With this structure in mind, we can see how a proton gradient can drive rotation of the **c** ring. Suppose that the Asp 61 residues of the two **c** subunits that are in contact with a half-channel have given up their protons so that they are in the charged aspartate form (Figure 18.35), which is possible because they are in relatively hydrophilic environments inside the half-channel. The **c** ring cannot rotate in either direction, because such a rotation would move a charged aspartate residue into the hydrophobic part of the membrane. A proton can move through either half-channel to protonate one of the aspartate residues. However, it is much more likely to pass through the channel that is connected to the cytosolic side of the membrane because the proton concentration is more than 25 times as high on this side as on the matrix side, owing to the action of the electron-transport-chain proteins. The entry of protons into the cytosolic half-channel is further facilitated by the membrane potential of +0.14 V (positive on the cytoplasmic side), which increases the concentration of protons near the mouth of the cytosolic half-channel. *If the aspartate residue is protonated to its neutral form, the **c** ring can now rotate, but only in a clockwise direction.* Such a rotation moves the newly protonated aspartic acid residue into contact with the membrane, moves the charged aspartate residue from contact with the matrix half-channel to the cytosolic half-channel, and moves a different protonated aspartic acid residue from contact with the membrane to the matrix half-channel. The proton can then dissociate from aspartic acid and move through the half-channel into the proton-poor matrix to restore the initial state. This dissociation is favored by the positive charge on a conserved arginine residue (Arg 210) in the **a** subunit. Thus, *the difference in proton concentration and potential on the two sides of the membrane leads to different probabilities of protonation through the two half-channels, which yields directional rotational motion.* Each proton moves through the membrane by riding around on the rotating **c** ring to exit through the matrix half-channel (Figure 18.36).

The **c** ring is tightly linked to the γ and ε subunits. Thus, as the **c** ring turns, these subunits are turned inside the $\alpha_3\beta_3$ hexamer unit of F_1. The exterior column formed by the two **b** chains and the δ subunit prevent the $\alpha_3\beta_3$ hexamer from rotating. Thus, the proton-gradient-driven rotation of the **c** ring drives the rotation of the γ subunit, which in turn promotes the synthesis of ATP through the binding-change mechanism. Recall that the number of **c** subunits in the **c** ring appears to range between 10 and 14. This number is significant because it determines the number of protons that must be transported to generate a molecule of ATP. Each 360-degree rotation of the γ subunit leads to the synthesis and release of three molecules of ATP. Thus, if there are 10 **c** subunits in the ring (as was observed in a crystal structure of yeast mitochondrial ATP synthase), each ATP generated requires the transport of 10/3 = 3.33 protons. For simplicity, we will assume that 3 protons must flow into the matrix for each ATP formed, but we must keep in mind that the true value may differ.

18.4.5. ATP Synthase and G Proteins Have Several Common Features

The α and β subunits of ATP synthase are members of the P-loop NTPase family of proteins. In Chapter 15, we learned that the signaling properties of other members of this family, the G proteins, depend on their ability to bind nucleoside triphosphates and nucleoside diphosphates with great kinetic tenacity. They do not exchange nucleotides unless they are stimulated to do so by interaction with other proteins. The binding-change mechanism of ATP synthase is a variation on this theme. The three different faces of the γ subunit of ATP synthase interact with the P-loop regions of the β subunits to favor the structures of either the NDP- or NTP-binding forms or to facilitate nucleotide release. The conformational changes take place in an orderly way, driven by the rotation of the γ subunit.

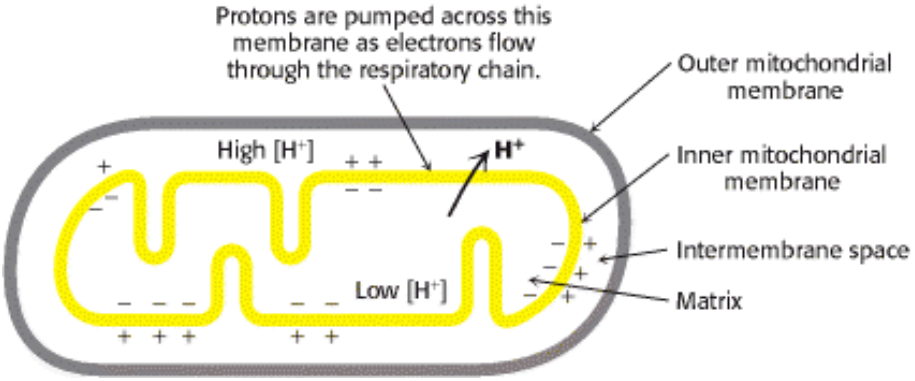

Figure 18.25. Chemiosmotic Hypothesis. Electron transfer through the respiratory chain leads to the pumping of protons from the matrix to the cytosolic side of the inner mitochondrial membrane. The pH gradient and membrane potential constitute a proton-motive force that is used to drive ATP synthesis.

