the enzyme. Thus, ratio of PEP and P_i regulates the activity of PFK, with PEP inhibiting and P_i promoting enzyme activity. This type of regulation is known as "bottom-up" regulation. It is significant in plants, since PEP is also metabolized by pathways other than glycolysis. This includes carboxylation of PEP by PEP carboxylase, which results in synthesis of OAA and malate. Malate may be transported to mitochondria. In plants instead of ATP and AMP, accumulation of PEP is the primary control point of glycolysis. PEP is the allosteric inhibitor of PFK, which is the secondary control point (Fig. 7.4). Activity of pyruvate kinase is also inhibited by intermediates of

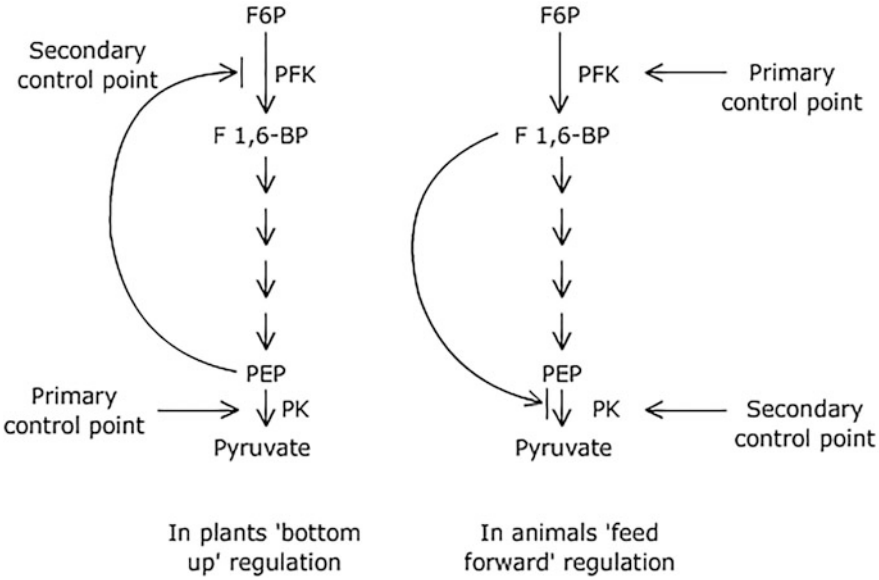

Fig. 7.4 Regulation of glycolysis in plants and animals

TCA cycle. Contrary to this, regulation of glycolysis in animals is "feed forward" in which the primary control point is the reaction catalyzed by PFK, which leads to accumulation of F 6-P. F 6-P inhibits the activity of pyruvate kinase. Thus, reaction catalyzed by pyruvate kinase is the secondary control point for glycolysis in animals. PEP carboxylase (or other enzymes that metabolize PEP) releases the inhibition of phosphofructokinase by PEP, hence allowing glycolysis to proceed. Reduced levels of cytosolic phosphoenolpyruvate result in elevated levels of fructose 2,6-bisphosphate, which affects the reaction catalyzed by PFK in both directions. Plant pyruvate kinase is not activated by fructose 1,6-bishosphate (which is seen in the case of animals). The advantage of "bottom-up" regulation of glycolysis in plants is that it allows them to control net glycolytic flux of pyruvate independent of related metabolic processes, such as the Calvin cycle and sucrose-triose phosphate-starch interconversion. Activity of hexokinase is allosterically inhibited by ATP or fructose 6-phosphate. However, no glucose 6-phosphatase has been identified in plants. Besides soluble cytosolic pool, a substantial pool of glycolytic enzymes bound to the surface of mitochondria exists under high respiratory rates. These are subjected to regulation by TCA cycle intermediates. Because of **substrate channeling** (Box 7.1), positioning of glycolytic intermediates on mitochondrial surface increases the efficiency of the process.

Box 7.1: Multienzyme Complex (Significance)
Enzymatic reaction rates are limited by the frequency at which enzymes collide with their substrates. If a series of reactions occurs within a multienzyme complex, the distance that substrates must diffuse between active sites is minimized; thereby achieving a rate enhancement. Multienzyme complex formation provides the means for substrate **channelling**, i.e., passing of metabolic intermediates between successive enzymes in a metabolic pathway, thereby minimizing diffusion of intermediates and avoiding side reactions. Enzymes in pyruvate dehydrogenase pathway are coordinated in multienzyme complex.

7.2 Oxidative Pentose Phosphate Pathway (OPPP)

In the 1930s Otto Warburg made an observation that in addition to glycolysis, an alternate metabolic pathway exists in living cells for glucose metabolism. The enzyme was isolated from yeast and erythrocytes and was named as *zwischenferment*. Instead of diphosphopyridine, DPN (now known as NAD^+), the enzyme used another coenzyme triphosphopyridine nucleotide TPN (now known as $NADP^+$) for oxidation of glucose to 6-phosphogluconate. However, details of the pathway, result of the work done by Horeckert et al., were presented in 1955. Enzymes, which catalyze the reactions, have been isolated, and regulatory mechanism involved has also been worked out. In plants, besides cytosol, the pathway also operates in plastids. It is not an alternate pathway but occurs in addition to glycolysis. Almost 10–15% of glucose is metabolized through this pathway. It consists of an oxidative phase and a non-oxidative phase. It is known as oxidative pentose phosphate pathway (OPPP), since first two irreversible reactions are oxidative, which are coupled with reduction of $NADP^+$ to NADPH. The subsequent reversible reactions are similar to those involved in regeneration of RuBP during Calvin-Benson cycle. Calvin-Benson cycle is also known as reductive pentose phosphate pathway since that is an assimilatory reductive pathway. OPPP is also called as **hexose monophosphate shunt (HMP)**, as hexose phosphates are regenerated during the cycle, or **phosphogluconate pathway** as first compound formed is phosphogluconate. The pathway provides cells with NADPH, which are required for biosynthetic anabolic reactions, and is especially significant in the plastids of the dark-grown plants or in animal cells (such as RBC) which do not have any other source of NADPH. NADPH is required for glutathione production which is essential for maintaining membrane integrity. Non-operation of this pathway, because of mutant forms of the enzyme glucose 6-phosphate dehydrogenase, causes hemolysis in animal cells.

OPPP includes an oxidative phase, which involves two irreversible reactions, while non-oxidative phase consists of reversible reactions.

7.2.1 Oxidative Phase

Oxidation of glucose 6-phosphate by the enzyme glucose 6-phosphate dehydrogenase results in the formation of 6-phosphoglucono-δ-lactone, an unstable intermediate which is spontaneously decomposed to form 6-phosphogluconate. This oxidative reaction is coupled with reduction of $NADP^+$ to NADPH since the enzyme is $NADP^+$-specific. It is highly exergonic reaction and is irreversible. This is the regulatory step of the pathway, which determines metabolic fate of glucose 6-phosphate, either through OPPP or glycolysis. The second reaction, the oxidative decarboxylation of 6-phosphogluconate, results in the formation of ribulose 5-phosphate. It is catalyzed by $NADP^+$-specific 6-phosphogluconate dehydrogenase which again is coupled with reduction of $NADP^+$ to NADPH, and one carbon is lost as CO_2 during the reaction (Fig. 7.5).

7.2.2 Non-oxidative Phase

Carbon atoms of ribulose 5-phosphate are reconstituted to regenerate glucose 6-phosphate during non-oxidative phase of the pathway which involves reversible reactions. Ribulose 5-phosphate is interconverted to ribose 5-phosphate and xylulose 5-phosphate through the action of ribose phosphate isomerase and ribulose phosphate epimerase, respectively. The two enzymes **transketolase** (TK) and **transaldolase (TA)** catalyze the interconversion of the intermediate sugars. The formation of sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate from ribose 5-phosphate and xylulose 5-phosphate is catalyzed by transketolase. Transaldolase is the characteristic enzyme of OPPP, which catalyzes the transfer of non-phosphorylated 3-carbon fragment from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate after hydrolyzing –C-C bond. As a result of this reaction, fructose 6-phosphate and erythrose 4-phosphate are synthesized. The next step also

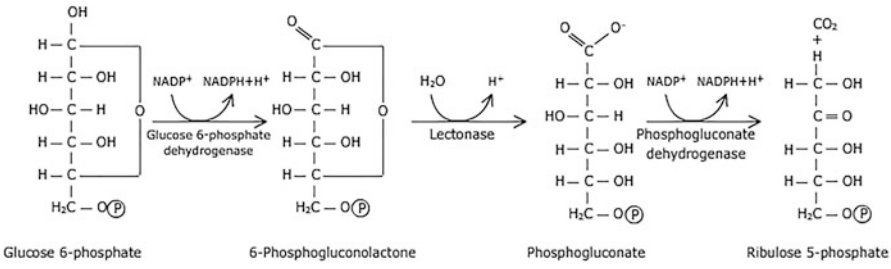

Fig. 7.5 Reactions of oxidative phase of oxidative pentose phosphate pathway

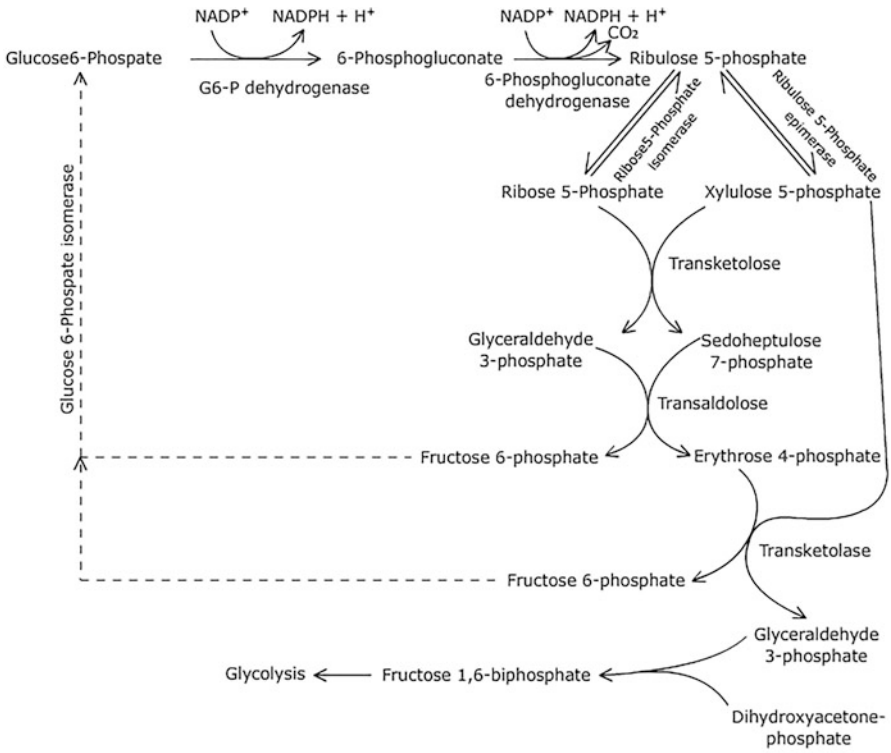

Fig. 7.6 Oxidative pentose phosphate pathway (OPPP)

involves transketolase, which results in the formation glyceraldehyde 3-phosphate and fructose 6-phosphate from erythrose 4-phosphate and xylulose 5-phosphate because the enzyme catalyzes transfer of 2-carbon fragment from xylulose 5-phosphate to erythrose 4-phosphate. The two triose phosphates condense together to form hexose phosphates (Fig. 7.6).

If removal of any of the intermediates is bypassed, overall reaction of the pathway can be written as:

$$6\text{Glucose } 6-\text{P} + 12\ \text{NADP}^{+} \rightarrow 5\text{Glucose } 6-\text{P} + \text{P}_{\text{i}} + 6\text{CO}_2 + 12\text{NADPH} + 12\ \text{H}^{+}$$

7.2.3 Significance of OPPP

1. In plants growing in dark or during greening of leaves when photosynthetic apparatus has not yet developed fully and the light generated NADPH is not available, OPPP is the sole source of NADPH, which is required for various

biosynthetic reactions. In plastids, it is required for fatty acid biosynthesis and nitrogen metabolism. Unlike plants, in animal cells, cytosolic OPPP is the only source for NADPH. NADPH is also required under oxidative stress as it is needed for the reactions which are involved in removing reactive oxygen species (ROS).
2. In plant cells, NADPH is also the source of ATP generation, since external NADPH dehydrogenase present on the cytosolic side of the inner mitochondrial membrane can oxidize NADPH, and the electrons move downhill through the electron transport chain.
3. NADPH produced during oxidation of glucose 6-phospate may also signal the sugar status in amyloplasts and may regulate enzymes of starch synthesis through thioredoxin system.
4. The pathway also provides ribose 5-phosphate which is the precursor for nucleotide biosynthesis as well as for ATP, NAD^+, and $NADP^+$.
5. Erythrose 4-phosphate along with phosphoenolpyruvate (a glycolytic intermediate) is required for the biosynthesis of plant phenolic compounds, such as lignin, flavonoids, phytoalexins, and aromatic amino acids (Fig. 7.3).

7.2.4 Regulation of OPPP

OPPP is mainly regulated by the redox status of the cell. Ratio of NADPH/$NADP^+$ regulates the activity of glucose 6-phosphate dehydrogenase, the first enzyme of the pathway. The enzyme is active if the ratio is low. Strong inhibition of the activity of glucose 6-phosphate dehydrogenase by NADPH indicates important role of OPPP in providing NADPH. In case not required, the nucleotide precursor ribose 5-P may enter glycolysis after it is converted back to glucose 6-P by the reversible non-oxidative reactions. If NADPH is not needed, ribose 5-P may be generated by alternate reactions than by OPPP. In plants regulation of OPPP is significant especially in plastids, where reductive pentose phosphate pathway (Calvin cycle) occurs. If both pathways operate simultaneously at the same place, fructose 6-phospate, an intermediate of the Calvin cycle, is oxidized through OPPP producing 2 NADPH and ribulose 5-phosphate. Ribulose 5-phosphate will enter Calvin cycle requiring 2 NADPH and 3 ATP for CO_2 assimilation. As a result, there is a net loss of 3 ATP molecules. NADPH produced during OPPP is also consumed. Such cycles are called **futile cycles**. Loss of ATP molecules is avoided since these two cycles operate at different times. Calvin cycle operates during daytime since the enzymes of the cycle are activated in light by the thioredoxin-ferredoxin system. On the contrary OPPP operates during dark since, unlike Calvin cycle enzymes, the first two enzymes of the cycle are inactive when reduced, which again is regulated by the same thioredoxin-ferredoxin system. Redox status of specific cysteine residues of OPPP enzymes plays an important role in their regulation. OPPP also shares some of the reactions with glycolysis. Operation of glycolysis and OPPP is interdependent since the two share some common intermediates and are operating at the same place at the same time. In both the cycles, phosphotrioseisomerase is one of the most active enzymes which catalyzes reversible interconversion of glyceraldehyde 3-phosphate

and dihydroxyacetone phosphate. In OPPP, the formation of dihydroxyacetone phosphate is favored which is transported out of the plastids by triose phosphate/P_i antiporters. On the contrary, glyceraldehyde 3-phosphate is favored in glycolysis and on the main pathway.

7.3 PEP Metabolism in Cytosol

The fate of PEP produced during glycolysis is determined by the cytosolic enzymes. Conversion of PEP to pyruvate is catalyzed either by pyruvate kinase or by PEP phosphatase. In the former case, ATP is synthesized, while in the reaction catalyzed by PEP phosphatase, there is no ATP synthesis (Fig. 7.7). Alternatively, PEP is metabolized by PEP carboxylase which catalyzes its carboxylation, resulting in the synthesis of oxaloacetate, which may either be transported to mitochondria as such or may be reduced to malate in cytosol using NADH. Malate may be transported to mitochondria. Thus reducing power (NADH) equivalents are also shuttled to mitochondria as malate. Once inside mitochondria, malate is oxidized to OAA coupled with reduction of NAD^+ to NADH. The reaction is catalyzed by malate dehydrogenase. In mitochondria, alternatively malate is converted to pyruvate by NAD^+ malic enzyme. Pyruvate enters TCA cycle through link reaction.

7.4 Pyruvate Metabolism

Pyruvate, the end product of glycolysis, is metabolized differently under aerobic and anaerobic conditions. Under aerobic conditions pyruvate is oxidized to acetyl-CoA in mitochondria, which enters the citric acid cycle, and the NADH formed in glycolysis is oxidized to NAD^+ through NADH shuttle system. NADH is not transported across inner mitochondrial membrane directly because of its transporters being absent. Under anaerobic conditions pyruvate is metabolized through fermentation.

