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Review

Light as a Signal Influencing the Phosphorylation Status of Plant Proteins[1]

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ABSTRACT

The phosphorylation-status of a number of plant enzymes has been shown to be altered in response to light. Phospho*enol*pyruvate carboxylase is phosphorylated (more active) in C_4 plants in the light but CAM phospho*enol*pyruvate carboxylase is phosphorylated (more active) in the dark. C_4 plant pyruvate, Pi dikinase is dephosphorylated (activated) in the light and sucrose phosphate synthase is less phosphorylated (more active) in the light. The mitochondrial pyruvate dehydrogenase is inactivated (phosphorylated) in the light. The reversal of these events occurs in the dark or when photosynthesis is inhibited. Phytochrome and blue light receptors also alter the phosphorylation-status of proteins. The evidence is rapidly increasing in support of signal transduction networks in plants that involve light reception.

Protein phosphorylation is an ubiquitous regulatory mechanism in biology and evidence for its occurrence and importance in plants is increasing rapidly. The interconversion of proteins between phosphorylated and dephosphorylated forms provides an effective mechanism for reversibly altering enzyme activity or protein function. There are a number of reports describing phosphorylation and dephosphorylation of plant proteins along with conditions and effectors that influence these processes. Additionally, there is increasing evidence that phosphorylation/dephosphorylation events are also involved in the network for transducing external signals to targeted processes within the cell.

Transduction of external signals is typically a chain of four major consecutive events: (a) The presence of the signal generated external to the cell. The signal may be environmental (*e.g.* light, temperature) or from another tissue or cell type (*e.g.* hormones, metabolites, ions). (b) The coupling of signal transduction to effectors or second messengers such as calcium, phospholipids, and polycations, which can regulate phosphorylation or dephosphorylation. (c) Changes in the phosphorylation-status of the target protein substrates. The phosphorylation status of a protein reflects the relative activities of the protein kinase and protein phosphatase which catalyze the interconversion processes. The effectors or second messengers alter the steady-state by either stimulating or inhibiting the protein kinase and/or protein phosphatase. (d) Alteration of target protein activity or function with a consequent change in cellular biochemistry and physiology. This chain of events is usually displayed as four levels of a cascade that amplify the original signal severalfold at each step. The result of such a cascade is that the environmental signal indirectly influences many downstream processes controlled by protein phosphorylation. While there is evidence for many such networks, few have been fully elucidated for either animals or plants. Knowledge of signal transduction in plants is particularly limited. In this brief review, we will focus on one part of the signal transduction network, *i.e.* those plant proteins and enzymes whose phosphorylation-status is altered by the external signal light, and some recent progress in this area. Plant protein phosphorylation has been recently reviewed (1, 16) along with plant protein kinases (2). We encourage the reader to consult these reviews for a more complete description of plant protein phosphorylation. The Proceedings of a Symposium on plant protein phosphorylation will be published by the fall of 1990 (17).

Approximately 30 different proteins in plants have been identified as targets for protein kinases (2, 17). The function and identity of these phosphoproteins is mostly unknown and the effect of phosphorylation on protein function and the initiating signal(s) are still uncertain in many cases. However, light has been implicated as the external signal for several protein phosphorylation events. In order for light to affect protein phosphorylation either directly or indirectly there must be a photoreceptor. The best known light receptors in plants are Chl and phytochrome. Chl is associated with the thylakoid membranes and photosynthesis, while phytochrome is ubiquitous in plant tissues as a controlling element of cellular development and gene regulation. Thus, it is not surprising that these two photoreceptors would be part of a regulatory network involving protein phosphorylation. A third photoreceptor, the blue light receptor is not well characterized, but is potentially involved in protein phosphorylation.

THYLAKOID PHOSPHOPROTEINS

Protein phosphorylation studies involving photosynthesis have described proteins involved in both energy conversion and enzymes involved in carbon metabolism. Within the

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chloroplast five thylakoid phosphoproteins have been identified, LHCP[3] and four PSII core proteins (5, 14). LHCP is the major antenna within the photosynthetic apparatus and it has been proposed that the phosphorylation of LHCP controls the distribution of the absorbed light energy between the two photosystems (21). Light regulates the reversible phosphorylation of LHCP through a thylakoid-bound protein kinase which responds to the light-dependent redox state of the intersystem plastoquinone pool and/or Cyt b_6/f complex. The consequence of phosphorylation is lateral movement of the LHCP from the appressed, grana-membrane region to the adjacent nonappressed, stromal membrane region (21). The four PSII phosphoproteins include the Chl *a* binding apoprotein and the 32 kD herbicide binding protein. While the sequences surrounding the phosphorylation sites have been established, a role or function for the phosphorylation of the PSII polypeptides has not been established (14). One suggested function is for protection against photoinhibition.

