Chapter 11
Aerobic Metabolism

No less than three different types of gradients make up respiration, representing the aerobic energy-exchange devices. Respiratory gradients are created, maintained, and exploited within mitochondria through the use of membranes. As will be shown, membrane-oriented energy exchange gradients differ tremendously as compared with anaerobic substrate-level phosphorylation. Membranes are constructed of lipid bilayers that act as a barrier separating areas of high (concentrated) energy from lower (dispersed) energy. Such separation defines the word gradient (a difference over a distance).

11.1 Mitochondria

Mitochondria are a double-membrane organelle once thought to be a type of ancient bacteria that began a symbiotic relationship with eukaryotic cells several billion years ago (mitochondria have an independent albeit limited DNA separate from that of the cells nucleus) (1–3). Taken literally this means that every cell in your body is composed of at least two life forms (in fact, several of your cell's organelles are thought to have originated as separate life forms). First seen as having a bean-shaped appearance, mitochondria were also found to congregate in those areas of the cell that require a sustained and or large energy (ATP) supply (4, 5), around a muscles contractile apparatus for example (6). It is now apparent that the membranes of a few mitochondria actively spread as a reticulum throughout a cell, amassing at sites of high energy demand (the surface area of a single mitochondria must be incredibly extensive). Far from having the sole role of primary energy supplier, mitochondria are important in cell signaling, the biosynthesis of iron compounds, calcium control within the cell, and apoptosis (programmed cell death). Figure 11.1 portrays a mitochondrian in a traditional bean-shaped form.

Depending on the cell type, mitochondria may occupy a volume of 1–50% of a cell's interior. The type and amount of structures found within a cell speaks loudly to what the primary function of a cell is. To place this in perspective, the zoologist

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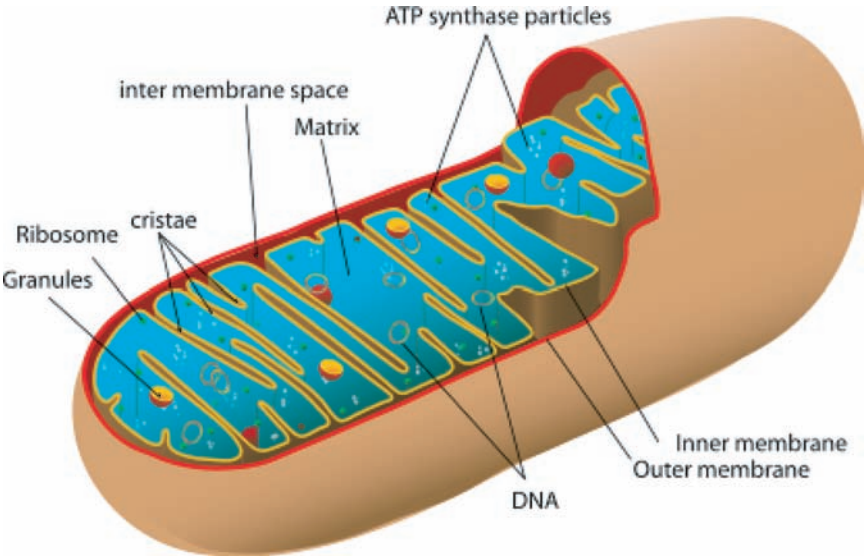

Fig. 11.1 A traditional pill-shaped portrait of a single mitochondrion is shown. Much of mitochondrial metabolism occurs within the mitochondrial matrix (where the enzymes of the Krebs cycle and fat oxidation are found) and the inner mitochondrial membrane (where electron transport chain resides) (Courtesy of Mariana Ruiz Villarreal, http://commons.wikimedia.org/wiki/Image:Diagram_of_an_animal_mitochondrion.svg)

Peter W. Hochachka (1937–2002) surmised that in a muscle cell, for example, three components make-up or fill the majority of the cells internal space: the sarcomere (the contractile apparatus), the sarcoplasmic reticulum (a double-membrane structure that contains the calcium needed to promote muscle contraction), and the mitochondria themselves (to supply the energy demands of contraction) (7). Fast twitch (white) muscle – the muscle of sprinters – is considered to be mostly anaerobic, containing perhaps a 1% volume of mitochondria but a drastically larger percentage of sarcoplasmic reticulum. Slow twitch (red) muscle – the muscle of long distance runners – is highly aerobic, whose volume is perhaps 50% filled by energy-producing mitochondria.