7.4.1 Fermentation

Plants are rarely exposed to anaerobic conditions. Anaerobic conditions prevail when plants are either completely submerged or O_2 is completely exhausted by respiration resulting in anoxia or in germinating seeds. O_2 is scarce or even absent in waterlogged soils, and, therefore, respiration is inhibited in roots. Because of mitochondrial electron transport not functioning in the absence of O_2, which is the terminal acceptor of electrons, NADH will not be oxidized. Failure to regenerate NAD^+ will leave the cell with no electron acceptor for the oxidation of glyceraldehyde 3-phosphate, resulting in stopping of energy yielding reactions of glycolysis. Continuation of glycolysis will require an alternate path for regeneration of NAD^+. In the absence of O_2, the end product of glycolysis, pyruvate, is metabolized either

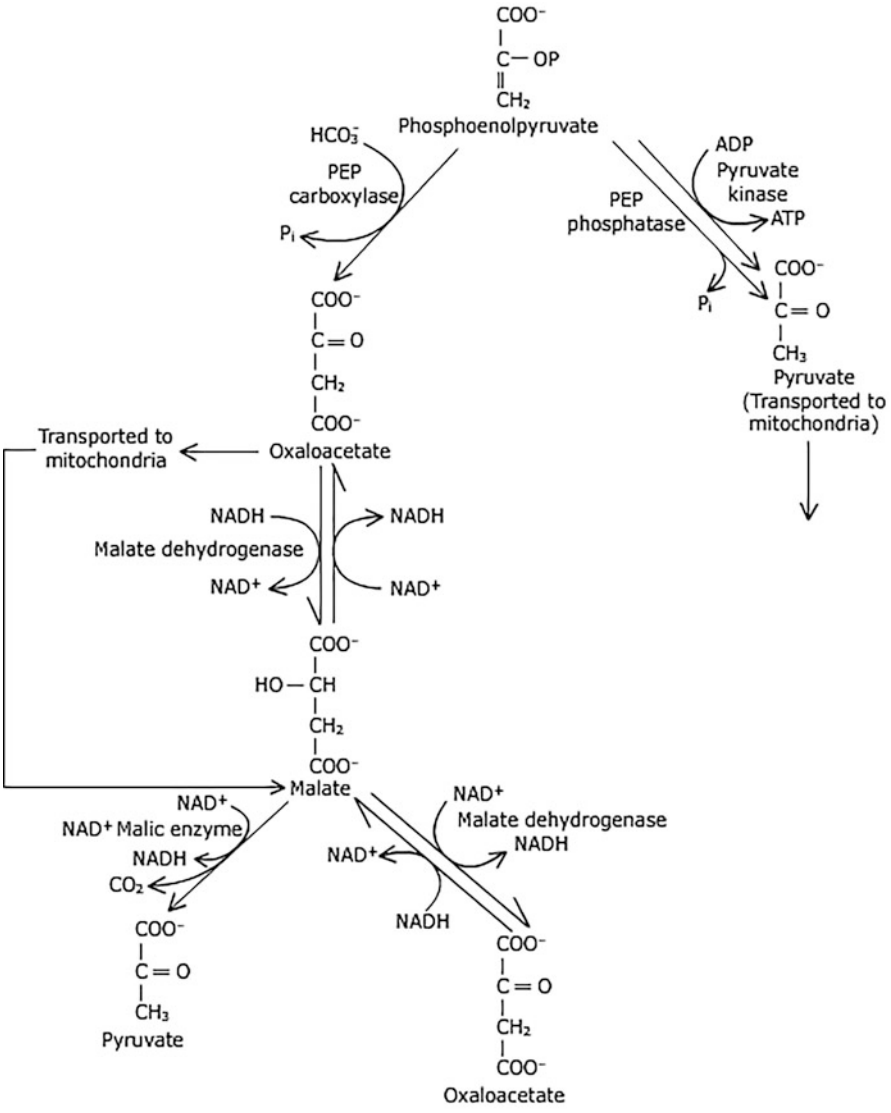

Fig. 7.7 During respiration, phosphoenolpyruvate is converted to either pyruvate or oxaloacetate in the cytosol which can also be transported to mitochondria. OAA is transported either as OAA or malate after it is reduced by the enzyme malate dehydrogenase. Inside mitochondria, malate is metabolized either by malate dehydrogenase or NAD^+ malic enzyme

through lactic acid or ethanol fermentation, resulting in oxidation of NADH to NAD^+. Breakdown of carbohydrates to ethanol and CO_2 is the main fermentative pathway in plants. Some lactate is also formed due to the activity of lactic acid dehydrogenase, but in contrast to lactic acid fermentation, ethanol fermentation can continue

for days in anoxic tissues since ethanol is nontoxic and can leach out of the plasma membrane. Initially oxygen deficiency induces synthesis of lactic acid dehydrogenase. Lactic acid formation lowers the cytosolic pH, resulting in inactivation of lactic acid dehydrogenase and upregulation of alcohol dehydrogenase. Oxidation of NADH to NAD^+ is coupled with reduction of pyruvate to either lactate or ethanol. There is no net change in oxidation state of carbon in glucose ($C_6H_{12}O_6$) when converted to lactate, and the H/C ratio also remains the same. Most of the energy is still retained in the molecule of lactate. During fermentation there is net production of 2 ATP for every molecule of glucose being converted to either lactate or alcohol.

In alcoholic fermentation, decarboxylation of pyruvate is catalyzed in an irreversible reaction by the action pyruvate decarboxylase. The enzyme requires Mg^{2+} and a tightly bound cofactor—thiamine pyrophosphate. Alcohol dehydrogenase catalyzes the reduction of acetaldehyde to ethanol using NADH, which is oxidized to NAD^+. End products of ethanol fermentation are ethanol and CO_2. CO_2 produced by pyruvate decarboxylase action in brewer's yeast is responsible for the characteristic carbonation of champagne. Modern-day brewing involves several enzymatic processes. Alcohol dehydrogenase occurs in organisms that metabolize ethanol which also includes human beings. In the liver the enzyme catalyzes oxidation of ethanol, followed by reduction of NAD^+ to NADH. This reaction is the reverse of production of ethanol during fermentation.

7.4.2 Pyruvate Metabolism in Mitochondria

Glycolysis is not the only energy-yielding catabolic pathway. Cells that rely exclusively on glycolysis to meet their energy requirement actually waste most of the potential energy of the carbohydrates. During fermentation glucose is converted to either lactate or ethanol which are relatively lesser reduced product and still retain most of the energy. The end product of glycolysis, pyruvate, is instead further oxidized, and the cell can recover considerably more energy. In the presence of oxygen, pyruvate is transported to mitochondria and is oxidized. Oxidation of an organic compound requires an electron acceptor such as NO_3^-, SO_4^{2-}, Fe^{3+}, or O_2, all of which are exploited as oxidants in different organisms. In aerobic organisms the electrons which are removed during oxidative metabolism are ultimately transferred to O_2. Studying pyruvate oxidation will involve reactions of conversion of pyruvate to acetyl-CoA, citric acid cycle, and oxidation of reduced intermediate compounds produced during TCA cycle through electron transport chain. Pyruvate derived from glucose is split into CO_2 and a two-carbon fragment in mitochondria that enters citric acid cycle for oxidation as acetyl-CoA. It is sometimes convenient to think of the citric acid cycle as an addition to glycolysis. However, it is really misleading to think of the citric acid cycle as merely a continuation of carbohydrate catabolism. The citric acid cycle is a central pathway for recovering energy from several metabolic fuels, including carbohydrates, fatty acid, and amino acids, especially in animals. TCA cycle also serves to provide precursors for a variety of

biosynthetic pathways. First of all we explore how acetyl-CoA, its starting compound, is formed from pyruvate followed by its metabolism through TCA cycle.

Oxidative Decarboxylation of Pyruvate: The Link Reaction Pyruvate produced in glycolysis is transported to mitochondria for further metabolism. The outer membrane allows passage of **metabolites** having molecular mass up to 1 kDa. However, free movement of proteins does not occur. Since the inner membrane does not allow free passage, the molecules need to be transported through various transporters. The inner mitochondrial membrane is infolded to form cristae and contains almost 50% of the mitochondrial proteins. The two membranes of mitochondria enclose a compartment known as matrix, and almost 50% of the molecules of mitochondria by weight are present in the matrix. Mobility is restricted since there is little water being present, and possibly the matrix proteins are organized in multiprotein complexes (**metabolons**) to facilitate substrate channeling. Pyruvate which enters mitochondria is converted to acetyl-CoA by oxidative decarboxylation. One carbon of the molecule is lost as CO_2. Oxidation is coupled to reduction of NAD^+ to NADH. The reaction is catalyzed by pyruvate dehydrogenase complex (PDC). In plants, PDC is present in plastids also, but there it plays a role in lipid biosynthesis. This reaction is also called link reaction since it joins glycolysis and TCA cycle.

$$\text{Pyruvate} + \text{CoA} - \text{SH} + \text{NAD}^+ \xrightarrow{\text{Pyruvate dehydrogenase complex}} \text{acetyl} - \text{CoA} + \text{NADH} + \text{CO}_2 + \text{H}^+$$

Pyruvate dehydrogenase complex (PDC) is a multienzyme complex consisting of three catalytic units. These are pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Conversion of pyruvate to acetyl-CoA is a multistep process which requires five cofactors along with the three enzymes. E1 is covalently linked to TPP, lipoic acid is covalently linked to E2, and FAD is the prosthetic group of E3. The other cofactors which are present as free molecules are NAD^+ and coenzyme A. Besides, the enzymes which regulate the activity of pyruvate dehydrogenase, are also present. In plants, the core of the PDC in mitochondria consists of 60 copies of E2 in 12 sets of 5, each having lipoic acid-binding region (studies from the purified form of PDC of mitochondria in maize) and binding site for E1. E1 is a tetraheteromer consisting of α and β subunits and is covalently linked with TPP. Phosphorylation of two conserved serine residues (at 300 and 306 position) in α subunit of the enzyme makes E1 inactive, while dephosphorylation of these residues converts the enzyme to active form. E1 subunits are covalently linked to the E2 core. E3, which is present as dimer, has got binding sites for FAD and NAD^+. E3 is also linked to E2 core toward the inner side of the complex. Besides this, the regulatory enzymes pyruvate dehydrogenase kinase (PDK), which catalyzes ATP-dependent phosphorylation of E1, and phosphopyruvate dehydrogenase phosphatase (PDP), which catalyzes dephosphorylation of the enzyme protein of E1, are also present as components of the complex. PDK and PDP are responsible for inactivation and activation of the enzyme, respectively.

Oxidative decarboxylation of pyruvate occurs in the following five steps (Fig. 7.8):

1. Pyruvate dehydrogenase (E_1-TPP), a TPP-requiring enzyme, decarboxylates pyruvate, with the formation of an intermediate hydroxyethyl-TPP. This reaction is identical with the one catalyzed by yeast pyruvate decarboxylase.

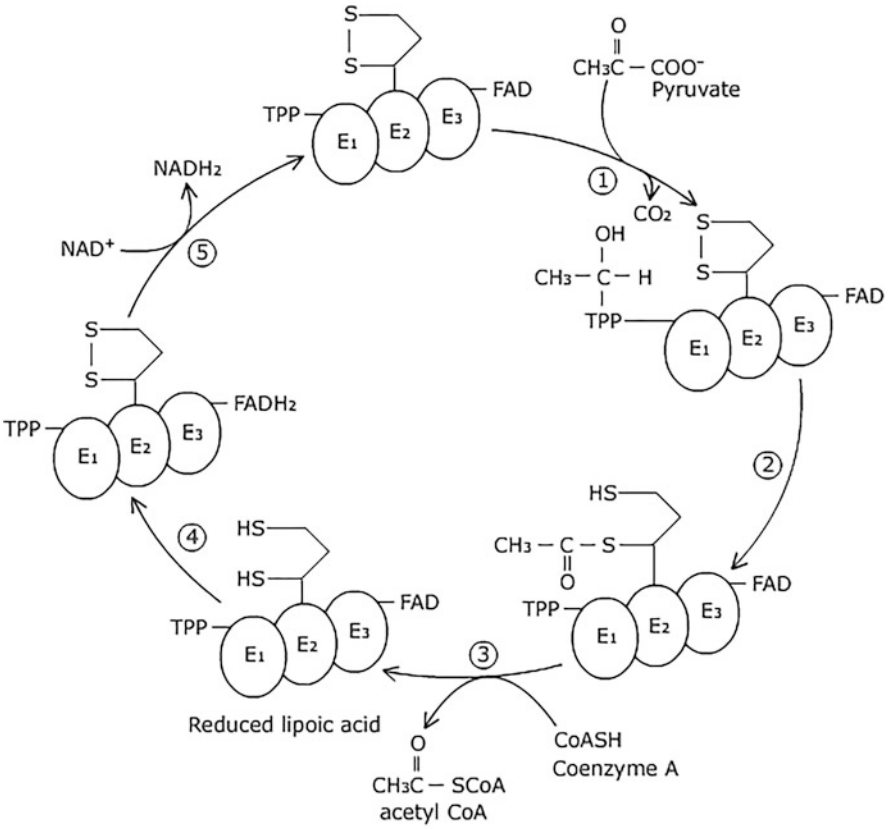

Fig. 7.8 Figure showing the reaction steps, which occur during conversion of pyruvate to acetyl-CoA catalyzed by pyruvate dehydrogenase complex (PDC). PDC is a multienzyme complex which consists of three enzymes, E1 (pyruvate dehydrogenase), E2 (dihydrolipoyl transacetylase), and E3 (dihydrolipoyl dehydrogenase), which require cofactors TPP, lipoic acid, and FAD, covalently linked to them, respectively. Besides these the other cofactors required are NAD^+ and CoA-SH. The reaction occurs in five steps: Step 1. E1 catalyzes decarboxylation of pyruvate generating acetaldehyde to produce hydroxyethyl-TPP complex. 2. Hydroxyethyl group forms acetyl-dihydrolipoamide, a thioester of lipoic acid of E2. 3. E2 catalyzes transfer of acetyl group from acetyl-dihydroipoamide to coenzyme A, resulting in the formation of acetyl-CoA and fully reduced dihydrolipoamide group of E2. 4. E3 catalyzes reoxidation of reduced dihydrlipoamide resulting in reduction of FAD to $FADH_2$. 5. $FADH_2$ is oxidized to FAD and is coupled with reduction of NAD^+ to NADH, and PDC is regenerated in original form

2. Unlike pyruvate decarboxylase, pyruvate dehydrogenase does not convert hydroxyethyl-TPP into acetaldehyde and TPP. Instead, the hydroxyethyl group is transferred to lipopic acid of the next enzyme dihydrolipoyl transacetylase (E2) of the complex, forming thioester bond. The reaction is coupled with the elimination of TPP and formation of acetyl dihydrolipoamide with simultaneous release of active E1. The hydroxyethyl is thereby oxidized to an acetyl group by the concomitant reduction of the lipoamide disulfide bond.
3. E2 catalyzes the transfer of the acetyl group from acetyl dihydrolipoamide to SH-CoA, yielding acetyl-CoA and dihydrolipoamide-E2.
4. Dihydrolipoyl dehydrogenase (E3, also called dihydrolipoamide dehydrogenase) reoxidizes dihydrolipoamide completing the catalytic cycle of E2. Oxidation of dihydrolipoamide is coupled with reduction of the covalently bonded cofactor of E3, i.e., FAD to $FADH_2$, and formation of the lipoamide disulfide bond (-S-S-).
5. Reduced $FADH_2$ is reoxidized by the simultaneous reduction of NAD^+ to NADH. E3 has got the binding site both for FAD and NAD^+.

As a result of sequence of these five reactions, the net result of oxidative decarboxylation of pyruvate is formation of acetyl-CoA and NADH and loss of one carbon as CO_2. This reaction is very significant and is regulated by various metabolites. Pyruvate dehydrogenase (PDH), the first enzyme catalyzing these set of reactions, is subjected to regulation. Ratio of ATP/ADP, which determines the metabolic status of the cell, has an important role to play in its regulation. The enzyme E1 is inactivated by ATP-dependent phosphorylation catalyzed by pyruvate dehydrogenase kinase, while a phosphatase removes the phosphoryl group making the enzyme active again (Fig. 7.9). Activity of PDH is also inhibited by the end products of the reaction, i.e., acetyl-CoA and NADH. Pyruvate activates PDH by inhibiting the activity of PDH kinase, thus removing the inhibition because of phosphorylation of the enzyme, while ammonium ions (NH_4^+) activate the activity of kinase, resulting in inhibition of the activity of PDH.

