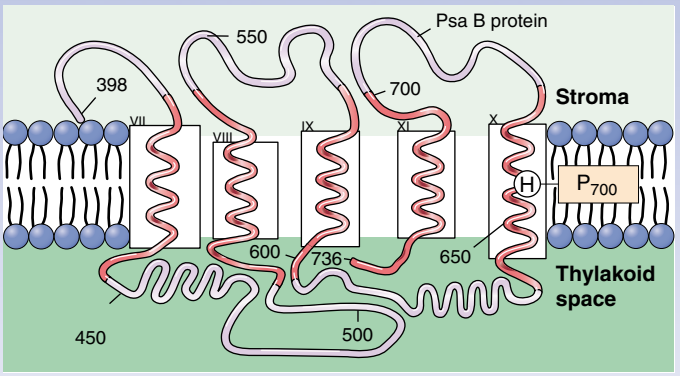
Part III

Energetics



The proposed antenna complex of the PsaB protein. Position 656 is a histidine (H) in the tenth pass (helix X) of the PsaB protein across the thylakoid membrane within chloroplasts. This histidine is where the PsaB protein makes contact with a P_{700} chlorophyll molecule.

How Do Proteins Help Chlorophyll Carry Out Photosynthesis?

Much public attention in recent years has been focused on high-profile science—headline-creating advances in the Human Genome Project, genetic engineering, and the battle against AIDS and cancer. Meanwhile, great advances have been made more quietly in other areas of biology. Among the greatest of these achievements has been the unmasking in the last decade of the underlying mechanism of photosynthesis.

In photosynthesis, photons of light are absorbed by chlorophyll molecules, causing them to donate a high-energy electron that is put to work making NADPH and pumping protons to produce ATP.

When researchers looked at the light-absorbing chlorophylls that carry out photosynthesis more closely, they found the chlorophylls to be arranged in clusters called photosystems, supported by proteins and accessory pigments. Within a photosystem, hundreds of chlorophyll molecules act like antennae, absorbing light and passing the energy they capture inward to a single chlorophyll molecule that acts as the reaction center. This chlorophyll acts as the primary electron donor of photosynthesis. Once it releases a light-energized electron, the complex series of chemical events we call photosynthesis begins, and, like a falling row of dominos, is difficult to stop.

Plants possess two kinds of photosystems that work together to harvest light energy. One of them, called photosystem I, is similar to a simpler photosystem found in photosynthetic bacteria, and is thought to have evolved from it.

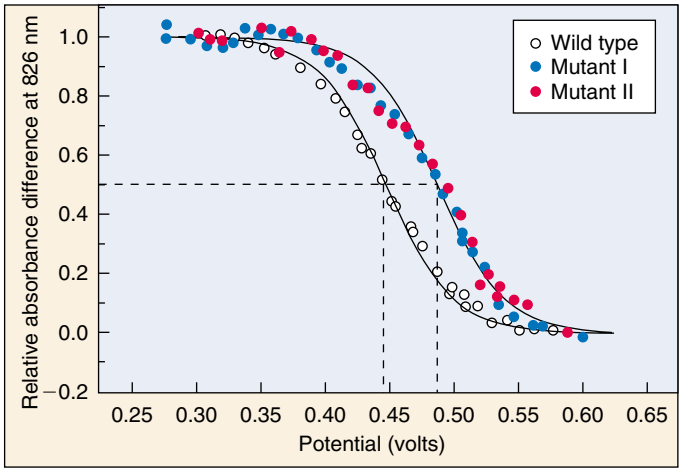
Photosystem I has been the subject of intense research. In its reaction center, a pair of chlorophyll molecules act as the trap for photon energy, passing an excited electron on to an acceptor molecule outside the reaction center. This moves the photon energy away from the chlorophylls, and is the key conversion of light energy to chemical energy, the very heart of photosynthesis.

Because the pair of chlorophyll molecules in the reaction center of photosystem I absorb light at a wavelength of 700 nm, they are together given the name P_{700}. The P_{700} dimer is positioned within the photosystem by two related proteins that act as scaffolds. These proteins, discovered less than 10 years ago, turn out to play a pivotal role in the photosynthetic process. Passing back and forth across the internal chloroplast membranes 11 times, they form a molecular frame that positions P_{700} to accept energy from other chlorophyll molecules of the photosystem, and to donate a photo-excited electron to an acceptor molecule outside the photosystem.

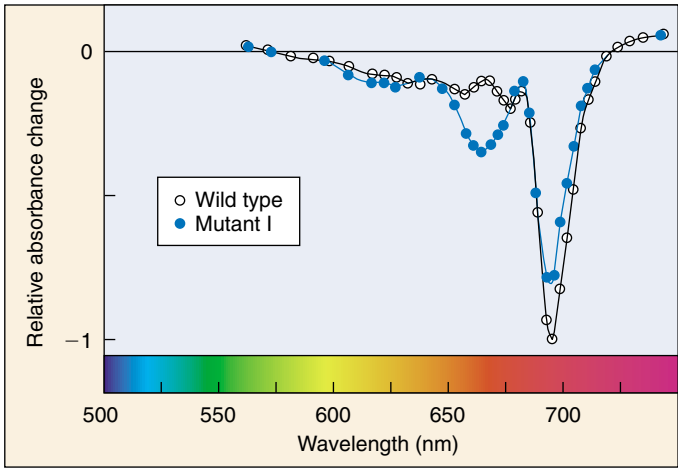
Recent research suggests that the role of these scaffold proteins, called PsaA and PsaB, is far more active than the passive support provided by a scaffold. Analysis of highly purified photosystems carried out in 1995 revealed that the distribution of electric charge over the two halves of the P_{700} dimer is highly asymmetric—one chlorophyll molecule exhibits a far greater charge density than the other. Because the two chlorophyll molecules of P_{700} are themselves identical, this suggests that the PsaA and PsaB proteins are actively modulating the physicochemical properties of the chlorophyll.