PYRUVATE, Pi DIKINASE, AND RUBISCO

Two chloroplast stromal phosphoproteins thus far identified are the PPDK and Rubisco. Phosphorylation of Rubisco has been reported by multiple investigators with some indicating exclusive phosphorylation of the small subunit, some indicating only large subunit phosphorylation and all indicating inconclusive data on light dependence and effect of phosphorylation (1, 11, 16).

PPDK is involved in CO_2 assimilation by plants exhibiting C_4 photosynthesis and its activity is modulated by reversible phosphorylation as a function of light (23). PPDK is rapidly activated in the light (dephosphorylated), inactivated in the dark (phosphorylated), and the phosphorylation state adjusted as a function of light intensity. The mechanism by which light controls the phosphorylation-status is unknown. Although pyruvate is a potent inhibitor of phosphorylation *in vitro* (4, 19), recent *in vivo* evidence seems to question whether light is acting through changes in metabolites, pH, or ions (19; Chollet in ref. 17 and references therein). One unique aspect of PPDK phosphorylation is that the phosphate donor is ADP as opposed to ATP or GTP, the usual nucleotide triphosphates. The phosphorylation and dephosphorylation of the regulatory threonine residue (Thr-456 in maize PPDK) are catalyzed by a rare single, bifunctional enzyme, termed PPDK regulatory protein with two distinct active-site domains (Chollet in ref. 17). Regulatory phosphorylation of PPDK in CAM plants has not yet been demonstrated; however, the light-dependent activation of the CAM PPDK reported by Sugiyama and Laetsch (22) is similar to the C_4 PPDK.

PEPCase AND SPS

Two cytosolic enzymes from green leaf tissue have been determined to undergo reversible phosphorylation: PEPCase and SPS. PEPCase is also involved in photosynthetic carbon assimilation in both C_4 and CAM plants. The C_4 plant PEPCase is regulated in the light such that it is less sensitive to feedback inhibition by malate, resulting in greater CO_2 fixation capacity (13 and references therein). Evidence supports the conclusion that the phosphorylation of PEPCase is the cause of this loss of malate-sensitivity, *i.e.* the enzyme extracted from light-treated tissue is more active and more phosphorylated than enzyme from dark-treated tissue. The PEPCase kinase is a soluble enzyme that phosphorylates a single serine residue (Ser-15 in maize PEPCase) on each subunit of PEPCase (13; Chollet in ref. 17). The mechanism and effector molecule(s) by which light controls the phosphorylation-status of the C_4 PEPCase has not been established but there is no supportive evidence linking regulation of phosphorylation to any cytosolic effector or autophosphorylation (Chollet in ref. 17). For CAM plants the phosphorylated form of PEPCase is the enzyme from dark tissue and again this is the form that is less sensitive to malate inhibition (15; Nimmo in ref. 17).

The second cytosolic enzyme that undergoes phosphorylation is SPS resulting in inactivation of the enzyme (12). The enzyme is more active in light-treated tissue (less phosphorylated) thus allowing for increased capacity of sucrose synthesis. Light apparently exerts its control of phosphorylation through metabolites of photosynthetic carbon metabolism since the effect of light and loss of phosphorylation are negated in the absence of carbon dioxide. The addition of mannose (a phosphate-sequestering agent) in the dark results in activation and dephosphorylation of SPS which potentially mimics the drop in cytosolic P_i during active photosynthetic carbon assimilation (12). SPS is phosphorylated *in vivo* at multiple sites and only some are of regulatory significance (at least two sites). Other sites are constituitively phosphorylated and while these sites stimulate activity when phosphorylated, they do not appear to play regulatory roles (Huber and Huber in ref. 17).