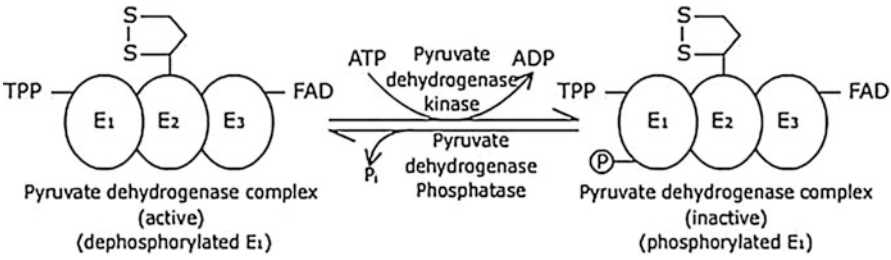

Fig. 7.9 Phosphorylation and dephosphorylation of E1 of the pyruvate dehydrogenase complex regulate the activity of pyruvate dehydrogenase complex

7.5 TCA Cycle

Acetyl group of acetyl-CoA is completely oxidized during citric acid cycle releasing the remaining energy. Released energy is conserved as reduced pyridine nucleotides, and the two carbons of the molecule are released as CO_2. The cycle is called as Krebs cycle after the name of the scientist who was responsible for putting together the various reactions which had been studied by different scientists. In fact this cycle was the first of series of reactions to have been put together to give the concept of cyclic reactions in which substrate of the first enzyme is regenerated. Hans Krebs demonstrated formation of citrate, a tricarboxylic acid, when OAA condenses with acetyl-CoA. The cycle was also named as citric acid cycle after the product of its first reaction, citrate. One complete round of cycle starting with acetyl-CoA yields two molecules of CO_2, three NADH, one $FADH_2$, and one high-energy compound (ATP/GTP in plants or animals, respectively). Citric acid cycle first came to light in the 1930s, when Hans Krebs proposed a circular reaction scheme for the interconversion of certain compounds containing two or three carboxylic acid groups. At that time, many of the citric acid cycle intermediates were already well-known plant products: citrate from citrus fruit, aconitate from monkshood (*Aconitum*), succinate from amber (*Succinum*), fumarate from the herb *Fumaria*, and malate from apple (*Malus*). Two other intermediates α-ketoglutarate and oxaloacetate are known by their chemical names because they were synthesized before they were identified in living organisms. Discovery of the citric acid cycle ranks as one of the most important achievements of metabolic chemistry, and Hans Krebs was awarded Noble Prize in 1953 for that.

7.5.1 General Features of the Cycle

1. It is a circular pathway during which acetyl groups from many sources, not just pyruvate, are oxidized. The citric acid cycle is often considered the hub of cellular metabolism.
2. The net reaction of citric acid cycle is

$$3NAD^+ + FAD^+ + ADP + P_i + \text{acetyl} - CoA \rightarrow 3NADH + FADH_2 + ATP + CoA + 2CO_2$$

Oxaloacetate that is consumed in the first step of the citric acid cycle is regenerated in the last step of the cycle. The citric acid cycle acts as multistep catalyst that can oxidize an unlimited number of acetyl groups. In eukaryotes, all the enzymes of the citric acid cycle are located in the mitochondria. All the products of the citric acid cycle must be consumed in the mitochondria or transported to the cytosol. Thus there is lot of exchange of metabolites between cytosol and mitochondria through various transporters located in the inner membrane (Fig. 7.10, Table 7.2).

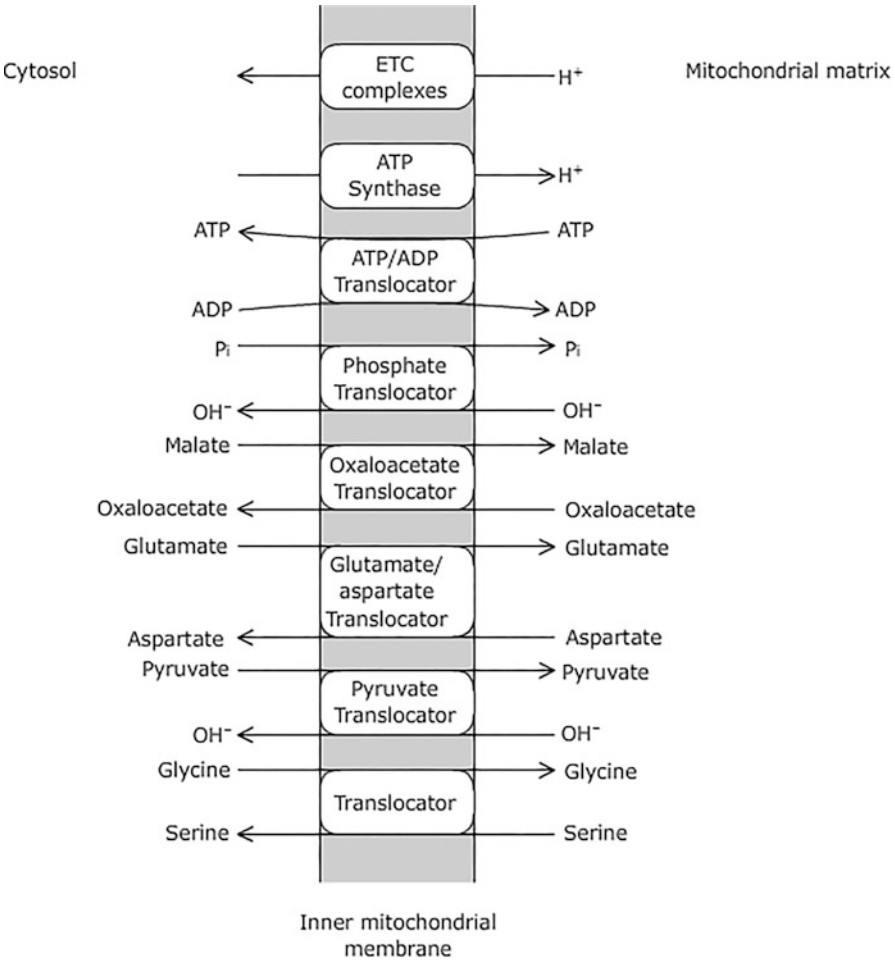

Fig. 7.10 Some of the transporters located in the inner membrane of mitochondria

3. The two carbon atoms of the CO_2 produced in one round of TCA cycle are not the same two carbons of the acetyl-CoA that began the round. These acetyl carbon atoms are lost in subsequent rounds of the cycle. However, the net result of each round of the cycle is the oxidation of one acetyl group to $2CO_2$.
4. Citric acid cycle plays **amphibolic role** since, besides playing a catabolic role, intermediates of the cycle serve as precursors for various biomolecules.
5. Oxidation of an acetyl group to $2CO_2$ requires the transfer of four pairs of electrons. Reduction of $3NAD^+$ to 3NADH accounts for three pairs of electrons; reduction of FAD to $FADH_2$ accounts for the fourth pair. Much of the free energy of oxidation of the acetyl group is converted in these reduced coenzymes. Energy is also recovered as ATP (or GTP in animals).

Table 7.2 Reactions of TCA cycle

	Reactions	Enzyme catalyzing the reaction	$\Delta G^{0'}$ of the reaction in $kJ.mol^{-1}$
1.	$Acetyl - CoA + OAA \rightarrow Citrate + CoASH + H^+$	Citrate synthase	−32.2
2.	$Citrate \leftrightarrow Isocitrate$	Aconitase	13.3
3.	$Isocitrate + NAD^+ \rightarrow 2 - oxoglutarate + NADH + CO_2 + H^+$	Isocitrate dehydrogenase	−17.5
4.	$2 - oxoglutarate + NAD^+ \rightarrow Succinyl - CoA + NADH + CO_2$	2-Oxoglutarate dehydrogenase complex	−33.5
5.	$Succinyl - CoA + ADP + P_i \leftrightarrow Succinate + ATP + CoASH$	Succinyl-CoA synthetase	−2.9
6.	$Succinate + FAD \leftrightarrow L - Fumarate + FADH_2$	Succinate dehydrogenase	0
7.	$L - Fumarate + H_2O \leftrightarrow L - malate$	Fumarase	−3.8
8.	$L - Malate + NAD^+ \leftrightarrow OAA + NADH + H^+$	NAD-malate dehydrogenase	−29.7

7.5.2 Acetyl-CoA Enters the TCA Cycle

Only a fraction of the energy stored in glucose is harvested in the form of ATP during glycolysis. Aerobic metabolism of pyruvate is the source for the majority of the ATP generated in respiration, when there is complete degradation and oxidation of glucose, resulting in release of both the carbons as two molecules of carbon dioxide. Oxidation during citric acid cycle includes a series of reactions (Fig. 7.11).

Condensation Reaction: The First Reaction of the Cycle The first reaction of the TCA cycle involves condensation of acetyl-CoA with oxaloacetate to produce citrate catalyzed by the enzyme citrate synthase (CS). There is an aldol condensation between methyl group of acetyl-CoA and carbonyl group of oxaloacetate, resulting in synthesis of citroyl-CoA. This reaction has an equilibrium constant near 1, but the overall reaction is driven to completion by subsequent hydrolysis of the high-energy thioester to citrate and free CoA. The overall $\Delta G^{0'}$ is -31.4 kJ.mol^{-1}, and under standard conditions the reaction is essentially irreversible. Although mitochondrial

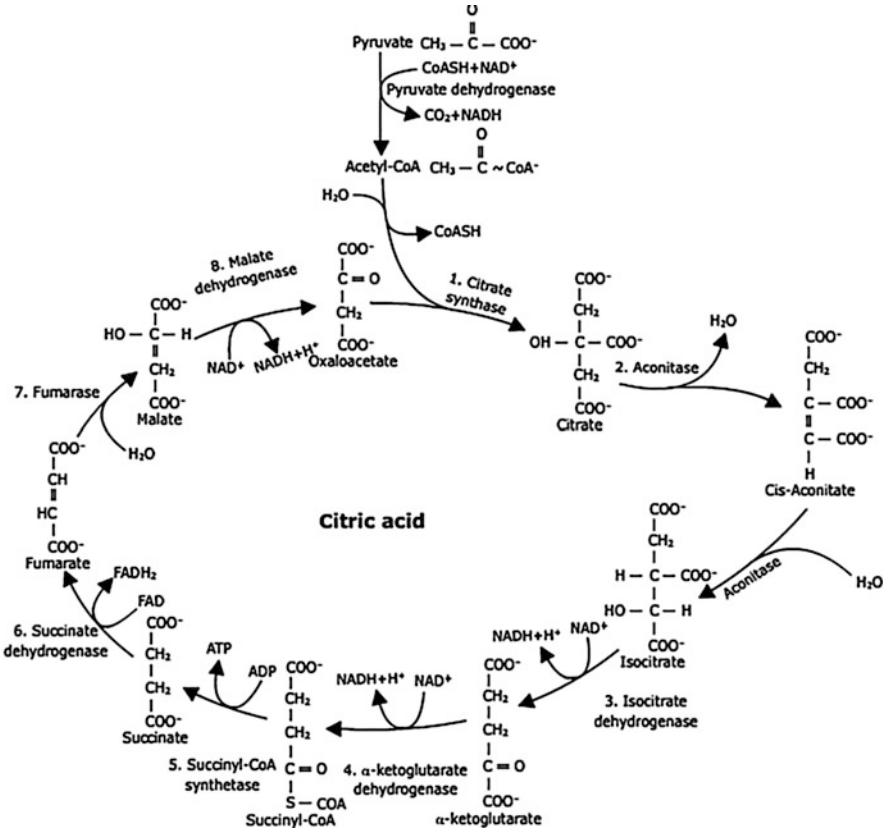

Fig. 7.11 Tricarboxylic acid (TCA) cycle

concentration of oxaloacetate is very low (much less than 1 M), the strong, negative $\Delta G^{0'}$ drives the reaction forward. Citrate synthase is a unique enzyme of mitochondria, but in plants this enzyme is also present in glyoxysomes which catalyzes reaction during glyoxylate cycle. The mitochondrial enzyme is homodimeric with each subunit having two domains—one large and rigid, while the other one is small and flexible. Active site of the enzyme is present in between the two domains. There is conformational change in the enzyme protein (induced fit) on binding of the first substrate, oxaloacetate, with small and flexible unit of the enzyme. As a result, the site for binding with second substrate acetyl-CoA is created, resulting the formation of an intermediate citroyl-CoA. This is followed by another conformational change resulting in the hydrolysis of the thioester bond. As a result acetyl-CoA and citrate are released. CS is inhibited by ATP, but this inhibition is not very significant since ATP concentration in mitochondria is very low. Reaction catalyzed by CS is the first step in this metabolic pathway having a large negative $\Delta G^{0'}$. As might be expected, it is a highly regulated enzyme. NADH, a product of the TCA cycle, is an allosteric inhibitor of citrate synthase, as is succinyl-CoA, the product of the fifth step in the cycle (which is also an analog of acetyl-CoA).

Citrate Is Isomerized by Aconitase to Form Isocitrate Citrate is isomerized to isocitrate by the action of aconitase in a two-step reversible reaction, involving cis-aconitate as an intermediate. The net effect is the conversion of a symmetrical tertiary alcohol, i.e., citrate to an asymmetric secondary alcohol, i.e., isocitrate. The active site of aconitase has iron-sulfur cluster. Cysteine residues of the enzyme protein form coordinate bonds with the three iron atoms, and the fourth iron is bound to one carboxylic group and non-covalently to hydroxyl group of citrate. In this reaction, water is first removed from citrate to yield cis-aconitate, which is then rehydrated resulting in interchange in positions of groups H- and HO- to produce isocitrate. The $\Delta G^{0'}$ of the reaction is 6.7 $kJ.mol^{-1}$. At equilibrium though only 10% isocitrate is present, its utilization in subsequent reaction drives the reaction toward right. Aconitase is present both in mitochondria and cytosol of the plant cell. In mitochondria it participates in TCA cycle, while in cytosol especially in germinating fatty seeds, it participates in glyoxylate cycle. Along with NADP-dependent isocitrate dehydrogenase in cytosol, it also plays an important role in providing 2-oxoglutarate for amino acid biosynthesis. Aconitase is responsible for catalyzing the first step in citric acid catabolism during fruit ripening. Fluoroacetate is an extremely poisonous agent that blocks the TCA cycle in vivo, although it has no apparent effect on any of the isolated enzymes. The action of fluoroacetate has been traced to aconitase in vivo.

Oxidative Decarboxylation of Isocitrate The next step of the TCA cycle involves oxidative decarboxylation of isocitrate, resulting in the formation of α-ketoglutarate (2-oxoglutarate). The reaction is coupled with reduction of NAD^+ to NADH and loss of one carbon as CO_2. It is catalyzed by the enzyme NAD-isocitrate dehydrogenase (IDH). Net $\Delta G^{0'}$ of the reaction is -8.4 $kJ.mol^{-1}$, which is sufficiently exergonic to pull the previous reaction catalyzed by aconitase forward. This two-step reaction

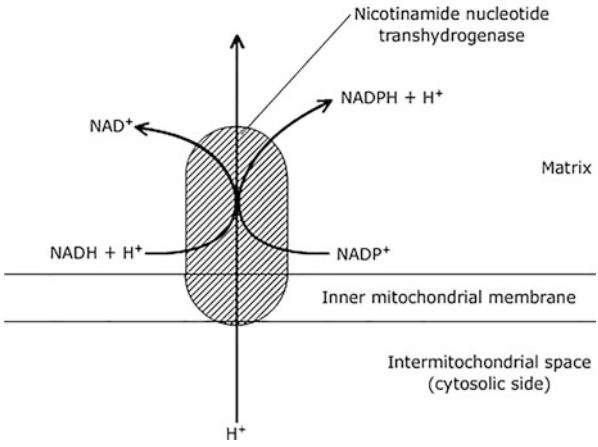

Fig. 7.12 NADPH generation in mitochondria

involves synthesis of an intermediate oxalosuccinate. The first step of the reaction results in the oxidation of the –OH group present at C-2 of isocitrate to form oxalosuccinate, which is followed by a decarboxylation of central carboxyl group, resulting in the formation of α-ketoglutarate. Decarboxylation of oxalosuccinate makes the reaction irreversible. Besides NAD-IDH, NADP-IDH is also present in mitochondria. In mitochondria NADPH may be generated either due to the activity of nicotinamide nucleotide transhydrogenase (Fig. 7.12) or because of the activity of $NADP^+$-specific isocitrate dehydrogenase ($NADP^+$-IDH). In plants it is the latter activity which is more significant. $NADP^+$ isoforms of isocitrate dehydrogenase are present in cytosol and plastids, while NAD^+-IDH isoform is present only in mitochondria. It is the NAD-IDH form which is important for TCA cycle. Citrate exported from mitochondria is metabolized by the cytosolic aconitase and cytosolic NADP-IDH, resulting in synthesis of α-ketoglutarate for amino acid metabolism. The main function of NADP-IDH enzyme both in mitochondria and cytosol may be to provide NADPH to be used in biosynthetic anabolic reactions. NADH produced by NAD-IDH is oxidized by the electron transport chain in mitochondria, which is the first reaction of the TCA cycle to be connected with oxidative phosphorylation. Oxidative decarboxylation of isocitrate is an important regulatory step of TCA cycle. Both NADH and ATP are allosteric inhibitors of NAD-IDH. Enzyme activity is also inhibited by NADPH. The product of the reaction, α-ketoglutarate, can enter nitrogen metabolism through biosynthesis of glutamic acid.