Mitochondria's outer membrane is roughly 50% protein and 50% lipid; it is an extremely porous structure that perhaps contributes to the mitochondrion's overall shape (8). The inner membrane has a protein content of almost 80% and is highly selective in its allowance of material exchange. The aerobic energy gradients are all found within or enclosed by this inner membrane (Fig. 11.1).

11.2 Krebs Cycle: Gradient 1

The aerobic biochemistry of substrate degradation is recognized by the metabolic pathway known as the Krebs cycle (named after its founder Hans Krebs,

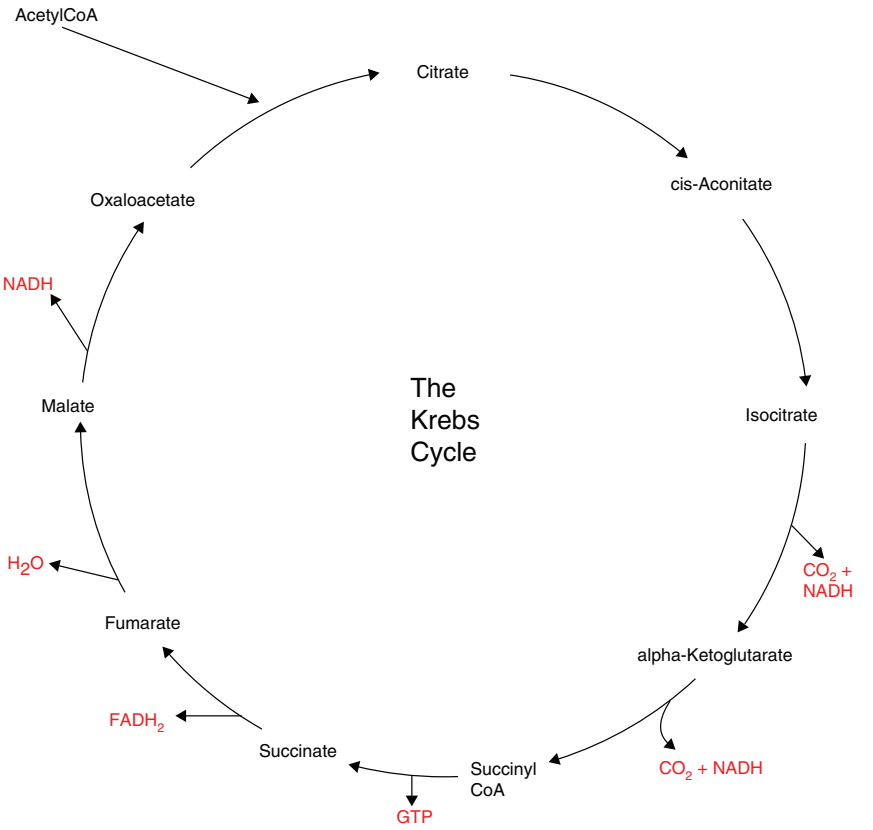

Fig. 11.2 The reactions of the Krebs cycle take place deep within the mitochondrial matrix, surrounded by the inner mitochondrial membrane. Acetyl co-enzyme A (CoA) enters the cycle from the *top right-hand corner*. Reactants are shown but the enzymes associated with these reactions are not (see Table 11.1). Products of the Krebs cycle are given in *red*. The purpose of the Krebs cycle is to supply the electron transport chain (ETC) with reducing equivalents