How can a protein pull off this physical-chemical sleight-of-hand? Just what are these proteins *doing* to the chlorophyll molecules? To look more closely at what is going on, you have to first figure out what part of the protein to look at. One way to get a handle on this problem is to compare the amino acid sequences of PsaA and PsaB with that of the bacterial photosystem from which they are thought to have evolved. It is likely that such an important part of the sequence would have been conserved and will be found in all three.

Several sequences are indeed conserved, but most of them prove not to interact directly with chlorophyll. One, however, is a promising candidate. A single amino acid in the helix X domain (that is, the tenth pass of the PsaB protein across the membrane), dubbed His-656, is conserved in all sequences, and is positioned right where the PsaB protein touches the P_{700} chlorophyll (see above). This amino acid, a histidine, has become the focus of recent efforts to clarify how proteins help chlorophyll carry out photosynthesis.



(a)



(b)

Effect of altering position 656. (*a*) When P_{700} interacts with normal and mutant forms of PsaB, the midpoint potentials are 447±6 mV in the wild type and 487±6 in the mutants, the mutant value being about 40 mV higher. (*b*) The bleaching band (dip in the absorbance) of P_{700} is shifted to the blue (left) and exhibits a new bleaching band at 667 nm when interacting with mutant forms of PsaB.

The Experiment

To determine the importance of His-656, and more generally of the helix X domain of the PsaB protein, Professor Andrew Webber of Arizona State University, working with his research team and the group of Professor Wolfgang Lubitz at Technische Universitat Berlin, has created site-directed mutations of His-656 in the photosynthetic protist *Chlamydomonas reinhardtii*. *C. reinhardtii* is widely used to study photosynthesis because of the ease with which lab experiments can be done.

Webber and his collaborators set out to change the amino acid located at position 656 of PsaB, and then to look and see what effect the change had on photosynthesis. If His-656 indeed plays a critical role in modifying the P_{700} chlorophylls, then a change at that position to a different amino acid should have profound effects.

Creating PsaB Proteins Mutant at Position 656. The first and key step in Webber's experimental approach was to genetically alter the chloroplast of *C. reinhardtii*, introducing a mutation of the PsaB gene at the His-656 position. To do this, the team employed site-specific mutagenesis to construct mutant plasmids pHN(B656) and pHS(B656), inserting a gene carrying either the Ser or Asn amino acids in place of His. The two mutation-carrying plasmids were then cloned into *C. reinhardtii*, cells carrying the mutant plasmids isolated, and presence of the mutated gene directly confirmed by sequencing the DNA.

Characterizing the Effects of 656 Mutations. Once researchers confirmed that the *C. reinhardtii* chloroplast DNA now contained the mutant forms of the PsaB gene, they proceeded to test the function of the mutated PsaB protein in coordinating P_{700}, examining interior thylakoid membranes isolated from the chloroplasts. To do this, the researchers measured the oxidation midpoint potentials of the P_{700} complexes, an indication of how tightly the chlorophyll molecules are holding onto their electrons.

The researchers further characterized the P_{700} complexes by measuring the changes in absorbance of the mutants versus the wild type to see if the mutations altered the spectral properties of the P_{700} chlorophylls.

The Results

The results of the examination of the oxidation midpoint potentials revealed that the influence of the PsaB protein on P_{700} had been profoundly altered by the mutations. The midpoint potential of P_{700} in the wild type was determined to be 447±6 mV, while the midpoint potential had increased to 487±6 mV in both the PsaB mutant I, HN(B656), and the PsaB mutant II, HS(B656) (see graph *a*). This increase in the oxidation midpoint potential by approximately 40 mV indicates that the mutations to the His residue significantly altered the redox property of P_{700} and, therefore, that His-656 is closely interacting with one of the chlorophyll molecules of the P_{700} dimer.

These results and this conclusion are further supported by changes observed in the spectral properties of the mutants and wild type (see graph *b*). There is a reduction and a slight shift in the 696 nm bleaching band (dip in absorbance) in PsaB mutant I toward the blue end of the spectrum and a new bleaching band appearing at 667 nm, both changes in the spectral properties of chlorophyll induced by the mutational changes in the PsaB protein.

Ultimately, the researchers conclude that the His-656 of PsaB directly coordinates the central magnesium atom of one of the two chlorophyll molecules of P_{700}. Their results are consistent with a model of photosystem I in which the first six spans of PsaB constitute an antenna domain for receiving energy from other chlorophylls and the last five membrane spans interact with the P_{700} reaction complex.

To explore this experiment further, go to the Virtual Lab at www.mhhe.com/raven6/vlab3.mhtml