Oxidative Decarboxylation of α-Ketoglutarate The next reaction of TCA cycle involves another oxidative decarboxylation during which α-ketoglutarate is converted to succinyl-CoA. The reaction is catalyzed by *α*-ketoglutarate dehydrogenase enzyme complex which also is a multienzyme complex. Similar to pyruvate dehydrogenase complex, it also consists of three enzymes, i.e., α-ketoglutarate dehydrogenase, dihydrolipoyl transsuccinylase, and dihydrolipoyl dehydrogenase. Reaction occurs in five steps which require five different coenzymes, i.e., TPP, lipoic

acid, CoASH, FAD, and NAD^+. Mechanism is analogous to the reaction catalyzed by pyruvate dehydrogenase complex. E1 of the α-ketodehydrogenase complex binds with α-ketoglutarate and differs from E1 of PDH complex in some of the amino acids; however, dihydrolipoyl dehydrogenase of both complexes is quite similar. Both the complexes seem to have the same ancestral origin. However, unlike PDH, E1 of this complex is not regulated by phosphorylation; rather AMP is the activator of the enzyme. Similar to pyruvate dehydrogenase reaction, NADH is also produced here along with a thioester product—in this case, succinyl-CoA. One carbon is lost as CO_2. The products of the reaction, succinyl-CoA and NADH, are energy-rich compounds which are important sources of metabolic energy. NADH provides ATP when oxidized through electron transport chain, while succinyl-CoA is metabolized through the following reaction.

Succinyl-CoA Is Converted to Succinic Acid Succinyl-CoA is a high-energy intermediate having negative free energy of hydrolysis. $\Delta G^{0'}$ of the reaction is –36 kJ. mol^{-1}, which is utilized to drive the phosphorylation ADP to ATP (in plants and bacteria) by means of substrate-level phosphorylation. In animals similar reaction results in phosphorylation of GDP resulting in synthesis of GTP. The reaction is catalyzed by succinate thiokinase also called succinyl-CoA synthetase (synthetases are the enzymes which catalyze condensation reactions involving nucleoside triphosphates, unlike synthase for which no nucleoside triphosphates are required). The free energies of hydrolysis of succinyl-CoA and GTP or ATP are similar. The net $\Delta G^{0'}$ of the reaction is -3.3 kJ.mol^{-1}. In addition to some of the reactions of glycolysis, this reaction is only such reaction in the TCA cycle, which is the source of substrate-level phosphorylation, in which a substrate (here succinyl-CoA), rather than an electron transport chain or proton gradient, provides the energy for phosphorylation.

Oxidation of Succinate to Fumarate Oxidation of succinate to fumarate is carried out by a flavoprotein-linked succinate dehydrogenase. This is the only enzyme of TCA cycle which is bound to inner mitochondrial membrane and is a component of the electron transport chain. Succinate-binding site of the enzyme is present toward the matrix. All other enzymes of TCA cycle are present as soluble proteins in mitochondrial matrix. Succinate dehydrogenase is also known as succinate ubiquinone oxidoreductase, a component of the electron transport chain. Succinate oxidation involves removal of electrons from succinate and passing them to UQ, a component of ETC resulting in the formation of trans-unsaturated fumarate. Succinate dehydrogenase is a dimeric protein, with subunits having molecular masses 70 and 27 kDa. The larger subunit of the enzyme protein is covalently linked to the cofactor FAD. The enzyme also contains non-heme iron, and the electrons removed from succinate pass through FAD and Fe-S centers before being passed to ubiquinone of the ETC. Activity of the enzyme has been demonstrated to be competitively inhibited by malonate in vitro. The enzyme in vivo is strongly inhibited by OAA but is activated by UQH_2, ATP, and ADP. Effect of ATP or ADP may be the indirect result of possibly activating the enzyme by releasing OAA. Activity of plant SDH

may be regulated by energy status of the mitochondrial membrane, physiological relevance of which is unclear, since there are reciprocal changes in the concentrations of ATP and ADP in the cell.

Trans-hydration of Fumarate to Form L-Malate The next reaction of TCA cycle involves trans-hydration of fumarate which requires addition of –H and –OH groups to the C=C double bond of fumarate resulting in synthesis of L-malate. Fumarase catalyzes the reversible reaction which has $\Delta G^{0'}$ value -3.8 kJ.mol^{-1}. The reaction is stereospecific, as in the reversible reaction, it is L-malate which is the substrate for the enzyme and not D-malate. Aconitase carries a similar reaction, i.e., trans-addition of -H and -OH occurs across the double bond of cis-aconitate.

Oxidation of L-Malate to Oxaloacetate This is the last reaction of TCA cycle in which malate is oxidized to regenerate OAA, thus completing the cycle. Oxidation of malate is coupled to reduction of NAD^+ molecule, the third one in the cycle. This energy-requiring reaction has $\Delta G^{0'}$ value 29.7 kJ.mol^{-1} which favors synthesis of malate rather than that of OAA. However, removal of OAA in subsequent reaction and oxidation of NADH to NAD^+ is responsible for the direction of the reaction toward right. Malate dehydrogenase is a ubiquitous enzyme, and its isoforms are found in mitochondria, cytosol, glyoxysomes, and peroxisomes. $NADP^+$-specific MDH is found in chloroplasts of C4 plants, where it is involved in production of malate from OAA during C4 pathway of CO_2 assimilation. It is NAD-MDH which participate in TCA cycle. Its isoform is also involved in exchange of reducing equivalents between cell compartments across the membranes (NADH shuttle).

7.5.3 Stoichiometry of TCA Cycle

Net reaction for complete oxidation of one molecule of acetyl-CoA through TCA cycle can be written as

$$\begin{aligned}&\text{Acetyl} - \text{CoA} + 3\text{NAD}^+ + \text{FAD} + \text{ADP} + \text{P}_\text{i} + 2\text{H}_2\text{O}\\&\quad\rightarrow 2\text{CO}_2 + \text{Co} - \text{ASH} + 3\text{NADH} + 3\text{H}^+ + \text{FADH}_2 + \text{ATP}\end{aligned}$$

The cycle is exergonic with a net $\Delta G^{0'}$ for one round of the cycle $= -40$ kJ.mol^{-1}.

Glucose metabolized via glycolysis produces two molecules of pyruvate, which in the presence of O_2 are converted to acetyl-CoA in mitochondria. 2NADH are produced during this conversion of 2 pyruvate to 2 acetyl-CoA. These two molecules of acetyl-CoA thus produced enter the TCA cycle. Combining glycolysis, link reaction, and the TCA cycle, the following net reaction is obtained:

$$\text{Glucose} + 2H_2O + 10NAD^+ + 2FAD + 4ADP + 4P_i \rightarrow 6CO_2 + 10\,NADH + 10H^+ + \ 2\,FADH_2 + 4\,ATP$$

All six carbons of glucose are released as CO_2, and a total of four molecules of ATP are produced as a result of substrate-level phosphorylation. This includes net production of 2 ATP during glycolysis and 2 ATP during two TCA cycles (for two molecules of acetyl-CoA). There are 12 reduced coenzymes produced which include 2 NADH produced during glycolysis, 2 NADH during link reaction, while 6 NADH and 2 $FADH_2$ during two TCA cycles. During oxidative phosphorylation, oxidation of NADH generates proton motive force sufficient enough for synthesis of 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. Thus during complete oxidation of a glucose molecule, 30–32 ATP molecules are produced (Table 7.3). This will be equivalent to 976 kJ.mol^{-1} (32 × 30.5 kJ.mol^{-1} for hydrolysis of ATP) which constitutes 34% of the potential energy of a glucose molecule (2840 kJ.mol^{-1}).

7.5.4 Amphibolic Role of TCA

TCA cycle occupies the central role in metabolism. Apparently TCA cycle is the terminal point at least in terms of carbon turnover. The cycle is important not only in catabolism but has **amphibolic** role since it has a significant role in anabolism as well (Fig. 7.13). Many intermediates of TCA cycle serve as precursors for various cellular compounds. Almost 50% of the carbon is retained by plant tissues through incorporation of intermediates of glycolysis, OPPP, and TCA for biosynthesis of the compounds required by the cell, instead of being released as CO_2. One of the important roles of the TCA cycle is to provide carbon skeleton for glutamate biosynthesis in the form of 2-oxoglutarate. There are transporters present in the inner mitochondrial membrane which facilitate exchange of 2-oxoglutarate with cytosolic malate. There are three sources of 2-oxoglutarate, which is required for glutamate biosynthesis. First is from TCA cycle in mitochondria, second is cytosolic NADPH-IDH, and third is in peroxisomes where it is generated during photorespiration by glutamate:glyoxylate aminotransferase. 2-Oxoglutarate is transported to chloroplasts where it is used for glutamate biosynthesis by GS-GOGAT enzymes. In the inner envelope of chloroplasts, there are transporters present for both 2-oxoglutarate and glutamate, which facilitate import of 2-oxoglutarate and export of glutamate. Another intermediate of TCA, i.e., citrate, also is transported out of mitochondria by tricarboxylic acid transporters present in the inner membrane of the organelle, and once in the cytosol, it is converted into isocitrate by the cytosolic aconitase. Isocitrate is the source of cytosolic 2-oxoglutarate, produced due to the activity of $NADP^+$-IDH. Export of tricarboxylate or dicarboxylate from mitochondria is accompanied with uptake of malate. Glutamate serves as the precursor of various other nitrogenous compounds, which include many other amino acids. Another intermediate of TCA, succinyl-CoA, is the source of carbons for various

Table 7.3 Stoichiometry of aerobic respiration of one molecule of glucose

	Reactions	ATP used or produced/reduced	ATP equivalent
	Glycolysis		
1.	Glucose → Glucose 6 − phosphate	ATP consumed	–ATP
2.	Fructose − 6 − phosphate → Fructose 1, 6 − bisphosphate	ATP consumed	–ATP
3.	2X3 − Phosphoglyceraldehyde → 2X1, 3 − Phosphoglycerate	2NADH produced	2 × 1.5 or 2.5[a] = +3 or 5 ATP
4.	2X1, 3 − Bisphosphoglycerate → 2X3 − Phosphoglycerate	2ATP produced	+ 2 ATP
5.	2Phosphoenolpyruvate → 2Pyruvate	2ATP produced	+ 2 ATP
	Link reaction in mitochondria		
6.	2 Pyruvate → 2 Acetyl − CoA	2NADH and 2ATP produced	2 × 2.5 + 2 = +7 ATP
	TCA cycle		
7.	2Isocitrate → 2X2 − oxoglutarate	2NADH produced	2 × 2.5 = +5 ATP
8.	2X2 − oxoglutarate → 2Succinyl − CoA	2NADH produced	2 × 2.5 = +5 ATP
9.	2Succinyl − CoA → 2Succinate	2ATP produced	+ 2 ATP
10.	2Succinate → 2Fumarate	2$FADH_2$produced	2 × 1.5 = +3 ATP
11.	2Malate → 2Oxaloacetate	2NADH produced	2 × 2.5 = +5 ATP
		Net production of ATP	**30–32 ATP molecules**

[a]The number of ATP produced depends on the nature of NADH shuttle, since NADH is transported to mitochondria for ATP generation. Those reactions have not been included in the table during which there is no ATP turnover

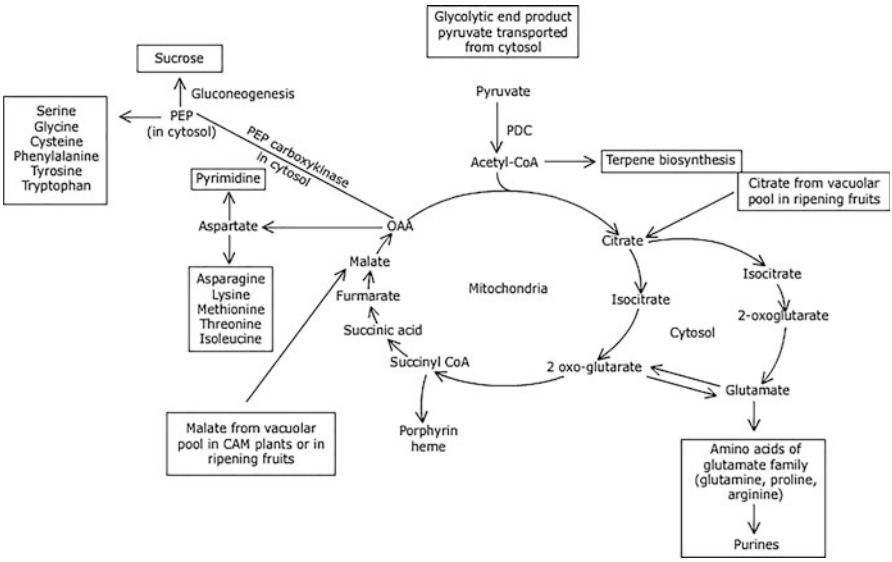

Fig. 7.13 Amphibolic role of TCA

porphyrin-containing molecules such a chlorophylls, cytochromes, etc. Oxaloacetate is transaminated to produce aspartate which itself is the precursor for asparagine, methionine, threonine, lysine, isoleucine, and pyrimidine nucleotides. On decarboxylation oxaloacetate produces phosphoenolpyruvate, which is the precursor of various aromatic amino acids including phenylalanine, tyrosine, tryptophan, and many other aromatic compounds. Besides PEP can be converted to 3-phosphoglycerate, which is required as the key precursor for biosynthesis of amino acids serine, glycine, and cysteine. In animals, citrate, exported from mitochondria, is responsible for synthesis of acetyl-CoA produced from oxaloacetate and acetyl-CoA by cytosolic ATP-citrate lyase. Oxaloacetate is transported to mitochondria as malate. In an animal cell, cytosolic acetyl-CoA is used as precursor for biosynthesis of fatty acids.

7.5.5 Anaplerotic Reactions

If there is no way of replenishing, removal of intermediates of TCA (which are to be used as precursor for anabolic pathway) will result in stopping the cycle. Various reactions, which act as "filling-up" pathways, are called as anaplerotic reactions (derived from Greek word *anaplerotikos* which means filling up) (Fig. 7.14). In plant cells one of the ways of replenishing the intermediates is by cytosolic oxaloacetate. Plant mitochondria are characterized by specific oxaloacetate transporters present in the inner membrane. Carboxylation of phosphoenolpyruvate generates OAA in the cytosol catalyzed by PEP carboxylase:

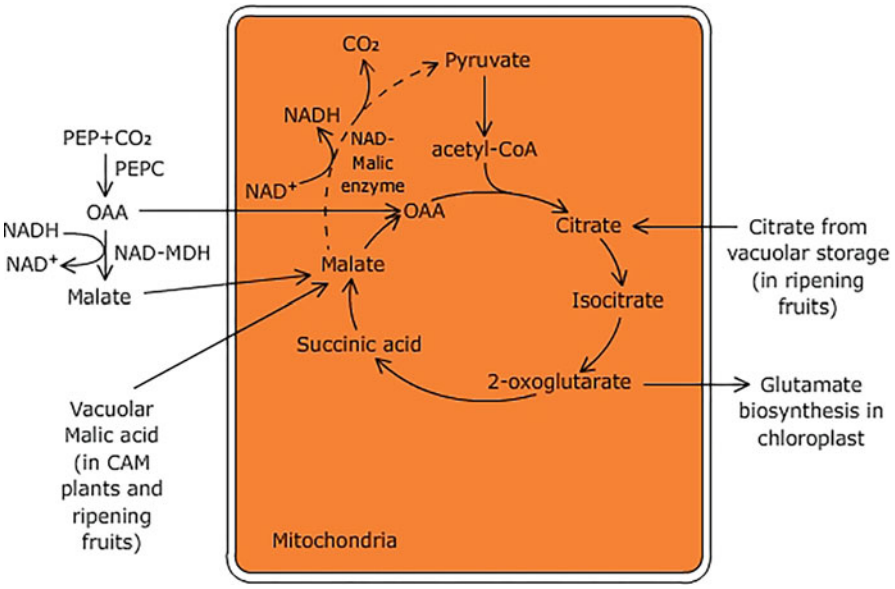

Fig. 7.14 Anaplerotic reactions. Some of the intermediates of TCA serve as precursors of some of the biomolecules. The intermediates of TCA are replenished by anaplerotic reactions. *PEPC* PEP carboxylase, *NAD-MDH* NAD-malate dehydrogenase

$$\mathrm{PEP} + \mathrm{HCO_3^-} \rightarrow \quad \mathrm{OAA} + \mathrm{P_i}$$

There is also a gain of one carbon in this reaction. Reduction of oxaloacetate by cytosolic malate dehydrogenase results in production of malate, which enters into the mitochondria. Malate may also be released and transported into the mitochondria from storage sites of the cell such as vacuoles through the translocators present in tonoplast and inner mitochondrial envelope. In mitochondria, malate is oxidized to OAA by NADH-malate dehydrogenase. Alternatively, OAA is decarboxylated to produce pyruvate by the catalytic action of **NAD-dependent malic enzyme.**

$$\mathrm{Malate} + \mathrm{NAD^+} \rightarrow \mathrm{Pyruvate} + \mathrm{NADH} + \mathrm{CO_2}$$

In this case requirement of glycolysis is substituted due to the formation of pyruvate by alternate reaction. In this case there is loss of carbon, first in the reaction catalyzed by NAD-dependent malic enzyme, secondly during conversion of pyruvate is acetyl-CoA. Though carbon is lost, the combined reactions of NAD-malate enzyme and PDC provide flexibility to the metabolism, since malate can be the source both for OAA and for acetyl-CoA. The resultant citrate can be oxidized to produce 2-oxoglutarate, which easily can be removed to provide carbon skeleton for glutamate biosynthesis without any requirement of pyruvate from glycolysis.