MITOCHONDRIAL PYRUVATE DEHYDROGENASE COMPLEX

It has recently been shown that the mtPDC complex from green pea leaves is reversibly inactivated (phosphorylated) *in vivo* in the light (3). This inactivation presumably results from the phosphorylation of the E-1α subunit which has been well documented *in vitro* and *in situ* (18). Inactivation (phosphorylation) of the mtPDC is favored *in situ* when pyruvate levels are low and when other substrates, particularly glycine, are being oxidized (18). The light-dependent *in vivo* inactivation of the mtPDC is most likely indirect. Inactivation of the photosynthetic apparatus by DCMU inhibits inactivation, as does inhibition of photorespiration. Inactivation was not observed when etiolated shoots were illuminated. It is proposed that the light-dependent inactivation (phosphorylation) of the green leaf mtPDC is, in part, a result of changing levels of photorespiratory metabolites in the mitochondrion. The result of linking photosynthetic and photorespiratory carbon metabolism to inactivation of the mtPDC would be to control carbon flow into the Krebs cycle without interfering with glycine oxidation and oxidative phosphorylation. This illustrates the potential length of the chain of events from the

[3] Abbreviations: LHCP, Chl *a/b* protein complex; HMG, high mobility group; PDC, pyruvate dehydrogenase complex; mtPDC, mitochondrial pyruvate dehydrogenase complex; PPDK, pyruvate, Pi dikinase; Rubisco, ribulose 1,5 bisphosphate carboxylase/oxygenase; PEPCase, phosphoenolpyruvate carboxylase; SPS, sucrose phosphate synthase.

initial signal to the response to protein phosphorylation. The light-dependent inactivation (and presumably phosphorylation) of mtPDC illustrates that reversible enzyme phosphorylation can either inactivate or activate an enzyme, *e.g.* phospho-PEPCase, more active; phospho-PPDK (C_4), inactive; phospho-SPS, less active. Light can initiate either phosphorylation or dephosphorylation events. In the reversible phosphorylation of mtPDC, PEPCase, PPDK and SPS, inhibition of photosynthetic electron transport and/or photophosphorylation inhibits or prevents the light-dependent event regulating these enzymes (3, 17).

The above studies linking external light to phosphorylation or dephosphorylation of plant proteins have mostly been done with normal daylight or growth chamber lighting. These phosphorylation or dephosphorylation activities are usually proportional to the light intensity at the subsaturating levels. Little, if any, investigation with the above enzymes, except PPDK (22), has focused on the quality of light or action spectrum for these events. We do know that plants are very responsive to certain components of light. The best example is the photoreceptor phytochrome and its reversible nature with red and far red light.

PHYTOCHROME AND NUCLEAR PROTEIN PHOSPHORYLATION

Phytochrome is known to regulate gene expression. A number of observations suggest a relationship between gene regulation and protein phosphorylation. Phytochrome and calcium have been implicated in regulating the phosphorylation of nuclear proteins as well as other proteins (7). For example, a recent report implicates phosphorylation and concomitant modulation of binding of a nuclear protein, AT-1, to DNA. AT-1 binds AT rich elements within promoters of certain photoregulated genes (6). Phytochrome is itself an apparent phosphoprotein (Lagarias in ref. 17). It is controversial whether it is also a protein kinase exhibiting autophosphorylation or is very tightly associated with a protein kinase (10). A nuclear protein kinase from maize has been described which phosphorylates HMG proteins (9). The HMG proteins are acidic, nonhistone proteins which have been suggested to regulate transcription. It is not known whether phytochrome regulates cell development or gene expression by affecting the phosphorylation of the HMGs. The role of phytochrome with regard to the phosphorylation of nuclear and nonnuclear proteins remains to be elucidated.

BLUE LIGHT ALTERED PROTEIN PHOSPHORYLATION

Brief *in vivo* blue light treatment decreases the *in vitro* phosphorylation of a 120 kD plasma membrane-associated protein orginating from the region of the epicotyl region of etiolated pea most sensitive to phototropic light stimuli (8, 20; Short *et al.* in ref. 17). *In vitro* blue light treatment of plasma membranes from the epicotyl region of etiolated pea results in enhanced phosphorylation of the 120 kD protein (Short *et al.* in ref. 17). This implies the existence of another light receptor that affects the phosphorylation status of an as yet unidentified protein. This last example serves to illustrate the state of infancy of studies on protein phosphorylation in plants.

OUTLOOK

There are many unanswered questions for all the systems thus far reported. What is the mechanism for transmembrane signal transduction? What is the identity of the numerous phosphoproteins observed? What is the effect of the phosphorylation or dephosphorylation of these proteins? For the enzymes described in this brief review, a very important question is the identity of the molecule(s) that link the light receptor to the particular protein kinase or phosphatase regulating these enzymes. The effects are not limited to changes in kinetic parameters of enzymes, *i.e.* phosphorylation may mark proteins for degradation, facilitate or prevent association of subunits or binding of control elements to promoter regions, *etc.* Unravelling the mysteries of protein phosphorylation will certainly cast critical light on the mechanisms by which plants regulate their development and metabolism. Such information is essential to our ability to understand plant metabolism and development and to engineer plants for our needs and a changing environment.

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