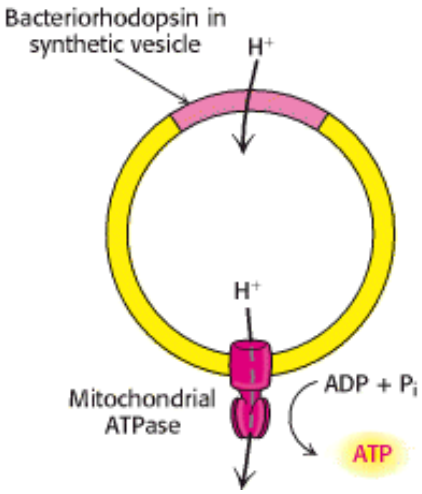

Figure 18.26. Testing the Chemiosmotic Hypothesis. ATP is synthesized when reconstituted membrane vesicles containing bacteriorhodopsin (a light-driven proton pump) and ATP synthase are illuminated. The orientation of ATP synthase in this reconstituted membrane is the reverse of that in the mitochondrion.

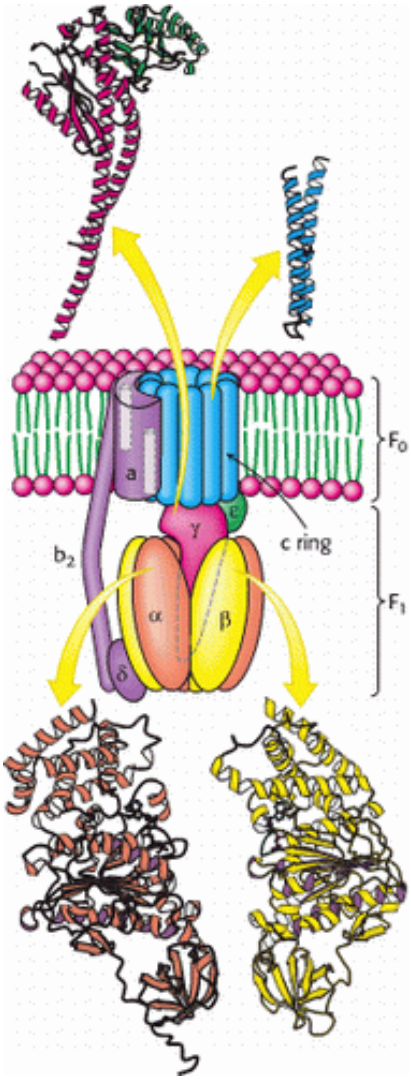

Figure 18.27. Structure of ATP Synthase. A schematic structure is shown along with detailed structures of the components for which structures have been determined to high resolution. The P-loop NTPase domains of the α and β subunits are indicated by purple shading.

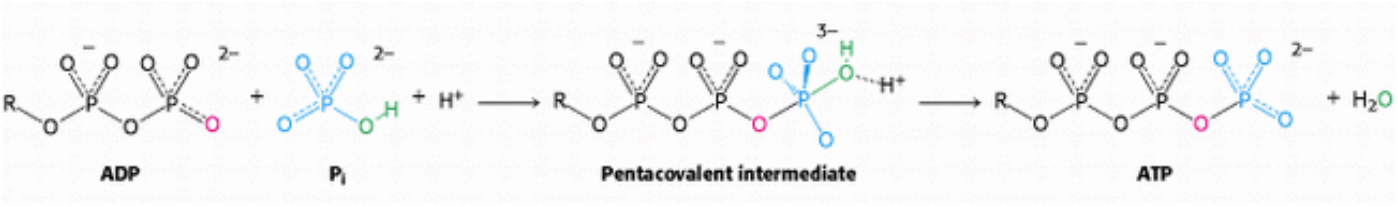

Figure 18.28. ATP Synthesis Mechanism. One of the oxygen atoms of ADP attacks the phosphorus atom of P_i to form a pentacovalent intermediate, which then forms ATP and releases a molecule of H_2O.

