Chapter 8

Phosphorylation of Thylakoid Proteins

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Summary

Application of novel techniques for the characterization of in vivo protein phosphorylation has revealed sixteen distinct phosphorylation sites in ten integral and two peripheral proteins in photosynthetic thylakoid membranes. In addition to phosphorylation of the photosystem II (PS II) proteins D1, D2, CP43, and PsbH, and the light-harvesting antenna polypeptides LHCII and CP29, phosphorylation has been found in photosystem I (PS I) protein PsaD and in two recently identified proteins TSP9 and TMP14. The accumulated knowledge favors an involvement of reversible phosphorylation in adaptive stress responses and cellular signaling, but not in direct regulation of photosynthetic activities like electron transfer or oxygen evolution. Enhancement of PS II protein phosphorylation by abiotic stress maintains the integrity of PS II before it migrates to the stroma regions of the thylakoids where dephosphorylation and subsequent protein turnover take place. Specific dephosphorylation of the D1, D2, and CP43 polypeptides is performed by a heat shock-inducible protein phosphatase intrinsic to the thylakoid membrane. The phosphatase activity is regulated by the lumenal peptidyl-prolyl isomerase TLP40. This regulation may coordinate the protein folding activity of TLP40 in the lumen with the protein dephosphorylation at the opposite side of the thylakoid membrane. Reversible phosphorylation of LHCII in vivo is under complex redox and metabolic control and is probably involved in regulation of the size of the PS II antennae. Cold- and high light-induced phosphorylation

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of CP29 may facilitate photoprotective energy dissipation by changing PS II-LHCII interactions under stress conditions. Phosphorylation of PsaD protein could be involved in regulation of PS I stability and ferredoxin reduction by PS I. The light-induced phosphorylation of TSP9, followed by its release from thylakoids, is implicated in plant cell signaling. The exact physiological roles of the protein phosphorylation events in thylakoids should be revealed by studies with appropriate mutants of plants and algae.

I. Introduction

Light- and redox-induced protein phosphorylation in chloroplast membranes was discovered by Bennett in 1977 (Bennett, 1977). The prevailing hypothesis during much of the last two decades has been that reversible phosphorylation of LHCII is involved in state transitions, i.e. in balancing the distribution of absorbed light energy between the two photosystems, PS II and PS I (Bennett et al., ; 1980; Allen et al., 1981; Allen, 1992, 2002, 2003; Allen and Forsberg, 2001). This hypothesis has further evolved through studies of the redox sensing that connects electron transfer and protein kinase activity in photosynthetic membranes (Allen, 1992; Vener et al., 1998; Aro and Ohad, 2003). Studies of the molecular aspects of redox-dependent thylakoid protein phosphorylation have revealed it to be an extremely complex process. A multiple factor-dependent regulation of LHCII phosphorylation has been demonstrated. In addition to the requirement of plastoquinone reduction for activation of LHCII kinase (Allen et al., 1981), the Qo site of the cytochrome bf complex operates as the redox sensor for induction of the kinase activity (Vener et al., 1995; Vener et al., 1997; Zito et al., 1999). Light-induced changes in LHCII also affect its phosphorylation (Zer et al., 1999; Zer et al., 2003) as does the thiol redox state and the ferredoxin-thioredoxin system of chloroplasts (Carlberg et al., 1999; Rintamäki et al., 2000). The latter mechanism for control of LHCII phosphorylation was uncovered largely due to the finding of an initially surprising irradiance-dependence for the amount of phospho-LHCII in vivo (Rintamäki et al., 1997). In plant leaves, LHCII was found phosphorylated only at light intensities lower than those during normal plant growth (Rintamäki et al., 1997; Rintamäki and Aro, 2001). These findings and the measurements of the excitation energy transfer between the two photosystems in plant leaves have seriously questioned the role of LHCII phosphorylation in state transitions (Elich et al., 1997; Haldrup et al., 2001; Rintamäki and Aro, 2001). Accordingly, the physiological function of LHCII phosphorylation remains an open question.

More than 1100 genes encode for protein kinases in the genome of *Arabidopsis thaliana* (The *Arabidopsis* Genome Initiative, 2000). At present there are five candidate *Arabidopsis* genes for membrane protein kinases that could phosphorylate thylakoid proteins: a family of three TAK kinases (Snyders and Kohorn, 1999, 2001) and two kinases homologous to Stt7 kinase from *Chlamydomonas reinhardtii* (Depege et al., 2003). The mechanism for the redox regulation of these kinases is elusive as is the identity and number of other possible thylakoid protein kinases. With respect to the genes for protein phosphatases operating in thylakoid membranes, the situation is even less clear. No gene or protein sequence information has yet been published concerning the enzymes involved in dephosphorylation of thylakoid phosphoproteins. The additional challenge in elucidating the redox-dependent system for thylakoid protein phosphorylation lies in the fact that it requires the integrity of the membrane and the electron transfer chain for operation. Nevertheless, there has been steady progress in the decoding of the molecular mechanisms for redox regulation of thylakoid protein phosphorylation, which has been periodically reviewed (Vener et al., 1998; Ohad et al., 2001; Rintamäki and Aro, 2001; Aro and Ohad, 2003; Zer and Ohad, 2003).

Three recent groundbreaking developments provided unprecedented possibilities for revealing the functions of protein phosphorylation in the regulation of photosynthesis. Firstly, the sequencing of plant genomes allowed the full-power application of proteomic approaches to study protein modifications in these species. Secondly, plant lines with knockouts of individual genes became commercially available. Thirdly, new analytical techniques permitted the detection of protein modifications in vivo in variable

Abbreviations: D1 – photosystem II reaction center protein; D2 – photosystem II reaction center sister protein; of 29 kDa – minor chlorophyll *a*/*b*-binding protein of photosystem II; of 43 kDa – chlorophyll *a* binding protein of photosystem II; LHCII – light harvesting chlorophyll *a*/*b*-binding proteins of photosystem II; PsbH – 9 kDa *psbH* gene product; PS I – photosystem I; PS II – photosystem II; PPIase – peptidyl-prolyl cis-trans isomerase; PP2A – protein phosphatase 2A; TLP20 – thylakoid lumen PPIase of 20 kDa; TLP40 – thylakoid lumen PPIase of 40 kDa; TMP14 – thylakoid membrane phosphoprotein of 14 kDa; TSP9 – thylakoid soluble phosphoprotein of 9 kDa