Besides sucrose, glutamate is also present in high concentration in the plant cells, which can be oxidized to 2-oxoglutarate by NADH-glutamate dehydrogenase located in mitochondrial matrix, which may join TCA. Photorespiratory glycine is also oxidized in mitochondria.

7.5.6 Role of TCA in Plants Under Stress Conditions

Under normal conditions, it is generally the sucrose which is translocated and metabolized in plants. However, reduced CO_2 assimilation under stress results in carbon starvation, especially when plants are exposed to prolonged darkness, low light conditions, low temperature, or drought. As a result, carbohydrate supply is reduced, and plants may need to metabolize other biomolecules, such as fatty acids or proteins. In plants fatty acids are oxidized by β-oxidation in peroxisomes, or in some of the case by α-oxidation, resulting in producing acetyl-CoA, which is metabolized through TCA cycle. Amino acids may be deaminated to produce α-keto acids, which are the intermediates of TCA cycle, e.g., glutamic acid may be oxidized and deaminated to produce 2-oxoglutarate by the NAD-dependent glutamate dehydrogenase. The non-protein amino acid such as proline accumulates under water stress conditions which is also oxidized to 2-oxoglutarate. Under carbohydrate starvation, oxidation of lysine, catalyzed by an enzyme lysine-2-oxoglutarate reductase, produces 2-oxoglutarate which enters TCA cycle. Various other amino acids including valine, isoleucine, and leucine are also thought to be degraded in mitochondria. In C3 plants, glycine produced during photorespiration is also metabolized in mitochondria. Glycine decarboxylase is responsible for glycine metabolism, and NADH generated during the reaction is either oxidized through ETC in mitochondria or is oxidized through OAA/malate shuttle system.

7.5.7 Regulation of TCA

Most important regulators of TCA are NADH and ATP. Ratio of NADH/NAD^+ and ATP/ADP is responsible for regulation of the cycle. These act as allosteric modulators of the TCA cycle enzymes. Enzymes are activated when the ratio falls below the threshold value. Under environmental stress, demand for ATP decreases in plants, resulting in increase in ATP levels. As a result, activity of some of the enzymes of TCA, such as citrate synthase, is inhibited. In plants there is a high ATP/ADP ratio in light since ATP is also generated during light reaction in chloroplasts. In that case ATP production in mitochondria is reduced because of which NADH is not oxidized. Accumulated NADH will ultimately either shut off TCA cycle or reduce it. The enzymes which are sensitive to inhibition by NADH include NAD-IDH and NAD-MDH. Since alternative mechanisms for oxidation of NADH are present in plants, the mechanisms for regulation of TCA cycle may be different and less rigidly controlled by NADH/NAD^+ ratio in comparison to that in animals. One of the important regulatory points is the entry of pyruvate into TCA

through link reaction. This reaction provides a balance between glycolysis and TCA. If TCA cycle is not operating, accumulated acetyl-CoA will inhibit the activity of PDC. On the contrary, supply of pyruvate from glycolysis will alleviate the inhibition. Pyruvate dehydrogenase complex is also regulated through phosphorylation and dephosphorylation of PDH. Major regulatory steps of TCA include reactions catalyzed by citrate synthase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase, succinic acid dehydrogenase, and fumarase. In plants another mechanism involved is regulation of enzyme activities by modulating their thiol redox status by cysteine-cysteine redox interconversion (-SH + HS^- ⇄ S - S-). It is well established for chloroplast enzymes. It is of considerable significance even with reference to the mitochondrial enzymes. Plant mitochondria are known to have thioredoxin system, which might be playing important role in regulating activity of TCA cycle enzymes. Activity of AOX has also been demonstrated to be regulated through thioredoxin system. Studies with Arabidopsis mutants have demonstrated that succinic acid dehydrogenase and fumarase are deactivated when reduced by thioredoxin, whereas cytosolic ATP-citrate lyase is activated. Activity of citrate synthase is also inhibited when oxidized. Similar to the mammalian system, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase activities are inhibited by NADH. However, knowledge about the regulation of TCA in plants is limited especially under in vivo conditions since most of the studies have been carried out with isolated enzymes (Fig. 7.15).

In addition to mitochondria, plants have another source for ATP, i.e., the light reaction in chloroplasts. Excess reducing power produced in the form of NADPH during light reaction needs to be transported out of the chloroplasts so as to protect thylakoids from excessive reduced conditions. OAA/malate shuttle is responsible for transporting reducing equivalents out of the chloroplasts. Once in cytosol malate may be transported to mitochondria. NADH/NADPH do not accumulate in mitochondria in plants, because of presence of alternate mechanisms for their

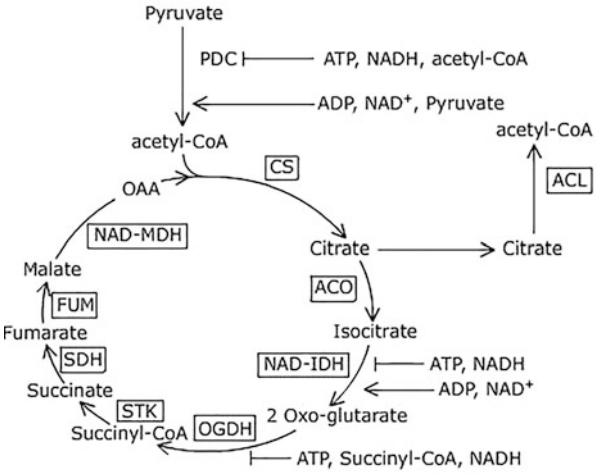

Fig. 7.15 Regulation of TCA cycle. Activities of citrate synthase (CS), succinic acid dehydrogenase (SDH), and fumarase (FUM) are inhibited when reduced by the thioredoxin system, while activity of ATP citrate lyase (ACL) is activated. *PDC* pyruvate dehydrogenase complex, *ACO* aconitase, *NAD-IDH* NAD-specific isocitrate dehydrogenase, *OGDH* 2-oxoglutarate dehydrogenase, *STK* succinic acid thiokinase, *NAD-MDH* NAD-malate enzyme

oxidation. Many studies have indicated reduction in TCA cycle enzyme activities, or TCA cycle does not operate in green parts of the plants under illuminated conditions. In mitochondria NAD-IDH is particularly sensitive to inhibition by light. NADH is also produced during oxidation of glycine by glycine decarboxylase complex during photorespiration. It is observed that glycine oxidation is coupled with decreased TCA cycle activity, may be because of inhibition by increase in levels of NADH. Ammonia released during oxidation of glycine inhibit PDC, thus inhibiting TCA at the entry point.

7.5.8 TCA Cycle and GABA Shunt

γ-Aminobutyric acid (GABA) is a non-proteogenic amino acid. Since GABA has been found to accumulate in plants in response to abiotic stresses, such as salt stress or low light intensities, its significance in metabolism has been studied. In animals role of GABA has been found to be that of neurotransmitter. Synthesis of GABA occurs as a result of bypass reactions of TCA which is also known as GABA shunt. GABA shunt is also known to provide interlinking of carbon and nitrogen metabolism. The intermediate of TCA, 2-oxoglutarate, is transaminated to glutamate, which is transported out of mitochondria. In the cytosol, glutamate is converted to GABA as a result of decarboxylation reaction catalyzed by glutamate decarboxylase. After GABA is transported back to mitochondria, it is converted to succinic acid semialdehyde (SSA) due to another transamination reaction which is catalyzed by GABA transaminase. The reaction is coupled with conversion of pyruvate to alanine. Succinic acid semialdehyde is oxidized to succinic acid with simultaneous reduction of NAD^+ to NADH. Reaction is catalyzed by succinic semialdehyde dehydrogenase (Fig. 7.16).

7.6 Oxidation of the Reduced Coenzymes Produced During TCA Cycle

Though discovered in the beginning of the century, it was in 1948 when Eugine Kennedy and Albert Lehninger discovered that mitochondria are the sites of oxidative reactions. 1100 proteins have been identified in mitochondria out of which functions of almost 300 proteins are not known. Reduced coenzymes, NADH and $FADH_2$, which are produced during glycolysis or TCA, need to be oxidized back to NAD^+ and FAD, respectively, so that glycolysis and TCA keep on functioning. In absence of the oxidized coenzymes, dehydrogenases in the oxidative pathways are inhibited. Energy released during oxidation of the coenzymes is conserved in the form of proton gradient across inner mitochondrial membrane which is coupled with ATP synthesis. ATP synthesis, coupled with oxidation of substrates, is termed as oxidative phosphorylation. It will be dealt in the chapter dealing with ATP synthesis. Electrons removed from NADH are passed through a number of electron carriers which are organized in the form of complexes. These are present in the inner

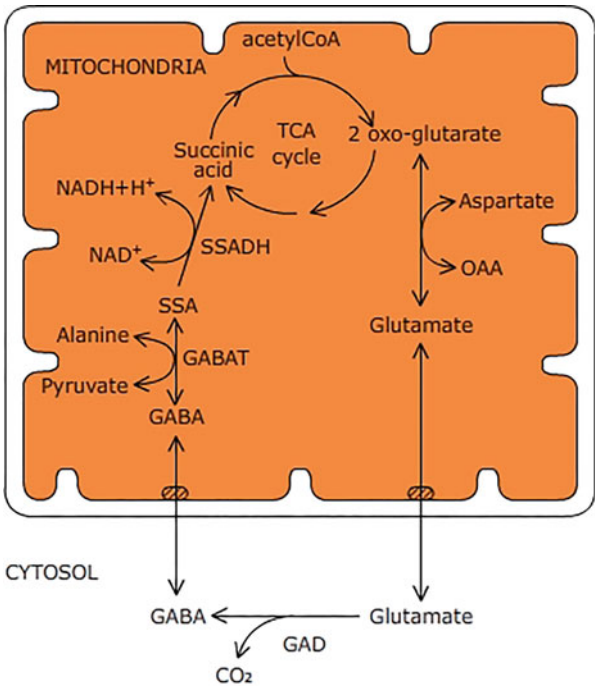

Fig. 7.16 GABA shunt—an alternative pathway in TCA cycle which gives flexibility against environmental changes. GABA is now considered a metabolite to play a significant role in C-metabolism in response to stress. *GABA* gamma-aminobutyric acid, *GAD* glutamate decarboxylase, *GABAT* GABA transaminase, *SSA* succinic semialdehyde, *SSADH* succinate-semialdehyde dehydrogenase, *OAA* oxaloacetate

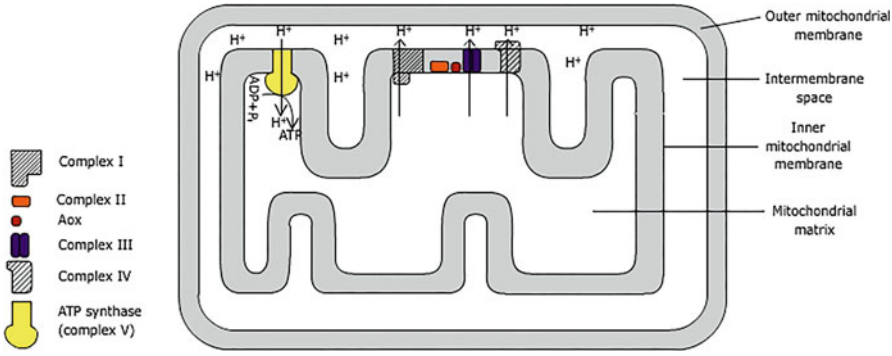

Fig. 7.17 Role of four complexes present in the inner mitochondrial membrane in generation of proton gradient. Protons accumulate in the intermembrane space and flow back through ATP synthase synthesizing ATP

mitochondrial membrane which divides the mitochondrial compartment in two spaces (Fig. 7.17). The space in between the outer and inner mitochondrial membrane is known as perimitochondrial space which directly is in contact with cytosol since **porins** present in the outer membrane allow free movement of the molecules having molecular weight up to 10 kDa, and most of the biomolecules can pass through these channels except the proteins. Unlike the outer membrane, the inner

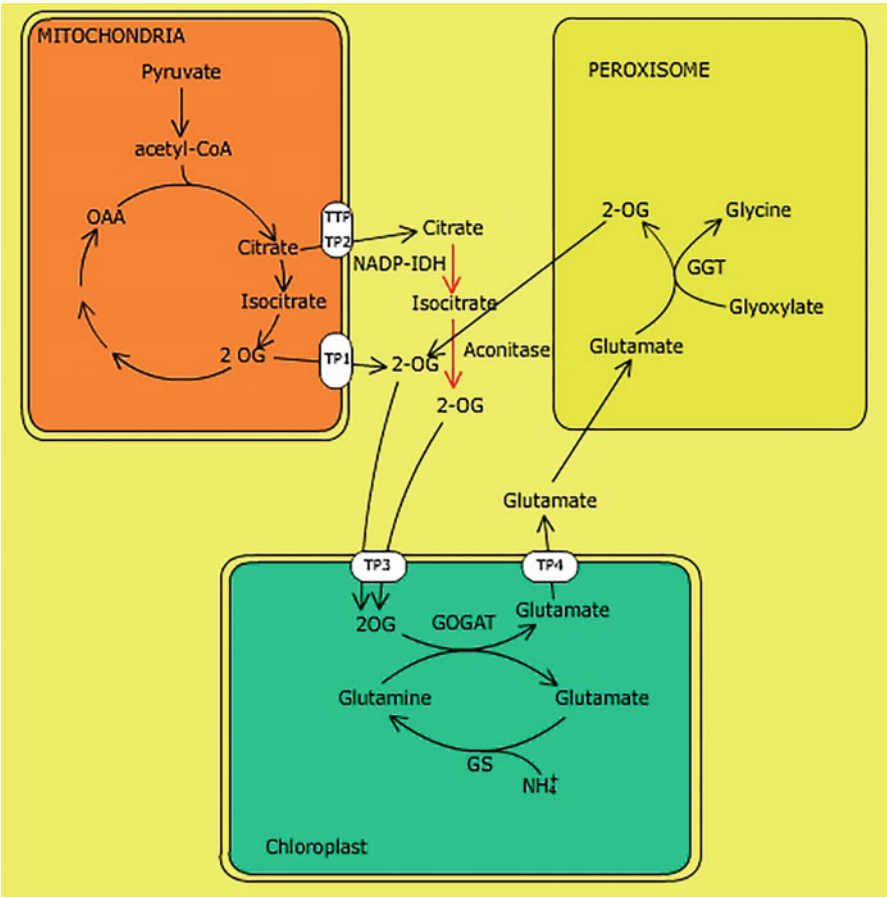

Fig. 7.18 Source of carbon skeleton of glutamate: *TP1* mitochondrial oxoglutarate transporter, *TTP* mitochondrial tricarboxylate transporter, *TP2* mitochondrial dicarboxylate transporter, *TP3* chloroplast 2-oxoglutarate transporter, *TP4* chloroplast glutamate transporter, *NADP-IDH* NADP-isocitrate dehydrogenase, *GS* glutamine synthetase, *GGT* gamma-glutamyltransferase

membrane is impermeable to most of the biomolecules, and movement of substance across the membrane is facilitated by transporters present in it which are responsible for exchange of various biomolecules, such as pyruvate, dicarboxylic and tricarboxylic acids (Fig. 7.18). ATP/ADP antiporters present in the membrane facilitate exchange of ATP with cytosolic ADP. The matrix side of the membrane is referred to as the N (negative) side, while the side facing the cytosolic side is known as P (positive) side according to the potential of the membrane which is created as a result of proton movement. Energy released during oxidation of NADH to NAD^+ can be calculated by their redox potentials. During oxidation final acceptor of electrons is O_2 which is reduced to H_2O. The two redox couples $NADH/NAD^+$ and O_2/H_2O have standard reduction potential ($E^{0'}$) −0.320 V and 0.815 V, respectively. Energy

released during movement of electrons in response to the redox potential is calculated by difference in redox potential of the two redox couples NADH/NAD^+ and H_2O/O_2:

$$\begin{aligned}\Delta E &= E_{H_2O/O_2} - E_{NADH/NAD^+} \\ &= 0.815 - (-0.320)\,V \\ &= +1.135\,V\end{aligned}$$

Free energy of any oxidative reaction can be calculated by using the following formula:

$$\Delta G = -nF\Delta E$$

where n represents the number of electrons transferred during the oxidation-reduction reaction, which is 2 in case of NADH oxidation, and F is the Faraday's constant, which is equal to 96.480 $kJ.V^{-1}mol^{-1}$. Free energy released during oxidation of NADH will amount to $\Delta G = -214\ kJ.mol^{-1}$. The energy is conserved as proton gradient across the inner mitochondrial membrane. It is released when protons move in response to the gradient. Since standard energy requirement for ATP synthesis is around 50 $kJ.mol^{-1}$, the energy released during oxidation of NADH is sufficient enough for synthesis of four molecules of ATP, but actually only 2.5 ATP are synthesized (which will be discussed in Chap. 4 dealing with ATP synthesis).