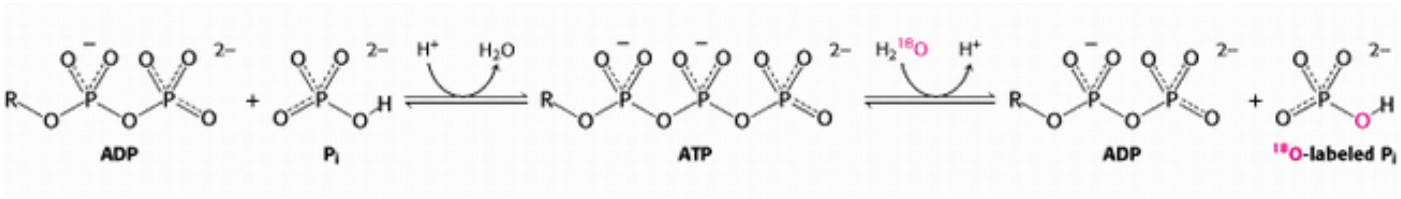

Figure 18.29. ATP Forms Without a Proton-Motive Force But Is Not Released. The results of isotope-exchange experiments indicate that enzyme-bound ATP is formed from ADP and P_i in the absence of a proton-motive force.

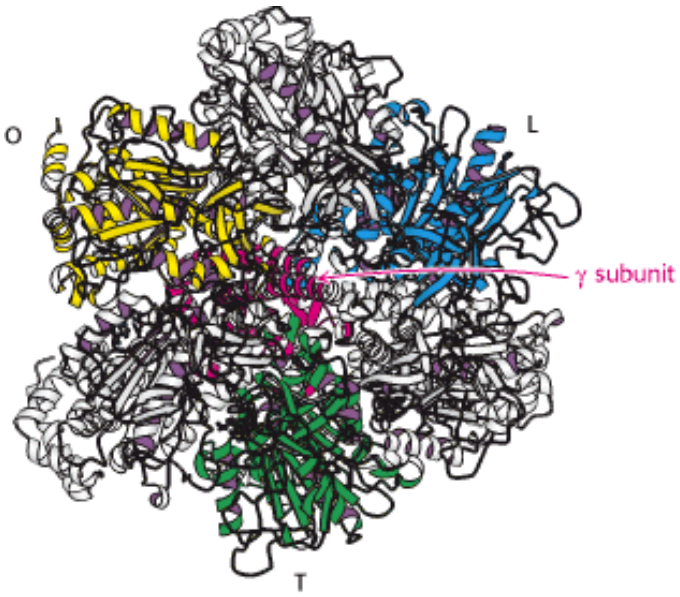

Figure 18.30. ATP Synthase Nucleotide-Binding Sites Are Not Equivalent. The γ subunit passes through the center of the $\alpha_3\beta_3$ hexamer and makes the nucleotide-binding sites in the β subunits distinct from one another.

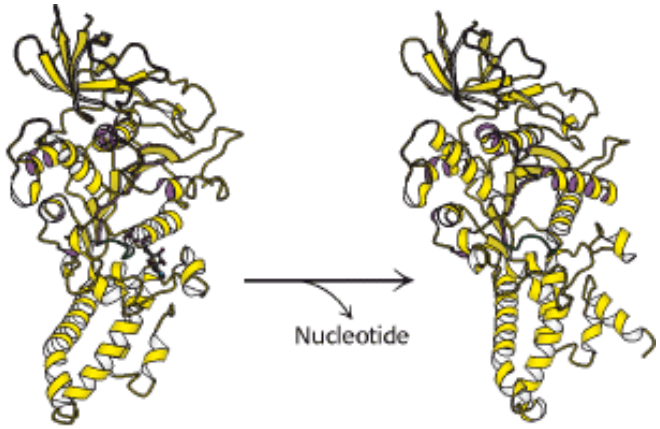

Figure 18.31. ATP Release From the β subunit in the open form. Unlike the tight and loose forms, the open form of the β subunit can change conformation sufficiently to release bound nucleotides.

