Light-Harvesting Complexes in Chlamydomonas reinhardtii:

Natali, A.

2017

document version
Publisher's PDF, also known as Version of record

Summary

The purpose of this thesis is to study in detail and unveil the differences within the single light-harvesting complex (LHC) present in the green alga *Chlamydomonas reinhardtii*. Due to this organism's simple life cycle, the ease with which mutants can be isolated from it, and the fact that its genome has been fully sequenced, it has been used for years as a model system to study fundamental process such as photosynthesis.

In a photosynthetic organism, LHCs have the crucial role of capturing photons and transporting the excitation energy to reaction centers in which light energy is converted into chemical energy. In vascular plant and green algae several complexes are present that belong to the LHC family. However, it is difficult to study these individually due to their highly similar biochemical and physical properties. To bypass this problem many researchers, during the past 20 years, have used an *in vitro* approach in which the apoprotein previously expressed in *E. coli* is folded *in vitro* in the presence of pigments. Chapter 2 describes in detail the *in vitro* reconstitution technique and analyzes the pros and cons of this method, giving also a detailed protocol for this technique.

LHCs are a protein family composed of several members with different functions. The green alga, *C. reinhardtii* contains nine genes (LHCBM1-9) encoding for the major light-harvesting complexes of Photosystem II. These antenna proteins are further divided into four groups based on their sequence homology: Type I (LHCBM3, LHCBM4, LHCBM6, LHCBM8, LHCBM9), Type II (LHCBM5), Type III (LHCBM2, LHCBM7) and Type IV (LHCBM1). As reported in Chapter 3, using the *in vitro* reconstitution procedure and exploiting the different isoeletric point of the proteins, we managed to isolate and analyze some of these complexes. We found that in general, all the LHCBMs share the same features, although small differences in their fluorescence decay kinetics are present, in particular for LHCBM5. In addition, we conclude that LHCBM2/7 tend to form homotrimers while LHCBM1 seems to be present in heterotrimers.

Another important feature present in these antennas is protein-protein interactions, which have a crucial role in regulating light-harvesting activity.

Many studies have been performed *in vitro* on isolated complexes in detergent micelles. However, these preparations represent an over-simplified system in which the complexes are solubilized in a non-native environment. In this condition is not possible to fully describe behavior during protein-protein interactions. To overcome this problem, complexes are studied in a system called a liposome. This makes it possible to combine the advantages of studying the LHCs in a thylakoid-like environment (lipid bilayer) while maintaining complete control over external conditions such as protein/lipid ratio. Based on this, in Chapter 4 we studied the interactions between multiple LHCII proteins of *C. reinhardtii* by varying the amount of antenna per liposome. We describe the tendency of LHCII to cluster in the membrane when the number of complexes per liposome is increased. This clustering also produces a shortening of the LHCII excited state lifetime, indicating that *in vivo* in their native membrane LHCIIs are usually clustered, which explains their shorter lifetime compared to isolated complexes.

Finally, Chapter 5 and 6 are focused mainly on photoprotection. In general, plants and green algae must always be able to cope with stress conditions, like high light, that can cause molecular damage. Photosynthetic organisms have developed several mechanisms of photoprotection, such as non-photochemical quenching (NPQ). In particular, *C. reinhardtii*'s capacity for the fast component of NPQ is due to a specific antenna called LHCSR3 that is able to sense the lumen acidification caused by high light condition. It has been demonstrated *in vitro* that the C-terminus of LHCSR3 is responsible for a pH-driven conformational change of the complexes in which it can go from a light-harvesting to a photoprotective state. Chapter 5 describes our work in which we replaced the C-terminus of LHCII with the C-terminus of LHCSR3. We demonstrated that the new "chimera" acquired the ability of LHCSR3 to sense the pH in the environment and, in addition, is able to switch from a light-harvesting state to a quenched state. In this way, we demonstrated that is possible to implement a new feature on LHCII. This can also open new ways to engineer a fast and reversible response to sudden variations in light-intensity in plants or green algae.

In contrast to the other LHCII, LHCSR3 is expressed only in stress conditions such as high light. Unfortunately, due to its sub-stoichiometry it is still unclear how this protein interacts with other complex *in vivo*. In Chapter 6 we demonstrated lines of *C. reinhardtii* that are able to express LHCSR3 constitutively. Our results indicate that the constitutive expression of this antenna leads to a stable accumulation in the thylakoid membrane. Although the amount is rather small, the presence of recombinant LHCSR3 in *C. reinhardtii* grown in normal light induces a fast rise of NPQ. This indicates

that the protein is active and doesn't need additional partners expressed in high light for its function. Finally, we also tested the ability of LHCSR3 to be active in a more evolved organism such as tobacco. In this case the accumulation of LHCSR3 seems to be so low that it has no effect on the phenotype and especially at NPQ level. Various assumptions in this regards are discussed in Chapter 6.