There are three ways of electron transport during reduction, transport of electrons coupled with proton transport ($H^+ + e^-$), transfer as hydride ion (H^-), or direct transfer of electron such as reduction of Fe^{3+} to Fe^{2+}. Most of the dehydrogenases which carry out oxidative reactions during TCA cycle are either **NAD-linked dehydrogenases** or **flavoproteins**. Oxidation by NAD-linked dehydrogenases involves removal of two electrons from the substrates along with removal of two hydrogen atoms. One of the hydrogen atoms is transferred to NAD^+ as the **hydride**, while the other one is released as H^+ in the medium. Though NADPH is used as reducing power for the anabolic reactions, plants have NADPH dehydrogenases which are capable of removing electrons from NADPH and channelizing them to the electron transport chain. Since NADH and NADPH are not able to cross the membranes, each cell compartment maintains its own pool, and there is exchange of reducing power equivalents. Additionally flavoprotein, such as succinic dehydrogenase, is also a component of the electron transport chain which has covalently bound flavin nucleotide. Unlike NADPH, standard reduction potential of flavin nucleotides is that of the flavoprotein and not of isolated FAD or FMN since it depends upon the proteins to which these are bound. Oxidized flavin nucleotide accepts either one or two electrons, yielding semiquinone form or $FADH_2$, respectively. Since flavoproteins can participate in either single- or two-electron transport, these mediate the electron transport in between the compounds which are oxidized on removal of two electrons and the compounds which are reduced on accepting single electron.

7.6.1 Electron Transport Chain

Besides NAD^+ dehydrogenases and flavoproteins, the other components of electron transport chain involve a quinone, heme proteins, and non-heme proteins. Quinone is a small hydrophobic molecule which is also known as **coenzyme Q (ubiquinone)** in ETC of respiration. It is an abundant mobile electron transfer component, which consists of a C_{45} to C_{50} prenyl side chain having a substituted *p*-benzoquinone head group. On accepting two electrons, it is reduced to quinol. Electron transport to quinone occurs in two steps. On accepting one electron, it is converted to semiquinone (UQ^{e^-}), while on accepting another electron also, it is reduced completely (UQ^{2e^-}) to quinol (UQH_2) on combining with two protons (H^+) from the medium. Both in fully oxidized and fully reduced forms, it is highly mobile. It acts at the junction of two-electron and single-electron transfer of ETC. Since its reduction involves both electrons and protons, it plays an important role in coupling electron flow with the proton movement.

Other components of the electron transport chain are **cytochromes**. These are heme proteins, and because of iron of the heme, which has variable valency, it can exist in oxidized forms, as Fe^{3+} which gets reduced to Fe^{2+} on receiving electron or vice versa. These participate in transfer of single electrons. Three categories of cytochromes are designated as a, b, and c which are characterized by their wavelength absorbing spectra. Cytochrome a absorbs the longest wavelength, i.e., near 600 nm, type b absorbs near 560 nm, while cytochrome c absorbs near 550 nm. Sometimes cytochromes are characterized by specifying exact absorption maxima such as cytochrome b_{562}. Reduction potential of the cytochromes is determined by the proteins to which the cytochromes are covalently attached through the cysteine residues of the apoenzyme. All cytochromes in mitochondria are integral parts of the inner membrane except cytochrome c which is present as soluble protein in the matrix and is associated with the inner membrane through electrostatic attractions. Non-heme proteins are also integral part of the electron transport chain. These are **iron-sulfur proteins** in which non-heme iron is present in association with inorganic sulfur or sulfur of the cysteine residues of the protein apoenzyme. Rieske iron-sulfur protein is a type of iron-sulfur protein in which, instead of binding with two cysteines of the protein, Fe is covalently bonded to two histidine residues of the proteins. These proteins participate in the transport of single electron at a time since only one Fe is oxidized or reduced at a time. Reduction potential of these proteins varies from −0.65 V to +0.45 V which depends upon the microenvironment of the protein in which it is working. Standard reduction potential of various components of electron transport chain is given in Table 7.4**.**

7.6.2 Electron Carriers Are Arranged in a Sequence

Electron carriers involved in transport of electrons from NADH to molecular O_2 were arranged in ordered sequence by using the following three approaches:

Table 7.4 Redox potential of some of the redox couples involved in respiratory chain complexes

Redox couple	$E^{0'}$ (volts)
$NAD^+/NADH$	−0.320
$FMN/FMNH_2$	−0.30
UQ/UQH_2	0.045
Cytochrome b (Fe^{3+}/Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}/Fe^{2+})	0.22
Cytochrome c (Fe^{3+}/Fe^{2+})	0.254
Cytochrome a (Fe^{3+}/Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}/Fe^{2+})	0.36
½ O_2/H_2O	0.815

1. By withholding the supply of O_2 which is the terminal electron acceptor in the electron transport chain, all components of the chain remain in reduced state. When O_2 is supplied rate and oxidation of the electron carriers occurs according to the sequence in which these are present. Components adjacent to O_2 are oxidized first, and the components last to be oxidized are the farthest away from O_2. Oxidized and reduced state of the compounds can be monitored spectroscopically since these two states of the molecules have different absorption maxima.
2. The second approach includes use of inhibitors. In presence of rotenone, NADH accumulates since it is not oxidized. However, addition of reduced coenzyme Q results in resumption of electron transport to O_2. Similarly, in presence of antimycin A, cytochrome b is not oxidized; addition of reduced cytochrome c1 results in resumption of electron transport. Cyanide inhibits the terminal step of electron transport which involves transfer of electrons to molecular O_2. In the presence of cyanide, all components of ETC accumulate in reduced state (Fig. 7.19).
3. The sequence of components of the electron transport chain, when arranged according to the above experimental approaches, is similar to when arranged according to their redox potentials. $NADH/NAD^+$ has the redox potential −0.320 V, while O_2/H_2O has the redox potential +0.815 V, and redox potential of the other components of ETC is between these is given in the Fig. 7.20.

7.6.3 Components of the Electron Transport Chain Are Present as Multienzyme Complexes

Oxidation of NADH releases 220 $kJ.mol^{-1}$ of energy. This will be wasted if all of the energy is released in a single step. However, multistep release of the energy is used for translocation of protons across membrane and is conserved as proton gradient. ATP synthesis occurs in response to the proton movement through ATP synthase. This process of ATP synthesis is known as oxidative phosphorylation. In 1962, Youssef Hatefi successfully isolated four different complexes (Complexes I–

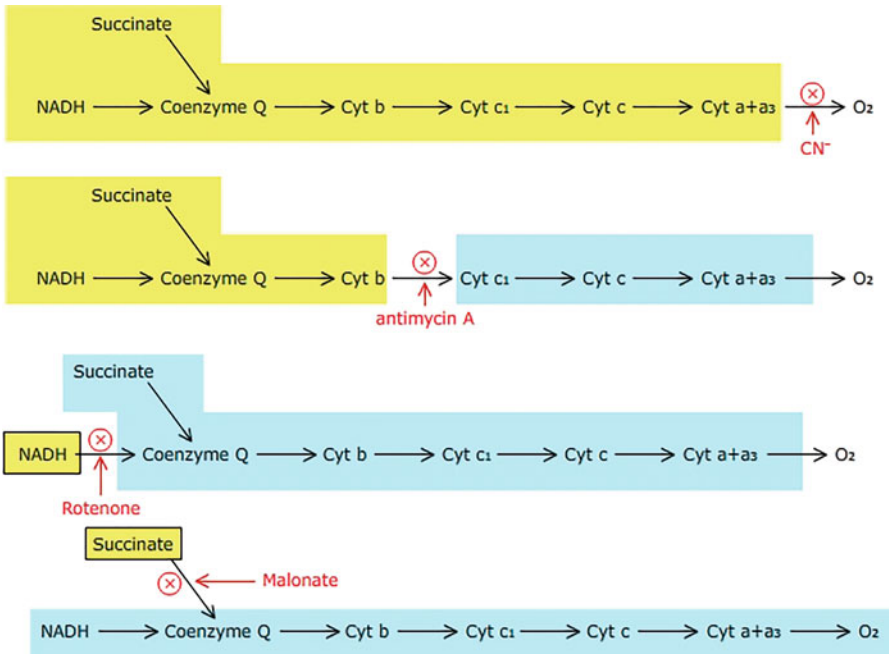

Fig. 7.19 The use of inhibitors in elucidating the sequence of components of electron transport chain. Yellow color indicates the components in reduced state, while blue color indicates the components in oxidized state

IV) while working with beef heart mitochondria, indicating that the components of the electron transport chain are associated into multienzyme complexes which are conserved in the mitochondria of all the organisms. Proteomic studies, using **peptide mass spectrometry,** have revealed that almost 85% of the proteins of these complexes are common in all eukaryotic systems. However, 15% of the proteins are plant specific or similar to those present in algae but are absent from fungi and animals. The role of these proteins has not been specified yet. Some of the proteins of these complexes are encoded in mitochondrial genome, while others are encoded in the nuclear genome. There are studies which indicate mitochondrial e-transport complexes to be associated to form supercomplexes which are called **respirosomes**. Complexes I, III, and IV have been found to be associated with different degrees and configurations, which suggest the possibility of channelling electrons from NADH to O_2 with higher transfer rate. The formation of supercomplexes may help in increasing the stability of the individual complexes as well as providing a regulatory mechanism that controls the passage of electrons through ETC (Fig. 7.21).

Complex I: NADH Dehydrogenase (NADH-Ubiquinone Oxidoreductase) This is the first supercomplex in the electron transport chain associated with NADH oxidation. It is a large multi-subunit complex consisting of about 45 polypeptides. It is similar to mammalian and fungal Complex I; however, there are a few proteins

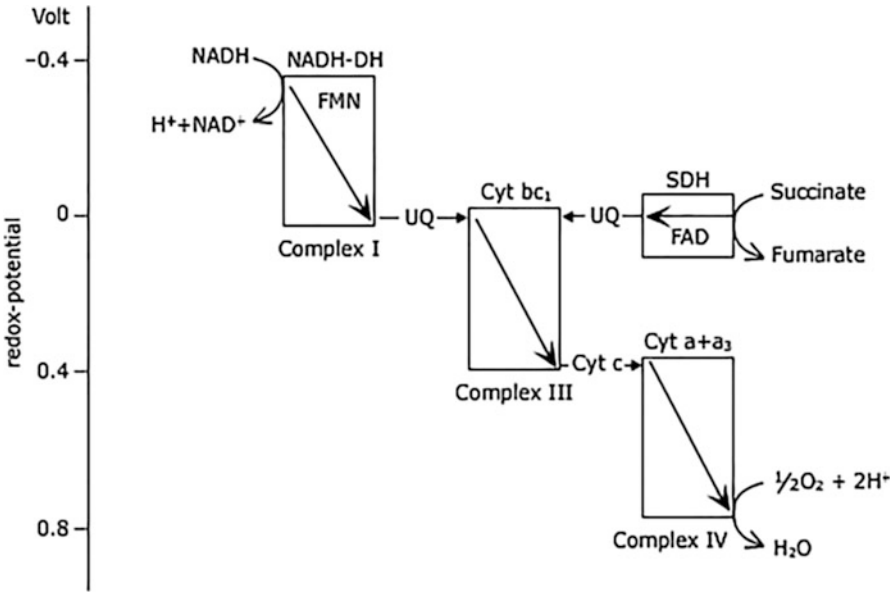

Fig. 7.20 Components of electron transport chain with their redox potentials. *NADH-DH* NADH dehydrogenase, *SDH* succinic acid dehydrogenase (constituent of Complex II)

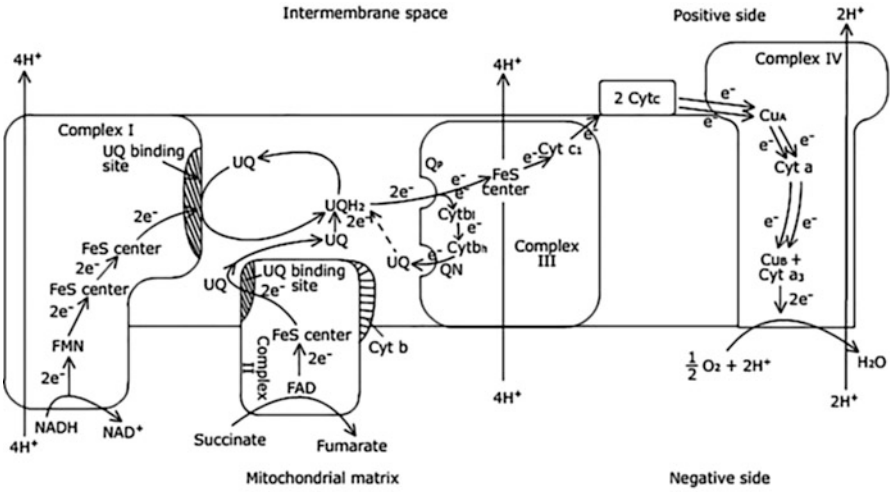

Fig. 7.21 Pathway of electron transport through four supramolecular complexes present in the inner mitochondrial membrane. Transport of two electrons removed from NADH is coupled with translocation of 10 H^+ across the membrane

which are plant specific. Plant mitochondrial genome may encode as many as nine components of Complex I. It has been found to be L-shaped in studies carried out with *Arabidopsis* mutants. The complex oxidizes NADH which is produced in mitochondrial matrix during TCA. The electrons removed from NADH are transferred to ubiquinone. While electrons are transferred to UQ, conformational changes occur in the antiporter proteins present in the membrane arm of the complex as a result of which four protons are translocated across the membrane. Besides presence of various proteins which make up this complex, the cofactors associated with the complex include FMN-containing protein and at least six Fe-S centers. Reaction catalyzed by the complex includes transfer of one hydride ion from NADH and one proton from the matrix to ubiquinone. It can be written as,

$$\mathrm{NADH} + \mathrm{H^+} + \mathrm{Q} \rightarrow \mathrm{NAD^+} + \mathrm{QH_2}$$

The two electrons and protons removed from NADH are transferred to FMN and then through a series of Fe-S proteins are transferred to ubiquinone which is bound to the ubiquinone-binding site of the complex. Transport of two electrons to UQ by the complex is coupled with transport of 4 H^+ across the membrane. Thus Complex I serves as the proton pump. The reaction can be written as

$$\mathrm{NADH} + 5\ \mathrm{H^+_N} + \mathrm{Q} \rightarrow \mathrm{NAD^+} + \mathrm{QH_2} + 4\mathrm{H^+_P}$$

The electron transfer can be inhibited by rotenone (a plant-derived flavonoid), amytal (a barbiturate drug), and piericidine (an antibiotic) which inhibit electron transfer from the Fe-S centers of the complex to UQ (Fig. 7.22). Plants are characterized by the additional presence of both external and internal NAD(P)H dehydrogenases which are insensitive to rotenone and are capable to oxidize both externally and internally generated NADH and NADPH (NADex and NADin). This again indicates flexible systems present in plants so that they are capable of adjusting according to their needs (Fig. 7.23).