1900–1981). This pathway also is known as the citric acid cycle and the tricarboxylic acid cycle. As shown in Fig. 11.2, the Krebs cycle does indeed operate in a cyclical format: its end-product oxaloacetate condenses with the entrance reactant acetyl co-enzyme A (CoA) to begin the cycle:

While typically represented in a cyclical format, it is understood that the Krebs cycle is a metabolic energy-exchange device with a higher-energy starting point and a lower-energy ending point. Thus, the metabolic pathway that is the Krebs cycle represents an energy-exchange gradient (Fig. 11.3).

ATP is not resynthesized in the Krebs cycle, though one act of substrate level phosphorylation is present that produces GTP (an ATP equivalent). The purpose of the Krebs cycle instead is to strip hydrogen ions (H^+) and electrons (e^-) from substrate, providing them to two specialized carriers: (1) nicotinamide adenine dinucleotide (NAD^+) and (2) flavin adenine dinucleotide (FAD). During one "spin"

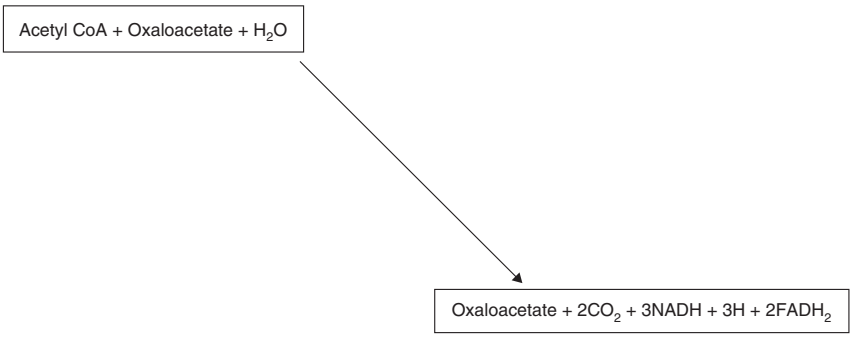

Fig. 11.3 Note that the initial reactants are fewer in number than the final products. These criteria hint that overall, the Krebs cycle gradient represents a spontaneous process

of the Krebs cycle, three NAD^+ and one FAD are required; two molecules of carbon dioxide (CO_2) are also formed and released. The nine enzymatic reactions of aerobic oxidation are provided in Table 11.1 (9).

The aerobic oxidation of substrate begins with the entrance of two carbon units into the Krebs cycle. Each 2-carbon unit is attached to a molecule called coenzyme-A (CoA). The linkage between the 2-carbon unit and CoA occurs via a "high-energy" thioester bond (sulfur linkage – S) to form acetyl CoA (some scholars think that the "high-energy" thioester bond predated ATP in the very first life forms on planet earth) (Fig. 11.4).

The 2-carbon unit of acetyl CoA condenses with the 4-carbon molecule oxaloacetate that is found as the last product of the Krebs cycle. The 6-carbon product

Table 11.1 Krebs cycle reactions (closed system), $\Delta G^\circ (\mathrm{kJ\,mol^{-1}})$

Reaction	Enzyme	ΔG°
1. Acetyl CoA + oxaloacetate + H_2O → citrate + CoA + H^+	Citrate synthetase	−31.4
2. Citrate → *cis*-aconitate + H_2O	Aconitase	+8.4
3. *Cis*-aconitate + H_2O → isocitrate	Aconitase	−2.1
4. Isocitrate + NAD^+ → α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase	−8.4
5. α-ketoglutarate + NAD^+ + CoA → succinyl CoA + CO_2 + NAD^+	α-ketoglutarate dehydrogenase	−30.1
6. Succinyl CoA + Pi + GDP → succinate + GTP + CoA	Succinyl CoA synthetase	−3.3
7. Succinate + FAD → fumarate + $FADH_2$	Succinate dehydrogenase	0
8. Fumarate + H_2O → L-malate	Fumarase	−3.8
9. L-malate + NAD^+ → oxaloacetate + NADH + H^+	Malate dehydrogenase	+29.7
		−41.0

Note that only standard Gibbs energy values are provided (ΔG°). Evidence suggests that the Krebs cycle enzymes are associated as a metabolon that channels substrate from enzyme-to-enzyme (10, 11). Table adapted from (9).