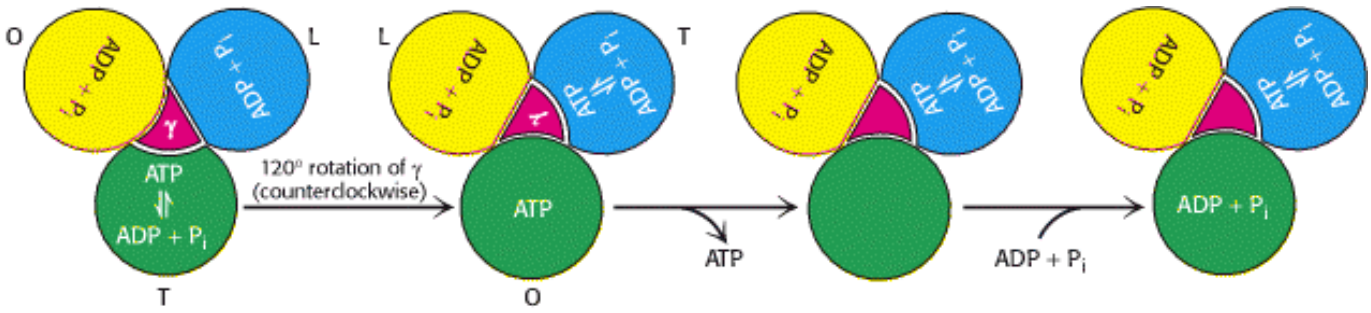

Figure 18.32. Binding-Change Mechanism for ATP Synthase. The rotation of the γ subunit interconverts the three β subunits. The subunit in the T (tight) form, which contains newly synthesized ATP that cannot be released, is converted into the O (open) form. In this form, it can release ATP and then bind ADP and P_i to begin a new cycle.

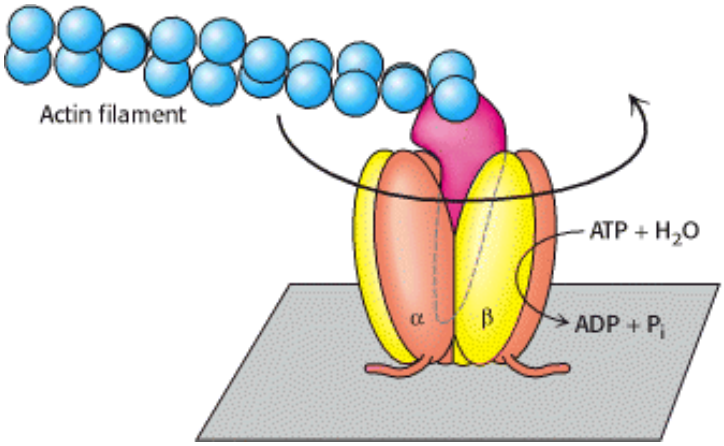

Figure 18.33. Direct Observation of ATP-Driven Rotation in ATP Synthase. The $\alpha_3\beta_3$ hexamer of ATP synthase is fixed to a surface, with the γ subunit projecting upward and linked to a fluorescently labeled actin filament. The addition and subsequent hydrolysis of ATP result in the counterclockwise rotation of the γ subunit, which can be directly seen under a fluorescence microscope.

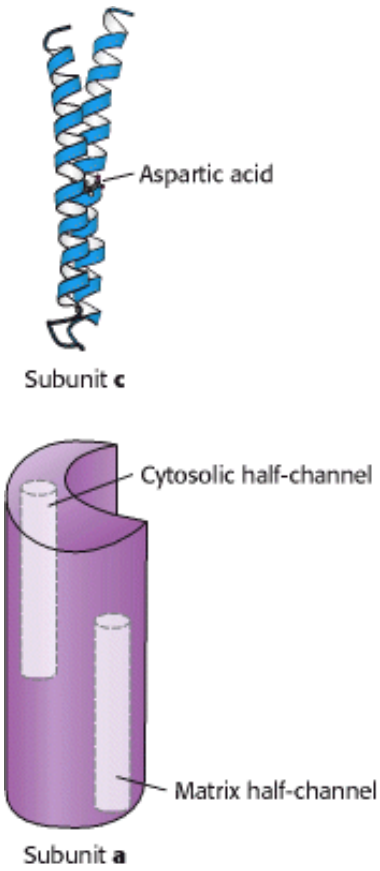

Figure 18.34. Components of the Proton-Conducting Unit of ATP Synthase. The **c** subunit consists of two α helices that span the membrane. An aspartic acid residue in the second helix lies on the center of the membrane. The structure of the **a** subunit has not yet been directly observed, but it appears to include two half-channels that allow protons to enter and pass partway but not completely through the membrane.