Complex II: Succinate Dehydrogenase (Succinate-Ubiquinone Oxidoreductase) This is the only membrane-bound enzyme of TCA cycle. It catalyzes oxidation of succinate to fumarate by removing two electrons and transferring them to ubiquinone which is reduced to UQH_2. Unlike Complex I, electron transport is not coupled with proton translocation across membrane, and this complex does not contribute to the proton gradient. This is the smallest complex of the electron transport chain, consisting of only four different protein subunits, which range in sizes from 13.5 to 70 kDa. In the majority of plants including angiosperms and gymnosperms, none of these proteins are encoded by the mitochondrial genome. Two of the smaller hydrophobic protein subunits are present as integral membrane proteins, while the other two large hydrophilic protein subunits extend in the matrix. Succinate-binding site, FAD, and three Fe-S centers are associated with the peripheral proteins which are extending toward the matrix of mitochondria. The two binding sites for ubiquinone are present in the hydrophobic integral proteins, which are also associated with

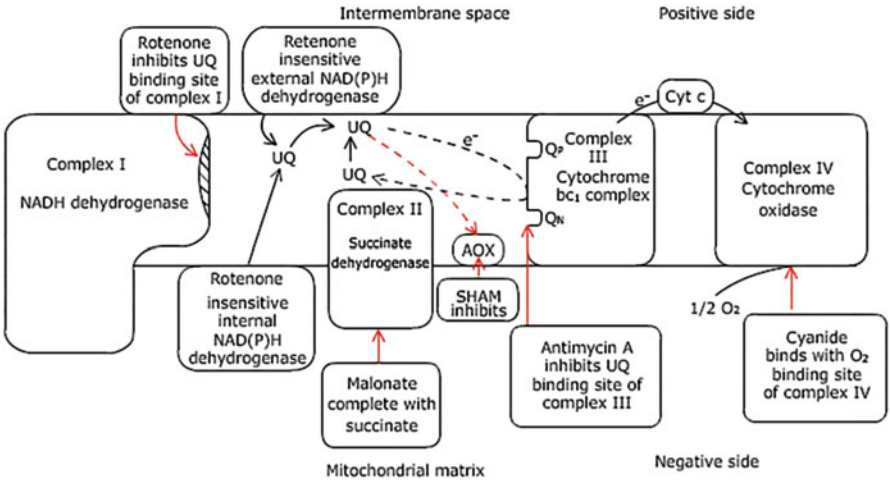

Fig. 7.22 Various inhibitors and their site of action on the transport of electrons

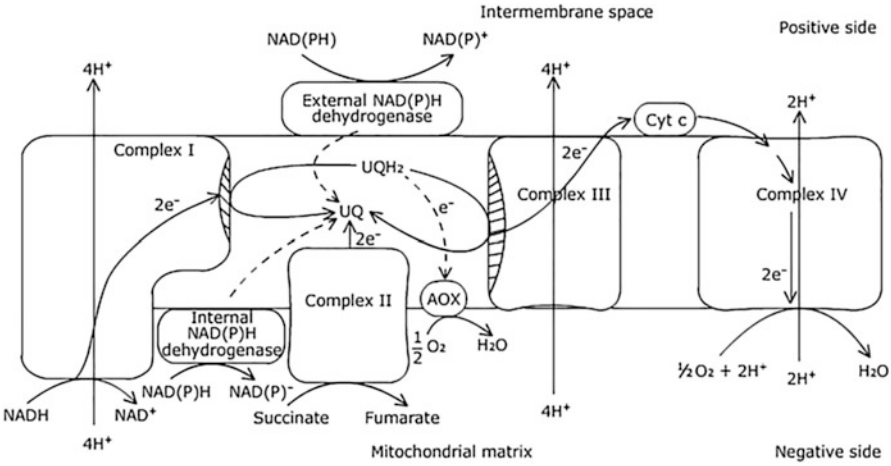

Fig. 7.23 Components of electron transport chain are present as four complexes: Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III and Complex IV (cytochrome oxidase). In plants, there are additional NAD(P)H dehydrogenases; both external and internal are present which oxidized NAD(P)H by transferring electrons directly to UQ, thus missing the first step of H^+ translocation. UQH_2 is oxidized by Complex III which is coupled with translocation of $4H^+$ across the membrane. In plants, there is also an alternate oxidase (AOX) present which can oxidize UQH_2 directly without transferring electrons to Complex III and Complex IV. From Complex III electrons are carried to cytochrome c and are delivered to Complex IV which pass on the electrons to O_2 which is reduced to water after receiving electrons combined with H^+ from mitochondrial matrix

cyt b. Electrons removed from succinate are transferred to UQ through FAD and three Fe-S centers. Electron transport by Complex II is inhibited by malonate which is an analogue of the substrate. Cytochrome b is not in the main pathway for electron transport; however, its role is believed to be in reducing the leakage of electrons to O_2 and thus in preventing the formation of reactive oxygen species.

Complex III: Cytochrome bc_1 Complex (Ubiquino-Cytochrome c Oxidoreductase)
Complex III is responsible for removing electrons from UQH_2 and transferring them to cytochrome c, which is a small protein present toward the N side of the membrane (inter-mitochondrial space). It is responsible for shuttling of electrons from Complex III to Complex IV. This is a huge complex whose complete structure was determined between 1995 and 1998 using X-ray crystallography. The functional unit of *cytochrome bc_1* has been found to be consisting of 11 polypeptide subunits, out of which only one is encoded in mitochondrial genome. This complex has the structure similar to cytochrome b_6f which is present in chloroplasts and is part of photosynthetic electron transport. It consists of cytochromes having heme of two b types, b_{566} (b_l) and b_{560} (b_h), and a cytochrome c_1, a Rieske-type Fe-S protein, and the other polypeptides. The complex has largest polypeptide in the center which is known as the core protein having a molecular weight ranging from 51 to 55 kDa. There are two binding sites present in the Complex III for binding with ubiquinone which are known as Q_P and Q_N sites, which indicate the Q-binding site present toward P (positive) side of the membrane and toward N (Negative) side of the membrane, respectively. The terminology is based on the fact that the intermembrane space of the mitochondria is the positive side because of accumulation of H^+, while the matrix side is negative because of lesser H^+ concentration. The complex is present at the interface of two-electron transfer and one-electron transfer. Two electrons are removed from UQH_2 at the P side of the membrane. One electron is passed on to cytochrome c_1 via Fe-S protein and then to cytochrome c which contains single heme. It is present toward the intermembrane space, held to the membrane by electrostatic attraction. It can move and deliver the electrons to Complex IV. The second electron removed from QH_2 is cycled through two hemes to UQ at the Q_N site reducing it partially to semiquinone state (UQ^{e^-}). The cycle is repeated twice; as a result UQ^{e-} will receive another electron in similar manner and will further be reduced to UQ^{2e-}, and then it picks up two H^+ from the matrix and will be reduced to UQH_2. In this form, it is freely mobile within membrane and is oxidized at the Q_P site. The two protons will be released in the intermembrane space. This cycle is known as Q-cycle which is significant for transporting protons across the membrane, thus adding to proton gradient. Q-cycle is similar to the one occurring during photosynthetic electron transport. For transport of two electrons in the chain, four protons are transported across the membrane (Fig. 7.24).

The net redox reaction of Q-cycle will be

$$QH_2 + 2 \text{ cyt } c_1(\text{oxidized}) + 2\ H^+{}_N \rightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4\ H^+{}_P$$

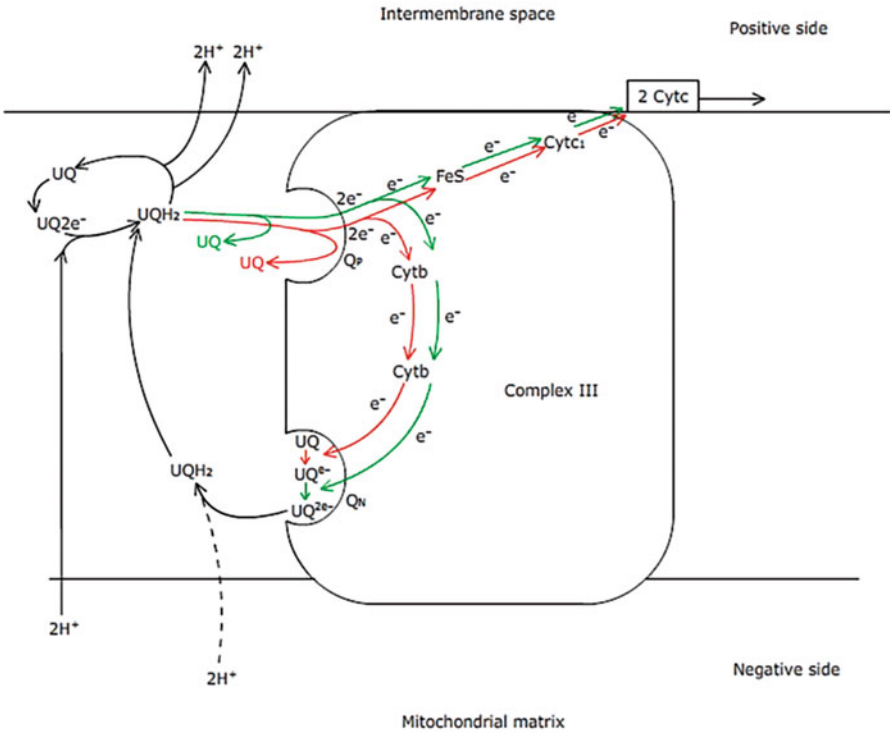

Fig. 7.24 Q-cycle. In the first cycle, out of the two electrons removed from UQH_2, one electron is transferred to cyt c, and one is cycle back to UQ via cyt b (demonstrated in red). Similar cycle is repeated from another molecule of UQH_2 (demonstrated in green). This results in reduction of UQ $^{e-}$ to UQ^{2e-}

The inhibitor antimycin A binds with the Q_N site of the complex, thus inhibiting reduction of UQ (Fig. 7.23).

Complex IV: Cytochrome Oxidase (Cytochrome c: O_2 Oxidoreductase) Reduced cytochrome c is positively charged, so it can diffuse on the negatively charged inner membrane of mitochondria toward the intermembrane space and deliver the electrons to cytochrome oxidase. Structure of the cytochrome oxidase has been worked out using X-ray crystallography. It consists of 13 protein subunits, out of which at least 3 are encoded in the mitochondrial genome. Redox centers of the complex consist of two "a" type cytochromes, i.e., cytochrome a and a_3, and two copper centers Cu_A and Cu_B. Redox centers are located in the mitochondrial encoded protein subunits. The complex consists of a large hydrophilic area, which protrudes in the intermembrane space and has got the binding site for oxidation of cytochrome c. The electrons removed from cytochrome c are transferred to two Cu_A atoms of the proteins which are held by their interactions with S of the cysteine of the protein. Electrons are then passed through heme of cytochrome "a" to another redox

center consisting of Cu_B, bound to histidine and cytochrome a_3. This second redox center probably acts as binuclear center, Cu_B and heme of $cyta_3$, which can accumulate two electrons. These electrons are then transferred to O_2, which on receiving the two electrons can bind with 2 H^+ from the matrix side and are reduced to water, completing the electron transport process.

$$[\,Fe^{+++}.Cu^{++}] + 2\,e^- \rightarrow [Fe^{++}.Cu^{+}]$$

$$½\,O_2 + 2\,e^- + 2\,H^+ \rightarrow H_2O$$

Both CO and CN^- can bind to the O_2-binding site of cytochrome a_3, thus compete with O_2, and act as inhibitor of the Complex IV. Thus, in the presence of CN^-, electron transport to O_2 is inhibited, and the whole process of electron transport is halted. However, in plants, respiration occurs even in presence of cyanide, which is known as cyanide-resistant respiration (Fig. 7.23).

7.6.4 Proton Translocation Creates Proton Motive Force (PMF)

There are three sites in the electron transport chain during NADH oxidation, which are coupled with transport of H^+ across the membrane:

1. Complex I, which is responsible for transport of 4 H^+ across the membrane coupled with the removal of two electrons from NADH
2. Complex III, which transports 4 H^+ coupled with transport of two electrons
3. Complex IV, which transports 2 H^+ coupled with transport of two electrons to O_2

Thus, energy released during movement of two electrons downhill from NADH to O_2 results in creating a gradient of 10 H^+ across the inner mitochondrial membrane, creating membrane potential also. As a result, there will be a positive side of the membrane toward the intermembrane space due to proton accumulation and negative side toward the matrix side of the membrane. These proton gradient and voltage gradient together generate *proton motive force* (PMF) which is responsible for backflow movement of protons from the cytosolic side toward matrix. Since the membrane is impermeable to protons, protons move through specific complex, Complex V or also known as F_o-F_1 or ATP synthase since these are associated with ATP synthesis (Fig. 7.23).

7.7 NADH Shuttles

"Redox status" of the cell is important for the cellular metabolism. Each compartment of the cell has got its own pool of pyridine molecules ($NAD(P)^+$) which may either be present as $NAD(P)^+$ or NAD(P)H. Many of the cellular reactions will require $NAD(P)^+$ in the oxidized state. In case $NAD(P)^+$ pool of the cell

compartments is exhausted, the cell metabolism will stop. Direct transport of NAD(P)H does not occur since transporters for NAD(P)/NAD(P)H are absent in the cell membranes. Transport occurs indirectly as exchange of reduced compound with the oxidized form. This exchange of reducing power indirectly is called NADH shuttle. In order to avoid **oxidative stress**, it is very important for the cells to modulate ratio of NAD(P)/NAD(P^+)H and is significant for maintaining redox homeostasis of the cell. ATP synthesis coupled with NADH oxidation occurs only in mitochondria because of the presence of electron transport chain. Various NADH shuttles operate in both plants and animals, which are responsible for reducing power to be exchanged in between different cell compartments. These include malate/oxaloacetate NADH shuttle, malate aspartate shuttle, and glycerol phosphate/dihydroxyacetone phosphate shuttle mechanism.

Glycerol phosphate/dihydroxyacetone phosphate shuttle: NADH produced during glycolysis is reoxidized to NAD^+ coupled with reduction of dihydroxyacetone phosphate to glycerol phosphate. Reaction is catalyzed by the cytoplasmic glycerol phosphate dehydrogenase. Glycerol phosphate diffuses through outer mitochondrial membrane and transfers electrons to FAD of the Complex II reducing it to $FADH_2$. The reaction is catalyzed by the mitochondrial glycerol phosphate dehydrogenase. Electrons from $FADH_2$ enter the electron transport chain at the coenzyme Q level (Fig. 7.25).

Malate/oxaloacetate shuttle: This shuttle is the most extensively studied redox exchange mechanism and is one of the most important NADH shuttles in plants. It is responsible for transfer of reducing equivalents in between chloroplasts, cytosol, mitochondria, and also peroxisomes. Oxaloacetate transporters are present in membranes which transport oxaloacetate in exchange of malate or vice versa. Excess NADPH produced during light reaction is transported out of chloroplasts in the form of malate, which is produced because of reduction of oxaloacetate. Malate is

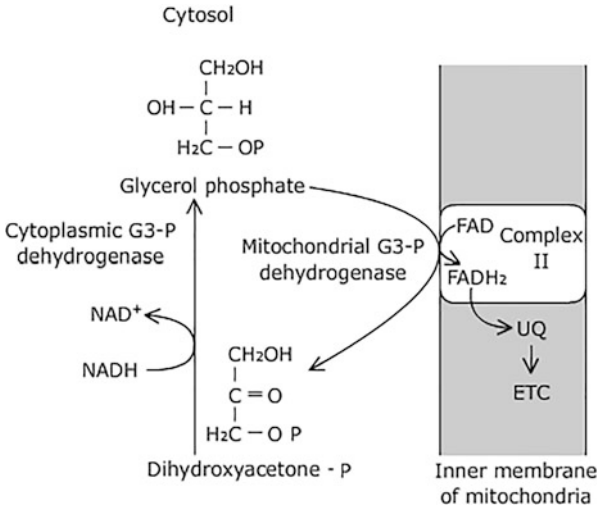

Fig. 7.25 Glycerol-3-phosphate shuttle

exchanged with cytosolic oxaloacetate, which is then transported to mitochondria in exchange of oxaloacetate through dicarboxylate transporters. Once inside mitochondria, malate is oxidized to oxaloacetate by NAD-malate dehydrogenase with simultaneous reduction of NAD^+ to NADH, which is oxidized through electron transport chain. In this way reducing power, NADPH, is transferred from chloroplasts to mitochondria as NADH. Direction of the shuttle depends upon the relative concentrations of the participating molecules. An alternative use of malate released from chloroplasts may be in generation of cytosolic NADH, which may be utilized for nitrate reduction. NADH, produced in mitochondria during photorespiration, may also be exported out to peroxisomes where it is consumed for reduction of α-hydroxypyruvate (Fig. 7.26).

Malate/aspartate NADH shuttle: This shuttle has been demonstrated in isolated mitochondria and is responsible for transporting cytosolic reducing equivalents in the form of malate. There are 2-oxoglutarate transporters and glutamate/aspartate transporters present in the mitochondrial membrane which facilitate the movement as malate and glutamate/aspartate, respectively. Oxidation of malate in mitochondria generates NADH which may be used for ATP generation through ETC. More ATP is generated through this shuttle than the glycerol phosphate/dihydroxyacetone phosphate shuttle. Exchange of glutamate with aspartate is electrogenic in nature through glutamate/aspartate carriers present in the mitochondrial membrane. This shuttle is significant especially in actively respiring mitochondria, when aspartate is driven out of mitochondrial matrix because of $\Delta\Psi$, mitochondrial matrix side being negative (Fig. 7.27).