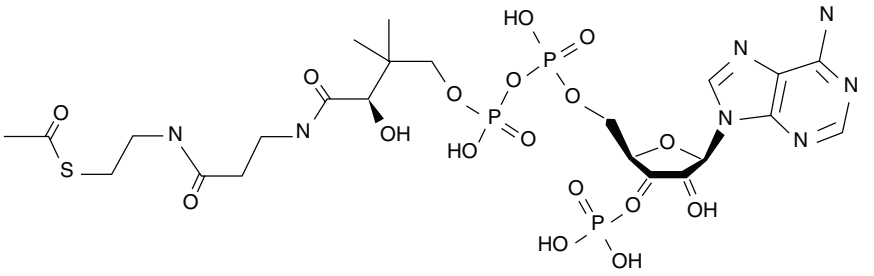

Fig. 11.4 A molecule of acetyl Co-A is shown. In this format, 2-carbon units begin the aerobic oxidation process. In this figure, the 2-carbon unit is located at the extreme *left* of the molecule, where one carbon forms a bond with sulfur (S)

formed from this reaction also provides another name to this cycle, the *citric acid cycle*. Recall that anaerobic glycolysis can only use glucose as substrate. Mitochondrial oxidation is unique in that two carbon units can be derived from glucose, fats, or proteins (amino acids). Moreover, carbohydrate substrates are oxidized much more completely, resynthesizing 18× more ATP as compared with anaerobic glycolysis, making mitochondria an ideal energy-exchange device within cells.

As substrate is oxidized in the Krebs cycle, NAD^+ and FAD are subsequently reduced; NAD^+ accepts one hydrogen ion (H^+) and two electrons ($2e-$) to become NADH; FAD is reduced by accepting $2e-$, 1 H^+, and one hydride ion ($:H^-$) to become $FADH_2$ (a hydride ion is a hydrogen atom that has two as opposed to one electron, hence the negative charge). Once reduced, NADH and $FADH_2$ deliver electrons to the electron transport chain (also known as the respiratory chain).

11.3 Electron Transport Chain: Gradient 2

The electron transport chain (ETC) represents another type of gradient that begins the two-part process of oxidative phosphorylation; this gradient is located within the lipid bilayer of the inner mitochondrial membrane. The word *oxidative* describes the association of the electron carriers with oxygen (phosphorylation is described later where ATP undergoes re-synthesis). Oxygen's attraction for electrons is great, providing the impetus for a gradient. As a metabolic gradient, oxidative phosphorylation describes driving forces that are exploited for useful purposes. Toward the top of the gradient are the electron carriers NADH and $FADH_2$, oxygen (O_2) is found at the bottom of the gradient (note that oxygen is not a part of the Krebs cycle, it is only found in the ETC) (Fig. 11.5).

NADH and $FADH_2$ enter the ETC at different places so that the attraction or electronegativity of oxygen for the electrons carried by NADH and $FADH_2$ is different. The electrical charge between NADH entering at the top of the ETC and oxygen at the bottom has been measured at 1.14 V ($\Delta G^\circ = -220\,\text{kJ mol}^{-1}$). $FADH_2$ enters the ETC at a lower point than does NADH, and so the energy differential is slightly less than that of NADH ($\Delta G^\circ = -200\,\text{kJ mol}^{-1}$).