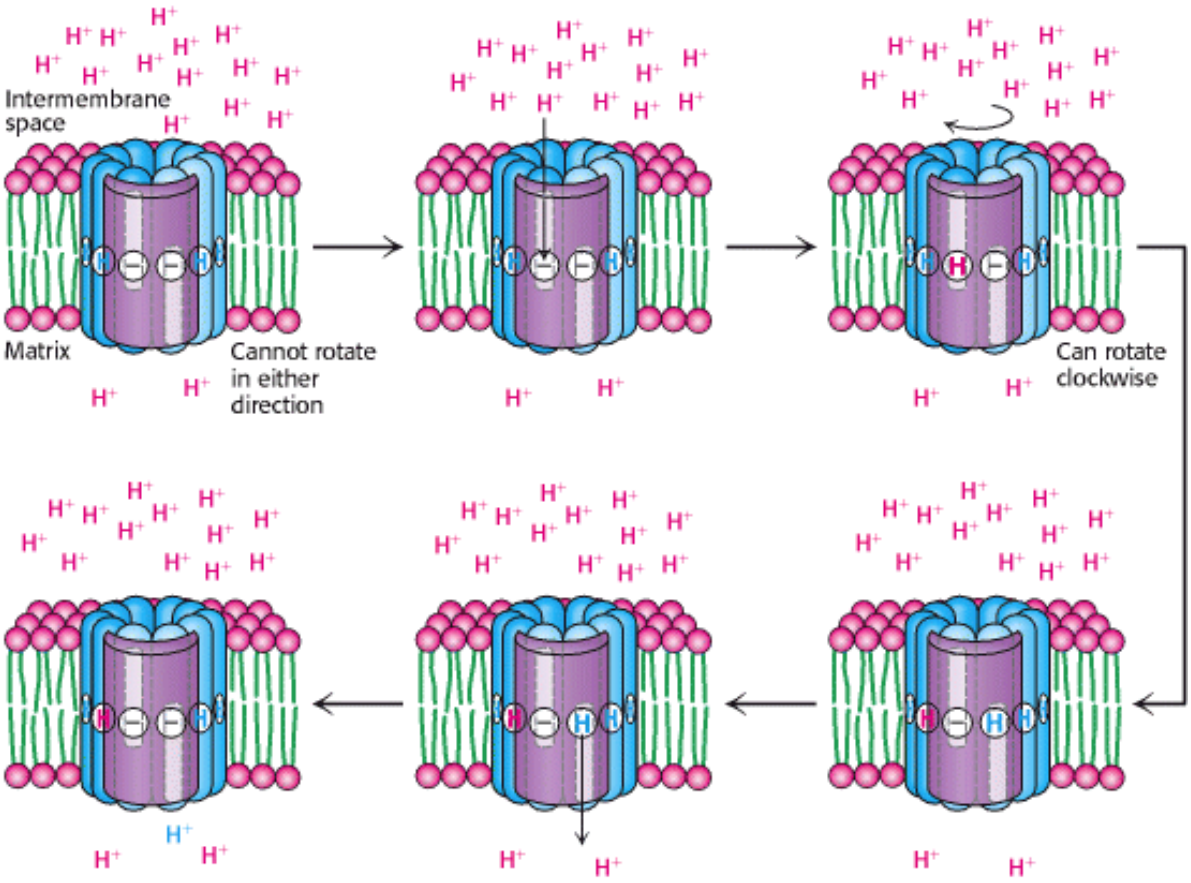

Figure 18.35. Proton Motion Across the Membrane Drives Rotation of the C Ring. A proton enters from the intermembrane space into the cytosolic half-channel to neutralize the charge on an aspartate residue in a **c** subunit. With this charge neutralized, the **c** ring can rotate clockwise by one **c** subunit, moving an aspartic acid residue out of the membrane into the matrix half-channel. This proton can move into the matrix, resetting the system to its initial state.

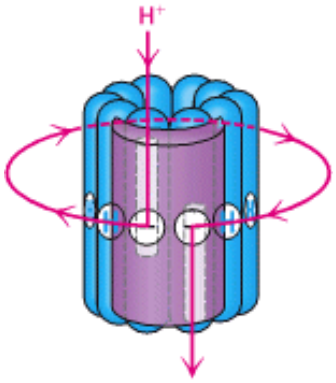

Figure 18.36. Proton Path Through the Membrane. Each proton enters the cytosolic half-channel, follows a complete rotation of the **c** ring, and exits through the other half-channel into the matrix.