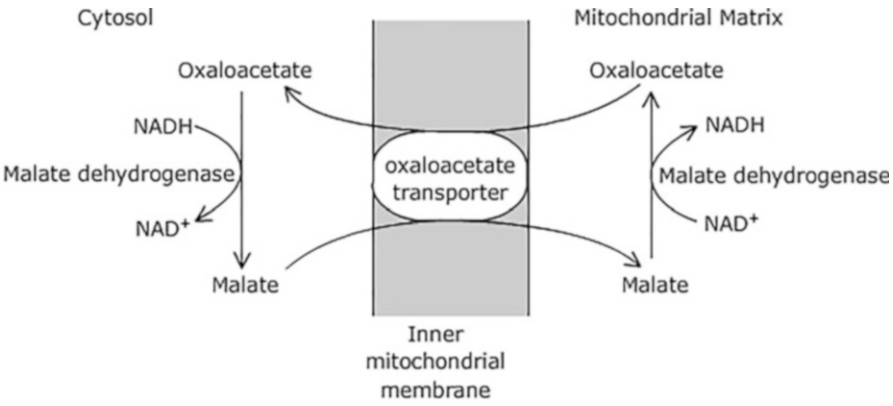

Fig. 7.26 Malate/oxaloacetate shuttle transports reducing equivalents from cytosol to mitochondrial matrix. Exchange occurs in the form of malate from cytosol to mitochondrial matrix and OAA from mitochondrial matrix to cytosol mediated by OAA transporters present in the inner mitochondrial membrane. Isoforms of malate dehydrogenase catalyze conversion of malate to OAA in cytosol and from OAA to malate in mitochondrial matrix. NADH produced in mitochondrial matrix can now be oxidized through ETC

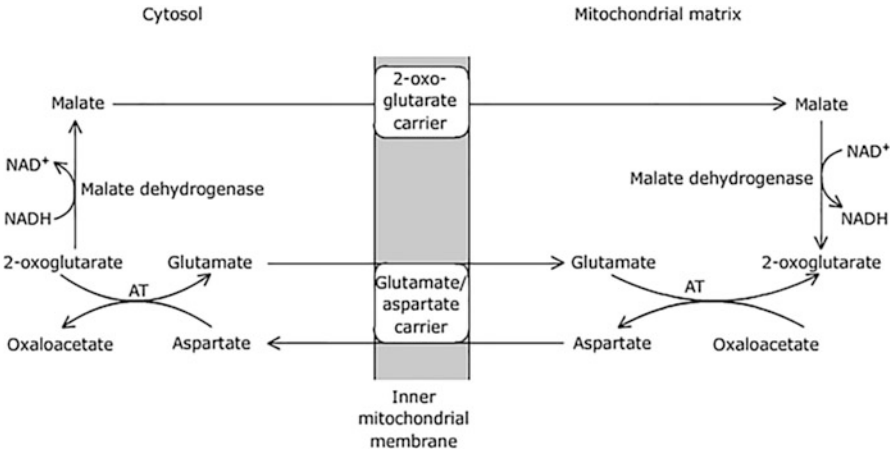

Fig. 7.27 Malate/aspartate NADH shuttle. Reducing equivalents are transported across inner mitochondrial membrane in the form of malate and aspartate. Isoforms of malate dehydrogenase and aspartate aminotransferase (AT) are found on both sides of inner mitochondrial membrane

7.8 Alternate Mechanisms of NADH Oxidation in Plants

Certain features of mitochondrial electron transport in plants are different from those in animals. In plants there are four more dehydrogenases present in the inner mitochondrial membrane, which are not inhibited by rotenone. Two of these rotenone-insensitive dehydrogenase, NADH and NADPH, are responsible for oxidizing cytosolic NADH and NADPH generated during glycolysis and oxidative pentose phosphate pathway, respectively. These pyridine nucleotides need to be oxidized for continuation of glycolysis and OPPP. These dehydrogenases donate electrons to ubiquinone directly bypassing the Complex I. Similarly the other two dehydrogenases NADH and NADPH are present in the inner mitochondrial membrane facing toward mitochondrial matrix, which also pass electrons directly to ubiquinone from mitochondrial NADH and NADPH. Activity of these dehydrogenases as well as their synthesis is increased in response to reduced levels of ADP and increased Ca^{2+} concentrations, because of increased transcription of the genes involved which occurs in response to stress. In mitochondrial matrix, NADH is produced in the TCA cycle, while NADPH is produced by **nicotinamide nucleotide transhydrogenase**, which is located in the inner mitochondrial membrane and acts both as an enzyme as well as transporter. It reduces $NADP^+$ to NADPH at the expense of NADH. Transhydrogenase involves only mitochondrial NAD^+ and $NADP^+$, and cytosolic pools remain unaffected. The enzyme reaction is coupled to translocation of proton.

$$NADH + NADP^+ + H^+_{(out)} \rightarrow NAD^+ + NADPH + H^+_{(in)}$$

Box 7.2: Mitochondrial Diseases Are Being Discovered

As can be understood, mitochondria are central to the metabolism in all the eukaryotes, since it is the hub where majority of ATP is produced. In plants however chloroplasts provide additional ATP especially in presence of light. Any dysfunctional mitochondrial disorder due to any of its defective enzymes, whether they are encoded in nucleus or mitochondria, results in various diseases in humans. The first mitochondrial disease to be understood was Leber hereditary optic neuropathy (LHON), a form of blindness that strikes in midlife as a result of mutations to the NADH-Q oxidoreductase component of Complex I. Some of these mutations impair NADH utilization, whereas others block electron transfer to Q. A knowledge about few of the diseases in human is there. However, more studies are required regarding diseases in plants due to defective mitochondrial functions. Respiratory chain complexes are composed of around 90 proteins, out of which almost 13 are encoded in the mitochondrial genome. Besides providing ATP, mitochondria are also the sites for ROS production especially when ubiquinone reduction exceeds its oxidation, and there is possibility of leakage of electrons to O_2 resulting in ROS generation. Little information is there regarding the role of mitochondrial ROS in plants. Studies carried out with Complex II mutants of *Arabidopsis*, SDH-1, have demonstrated that these exhibited reduced response to stress.

Since the electrons are directly donated to ubiquinone and Complex I is bypassed, less proton gradient is created. Thus, the main role of these dehydrogenases is in oxidation of extra NADH and NADPH, so that the components of electron transport chain remain in oxidative condition rather than in reduced form and ROS formation is avoided under **oxidative stress**. At times NADH is not required, but TCA occurs to provide precursors for various anabolic pathways. These dehydrogenases play an important role in oxidation of NADH for continuation of TCA. It is evident that mitochondria are the hub of metabolic activities. Impaired mitochondrial metabolism leads to various diseases both in animals and plants (Box 7.2).

7.9 Cyanide-Resistant Respiration

In animals respiration is completely inhibited by cyanide, which blocks the electron transport between O_2 and the Complex IV. However, in plants respiration is inhibited 10–100% by cyanide, depending upon the species variations. There is an **alternate oxidase (AOX)** present in the inner mitochondrial membrane, which facilitates transfer of the electrons from reduced ubiquinone (semiquinone or quinol) directly to O_2 bypassing the Complex III and Complex IV (Figs. 7.22 and 7.23).

Respiration which occurs in presence of cyanide is known as cyanide-resistant respiration. Unlike alternative dehydrogenases in which branch point of electron transport is before reducing ubiquinone, AOX provides the branch point after ubiquinone has been reduced, and electrons instead of being transported through cytochromes oxidases are directly transferred to O_2. As a result, proton gradient is not created across inner mitochondrial membrane, and ATP is not produced. Energy of the electron transport is dissipated as heat, and ATP yield decreases by up to 60%. The pathway is also believed to be associated with **thermogenic** events of the plants. An increase in as much as 25 °C has been observed in voodoo lily (*Sauromatum guttatum*) at the time of inflorescence (spadix) formation. Increase in temperature is associated with volatilization of various compounds, such as amines, which give putrid smell and serve to attract pollinators. Cyanide-resistant respiration may also be responsible for generating heat in plants growing in cold climate. Besides having thermogenic significance, cyanide-resistant respiration may have a role in metabolism of plants subjected to oxidative stress. Inhibition of electron transport under stress conditions results in increased ratio of $NADH/NAD^+$, which will result in slowing of TCA cycle. Alternate oxidase catalyzes oxidation of NADH. The formation of salicylic acid is induced under stress, which leads to synthesis of AOX. CN-resistant respiration is considered as "spill-over" mechanism or can be compared to a short circuit. It is considered significant in plants growing under high light intensities or other kinds of stress. ROS accumulate in AOX mutants of *Arabidopsis* when exposed to adverse conditions. Two forms of AOX have been reported in dicots (AOX1 and AOXII), while in monocots there is only one form, i.e., AOXI. AOX is a homodimer with each subunit having the molecular mass of 36 kDa, which are tightly bound to the matrix side of the inner mitochondrial membrane. Cysteine residues, located at the active sites of AOX, are exposed to matrix. When oxidized they are bonded to each other because of the disulfide bonds (-S-S-), making the enzyme inactive. When reduced (-SH) these are not bonded to each other, and the enzyme remains active. This interconversion of redox state of AOX is regulated by the mitochondrial NADP-dependent thioredoxin system, which also regulates activity of many of TCA cycle enzymes. Regulation of redox status of Calvin cycle enzymes is by ferredoxin-dependent thioredoxin system.

$$\mathrm{NADPH} + \mathrm{Thioredoxin_{ox}} \rightarrow \mathrm{NADP^+} + \mathrm{Thioredoxin_{red}}$$

$\mathrm{Thioredoxin_{red}}$ is responsible for reduction of the cysteine residues, which makes the enzyme susceptible to activation by pyruvate, and also it has more affinity with reduced UQH_2. Increased ratio of $NADH/NAD^+$ will decrease the TCA cycle. As a result of this, pyruvate accumulates which in turn increase activity of AOX and NADH is oxidized. **Salicylhydroxamic acid (SHAM)** interferes with the electron transport to AOX; that is why the pathway is also known as **SHAM pathway**.

Summary

- Respiration is a multistep catabolic process catalyzed by enzymes. Energy released in some of the steps is sufficient enough to be used for ATP synthesis. In all living beings, carbohydrates are the main source of energy. In plants sucrose or starch is the main source of glucose which is primarily metabolized during respiration.
- Initial steps of glucose catabolism, known as glycolysis, are similar both in the presence or absence of O_2. Many scientists have contributed in elucidating the path of glycolysis. Unlike in animals, glycolysis occurs both in cytosol and plastids in plants, and there are various isoforms of the glycolytic enzymes. Regulation of glycolysis in plants is different from that of animals. In plants, there is "bottom up," while in animals it is "feed-forward" regulation of glycolysis.
- In the absence of O_2, pyruvate is metabolized through fermentation in cytosol. It is converted either to lactic acid or ethanol. For continued production of 2 ATP per glucose molecule, in absence of O_2, fermentation is necessary so that NADH is oxidized to NAD^+ to be available for glycolysis. Besides energy, glycolytic pathway also provides precursors to be used for biosynthesis of various biomolecules.
- Additionally, glucose is metabolized through oxidative pentose phosphate pathway (OPPP). OPPP consists of an irreversible oxidative phase, during which two molecules of NADPH are produced per molecule of glucose 6-phosphate and one carbon is lost as CO_2 and non-oxidative reversible phase which results conversion of ribulose 5-phosphate back to glucose 6-phosphate. Both in plants and in animal cells, OPPP operates in cytosol. However, in plants the pathway occurs in plastids also, where OPPP operates in dark, while reductive PPP operates in light. The enzymes of both pathways are regulated by light through the thioredoxin-ferredoxin system. OPPP plays a significant role in providing NADPH especially in fungi, in animal cells, and in plants during dark. Various intermediates of the pathway also serve as precursors for other pathways, such as ribose 5-phosphate is used for nucleotides biosynthesis, and erythrose 4-phosphate along with glycolytic intermediate PEP is a source of phenolic compounds.
- In the presence of O_2, pyruvate is converted to acetyl-CoA in mitochondria by oxidative decarboxylation and catalyzed by pyruvate dehydrogenase complex (PDC), which is a complex reaction and involving five steps. PDC is a multienzyme complex of three enzymes and five cofactors.
- Acetyl-CoA condenses with oxaloacetate to form citrate, which is the first reaction in citric acid cycle. Hans Krebs had put together the works of many scientists and proposed the reactions to be cyclic in nature and proposed citric acid cycle. TCA cycle is primarily regulated by NADH/NAD^+ and ATP/ADP ratio. Besides providing NADH, which is oxidized by electron transport chain to generate ATP,

TCA cycle also plays an anabolic role. Various intermediates of the cycle serve as precursors for many biomolecules. As some of the intermediates of TCA are consumed, there are anaplerotic reactions, which replenish the cycle intermediates.

- Reduced coenzymes, NADH and $FADH_2$, are oxidized through electron transport chain. Energy released during downhill movement of electron is conserved as the proton gradient across inner mitochondrial membrane, which is coupled with ATP synthesis. Components of the electron transport chain are present in four complexes, Complexes I, II, III, and IV. Movement of electrons between these complexes is facilitated by the mobile carriers, ubiquinone and cytochrome c. O_2-binding site is present in Complex IV.
- Plants have unique features like the presence of external and internal NAD(P)H dehydrogenases in addition to the electron transport chain, which are rotenone insensitive. There is an alternate oxidase (AOX) present in plants, which accepts electrons from ubiquinone directly bypassing Complexes III and IV. This is called as cyanide-resistant respiration, since the site of action of cyanide, which is present in Complex IV, is bypassed during the electron transport. Cyanide-resistant respiration does not result in any ATP generation. It is associated with dissipation of extra energy and might be associated with coping mechanism in plants when subjected to stress.

Multiple-Choice Questions

1. While studying glycolysis, adding iodoacetate to the medium leads to accumulation of:
   (a) Fructose 6-phosphate
   (b) 3-Phosphoglyceraldehyde
   (c) 1,3-Bisphosphoglycerate
   (d) Fructose 1,6-bisphosphate
2. Net gain of energy during conversion of one glucose molecule to two molecules of pyruvate in glycolysis is:
   (a) 2 ATP + 2 NADH
   (b) 4 ATP
   (c) 2 NADH
   (d) 2 ATP
3. Which of the following reaction in glycolysis is not reversible?
   (a) Glucose 6-phosphate to fructose 6-phosphate
   (b) Glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate
   (c) 2-Phosphoglycerate to 2-phosphoenolpyruvate
   (d) Fructose 6-phosphate to fructose 1,6-bisphosphate

4. Under anaerobic conditions in the cell, fermentation is necessary because:
   (a) Lactate is produced.
   (b) Ethanol produced leaches out of the cell.
   (c) NADH is oxidized to NAD^+.
   (d) ATP is produced.
5. Pyruvate dehydrogenase is active when the enzyme is:
   (a) Dephosphorylated
   (b) Phosphorylated
   (c) Reduced
   (d) Oxidized
6. TCA has got amphibolic role in cell metabolism because:
   (a) Both ATP and NADH are produced in the cycle.
   (b) It is the main pathway for generation of metabolic form of energy.
   (c) It is responsible for oxidative as well as reductive reactions.
   (d) Precursors of various pathways are also produced during the cycle besides their oxidation.
7. Glycolysis in plants differs from that of in animals in the presence of:
   (a) Fermentation in animals
   (b) PP_i-dependent phosphofructokinase
   (c) Transport of glycolytic NADH to mitochondria
   (d) Synthesis of pyruvate from PEP in animal cells
8. Antimycin A inhibits electron transport at the:
   (a) UQ-binding site of Complex I
   (b) UQ-binding site of Complex III
   (c) O_2-binding site of Complex IV
   (d) With Complex II
9. The inhibitor for cyanide respiration is:
   (a) Rotenone
   (b) Antimycin A
   (c) Salicylhydroxamin
   (d) Cyanide
10. The number of ATP equivalents produced during complete oxidation of one glucose molecule is:
   (a) 30–32
   (b) 32–34
   (c) 34–36
   (d) None of the above

Answers

1. d 2. a 3. d 4. c 5. a 6. d 7. b
8. b 9. c 10. a

Suggested Further Readings

Browsher C, Steer M (2008) In: Tobin A (ed) Plant biochemistry. Garland Science, Taylor & Francis Group, New York, pp 143–192

Campbell MK, Farrell SO (2012) Biochemistry, 7th edn. Cengage Learning, Stanford, pp 481–506

Nelson DL, Cox MM (2017) Lehninger principles of biochemistry, 7th edn. WH Freeman, MacMillan Learning, New York, pp 533–570

Taiz L, Zieger E, Moller IM, Murphy A (2015) Plant Physiology and Development, 6th edn. Sinauer Associates, Inc. Publishers, Sunderland, pp 317–339