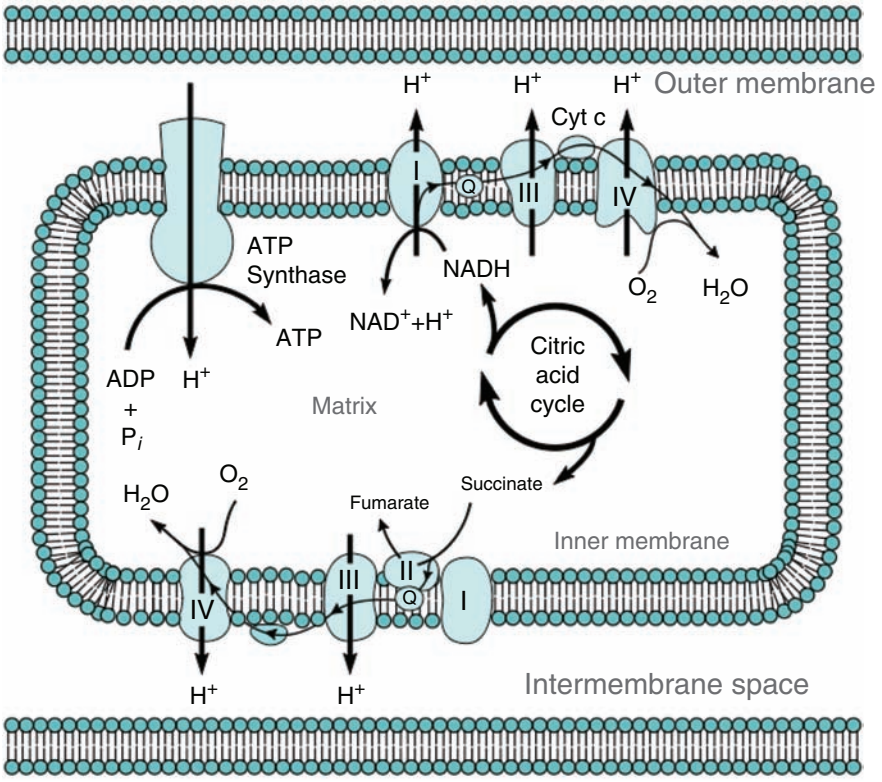

Fig. 11.5 The electron transport chain (ETC) is depicted above within the mitochondria's inner membrane as I, Q, III, Cyt c, IV. Hans Krebs' Citric Acid Cycle supplies electrons to the ETC. An ATPase is also shown. Overall ΔG° spanning the complete ETC is $-220\,\mathrm{kJ\,mol^{-1}}$ (9). Oxygen is reduced with both electrons and protons to form water (courtesy of Tim Vickers, http://commons.wikimedia.org/wiki/Image:Mitochondrial_electron_transport_chain%E2%80%94-Etc4.svg)

The word *phosphorylation* describes the attachment of Pi to ADP, but similar to the Krebs cycle, actual ATP resynthesis is not directly associated with the ETC. The energy exchange of electron transfer is instead made available to pump protons (H^+) from one side of mitochondria's inner membrane to another. As a result, a proton-motive force is created.

11.4 Proton-Motive Force: Gradient 3

Humans and animals (including scientists!) often learn by example. But this is not always the case. Take for instance the birth of biochemistry where glycolysis was

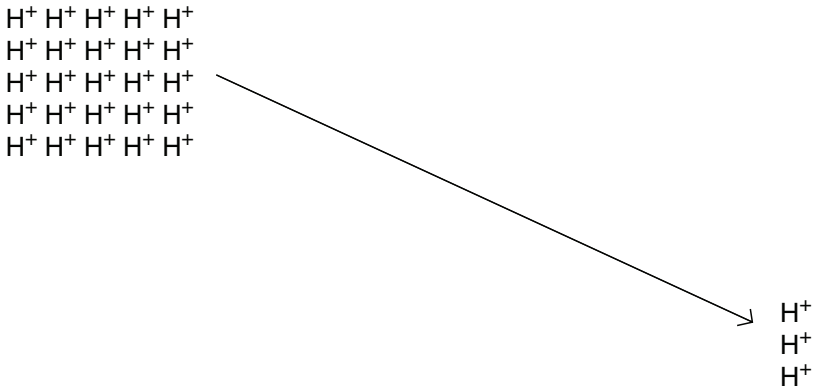

Fig. 11.6 The proton-motive gradient invokes a force, what Peter Mitchell described as chemiosmosis. This is the third gradient of aerobic energy exchange (the Krebs cycle and ETC representing the other two gradients)

discovered as a form of substrate-level phosphorylation in the re-synthesis of ATP. From such a perspective it would appear natural to search for a similar means in the quest to uncover the mitochondrial mechanism of ATP resynthesis. Based on this line of reasoning, it was thought that mitochondrial proteins (enzymes) were directly involved in phosphate (Pi) shifts and transfers, just as they were for anaerobic glycolysis. Mitochondria were discovered as the source of aerobic metabolism in the mid-1940s and subsequently dissected to reveal anatomical structure in the 1950s. Biochemical analyses followed suite. Many proteins were found, yet the search for the mechanisms of aerobic substrate-level phosphorylation proved futile, they simply were not there. A novel approach to aerobic energy exchange was seemingly called for. In 1961 a scientist by the name of Peter Mitchell (1920–1992) came up with a hypothesis that even today is still referred to as a radical mechanism of ATP resynthesis. Over the years disproof of his idea was never found; he was rewarded with a Nobel Prize in 1978.

Mitchell elucidated how electron transfer within the ETC provided the energy to physically pump protons (H^+) from the inside to the outside of the inner mitochondrial membrane. With this pumping a buildup of H^+ takes place, being greater on the outside as compared with the inside of the mitochondrial inner membrane. For every pair of electrons provided by NADH, 10 H^+ are pumped; for every pair of electrons provided by $FADH_2, 6H^+$ are pumped (10). A gradient is created that is both chemical (H^+) and electrical (charge separation across a membrane) in nature (Fig. 11.6).

Residing across the inner mitochondrial membrane is a protein-derived proton portal known as mitochondrial ATPase. The buildup of hydrogen ions created by the ETC literally "fall" down their gradient through this portal and the energy exchange of this transfer is captured by the ATPase to resynthesize ATP from ADP and Pi. In

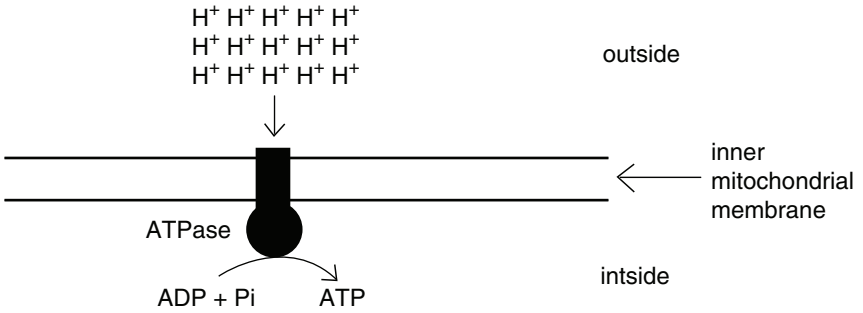

Fig. 11.7 The mitochondrial ATPase is shown in *black* above spanning the inner mitochondrial membrane. Mitochondrial ATPase is a rotary engine of sorts whose protein components spin as protons pass through. This rotational energy is captured and utilized to resynthesize ATP from ADP and Pi

reality, H^+ do not pass through the membrane in a linear fashion but instead travel along a complicated pathway created by the protein structures of the mitochondrial ATPase (12, 13). At least two H^+ pathways are present in the mitochondrial ATPase; one that is associated with the degradation of the proton-motive force, the other with the reduction of oxygen to form water (13). Current evidence suggests that passage of 3 H^+ through the mitochondrial ATPase is required for each ATP resynthesized (see Fig. 11.7).

The passage of protons down the chemiosmotic gradient is said to be tightly coupled when each H^+ travels through the mitochondrial ATPase. However, as with most energy-exchange devices this coupling is not perfect (inefficient) and the chemiosmotic gradient is no exception. Membranes are not perfect barriers. When protons traverse the inner mitochondrial membrane without resynthesizing ATP, respiration is said to be uncoupled. In some tissues this is a natural phenomenon, producing heat rather then ATP in the process. Brown adipose tissue (BAT) for example, found in small mammals and infant (but not adult) humans, acts as a type of furnace producing heat for protection from the cold. The presence of specific proteins called uncoupling proteins (UCPs) seems to on the one hand encourage inefficiency in terms of ATP production; on the other hand, in terms of heat output rather than heat loss, this is an effective mechanism.

11.5 The Creatine Phosphate Shuttle

The ATP that undergoes resynthesis within mitochondria is not the ATP that undergoes hydrolysis in other areas of the cell. In muscle, for example, a shuttle system is thought to exist between the sites of energy supply (i.e., mitochondria) and energy demand (e.g., the muscle sarcomeres) – the creatine phosphate (CP) shuttle – to help maintain high levels of ATP and low levels of ADP at both sites (14). Recall that it

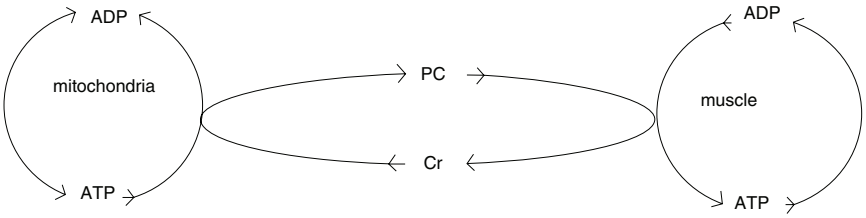

Fig. 11.8 The creatine phosphate shuttle. In this scenario the two energy-exchange devices – mitochondria and muscle – are separated in an apparent attempt to minimize the effect one system has on the other while matter and energy fluxes continuously take place within and without both. Adapted from (14)

is critical to the Gibbs energy availability of ATP to maintain high [ATP] and low [ADP] concentrations (see Fig. 7.5). Also recall that the two systems, mitochondrial energy exchange and muscle contraction energy exchange, are open systems. The CP shuttle is hypothesized to act as a buffer between the two systems, possibly preventing drastic changes in both systems from taking place in response to those fluxes taking place within and without any one system (Fig. 11.8). ATP undergoes hydrolysis at the myosin–actin interface of the sarcomere forming ADP and Pi. That ADP is immediately re-phosphorylated by CP. The left-over creatine (Cr) is shuttled to the mitochondria where ATP re-phosphorylates Cr to form CP.

11.6 ATP Tally

Net ATP resynthesis is now derived from the complete anaerobic and aerobic oxidation of glucose (Table 11.2) (15).

Table 11.2 ATP resynthesis from anaerobic and aerobic sources

Source	Metabolism	ATP
Substrate-level phosphorylation	Glycolysis	2
$2NADH + H^+$	Glycolysis	4
$2NADH + H^+$	Pyruvate – acetyl CoA	6
Substrate-level phosphorylation	Krebs cycle	2
$6NADH + H^+$	Krebs cycle	4
2FADH2	Krebs Cycle	18
		36

The values are estimates and vary slightly depending on the source (the exact amount of ATP resynthesized in regard to the amount of oxygen consumed likely varies slightly among cell types and under different conditions (e.g., the extent of uncoupling proteins differs among cells) (adapted from (15)).

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