ACID-LABILE, HISTIDINE PHOSPHOPROTEINS IN CHLOROPLASTS AND MITOCHONDRIA: POSSIBLE CANDIDATES FOR REDOX SENSOR KINASES

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1. Introduction

"Bacterial" two-component regulatory systems (1) have recently been found in the eukaryotes yeast (2) and *Arabidopsis* (3). The two components are a sensor and a response regulator. The sensor, typically a membrane protein, undergoes autophosphorylation on a histidine side chain. Phosphorylation occurs in response to an environmental signal, which may be changes in phosphate, nitrate, ammonium or oxygen concentrations, osmotic potential, molecules eliciting chemotaxis, and a variety of other factors. The sensor then interacts with the second component, the response regulator, through a phosphotransferase reaction. The response regulator then becomes phosphorylated on an aspartate side chain, and this initiates the appropriate response to the original environmental signal.

Sensors and response regulators that respond to oxidation-reduction reactions (4) are of particular relevance for bioenergetic systems. The redox sensor ArcB is inserted into the respiratory chain of *Escherichia coli*, and its cognate redox response regulator, ArcA, regulates transcription of genes encoding components of the alternative oxidases that are deployed in anaerobic respiration (5). The redox sensor RegB (6,7) of the purple non-sulphur bacterium *Rhodobacter capsulatus* is required for anaerobic induction of the photosynthetic apparatus, through its action on the redox response regulator RegA (8). Figure 1 depicts schematically a general two-component redox regulatory system that regulates transcription.

Chloroplasts and mitochondria are clearly of prokaryotic ancestry (e.g. 9), and it is reasonable to ask if they have retained two-component redox regulatory systems that might function in molecular redox signalling (10). Here we present data that suggests the presence of proteins phosphorylated on histidine in chloroplasts and mitochondria: both organelles contain proteins whose phosphorylation is acid-labile, a strong indication of phosphohistidine sensors (11, 12). Proteins with phosphohistidine may have been overlooked in previous studies of protein phosphorylation in chloroplasts (13), since treatment of samples with TCA and of gels with acetic acid are routine in such investigations. The possible functions and evolutionary significance of chloroplast and mitochondrial histidine phosphoproteins are discussed.

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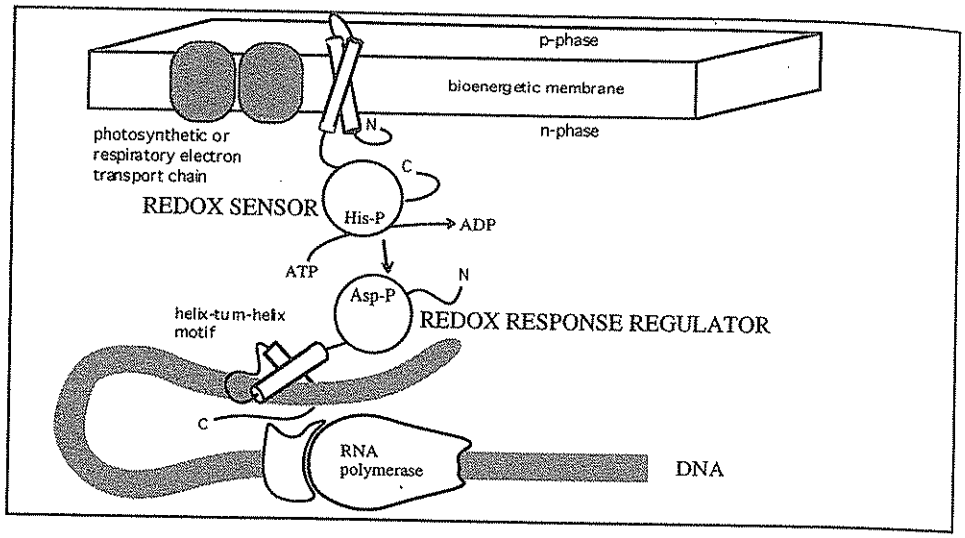

Figure 1. Two-component redox regulation of transcription.
A redox sensor is a membrane phosphoprotein that becomes phosphorylated on a histidine side chain when oxidised or reduced by components of an electron transport chain. Its substrate, the redox response regulator, is a sequence-specific DNA-binding protein that becomes phosphorylated on aspartate, regulating transcription.

2. Materials and Methods

Intact chloroplasts and mitochondria were isolated from pea seedlings as previously described (14). Intact chloroplasts were incubated at room temperature with [^{32}P]P_i or [γ-^{32}P]ATP, as indicated, and mitochondria were incubated with [γ-^{32}P]ATP. The reactions were terminated by adding neutral electrophoresis sample buffer. Acid lability of chloroplast phosphoproteins was investigated by inclusion and omission of the staining and destaining procedure in treatment of otherwise identical SDS-PAGE gels. Acid and alkali stability of mitochondrial phophoproteins according to the method of Oda and Hasunuma (12) as described in the legend of Figure 3.

3. Results and Discussion

Some or all of the acid-labile chloroplast phosphoproteins (Fig. 2) may prove to show redox-regulation as shown for other types of phosphoproteins in mitochondria (15, 16). This makes them possible candidates for redox-sensors. The possibility of redox regulation of the chloroplast acid-labile phosphoproteins is currently under investigation. One possibility, discussed elsewhere (13, 17) is that a plastoquinone pool redox sensor underlies the redox regulation of LHC II phosphorylation. In this case, the cognate response regulator may be the LHC II kinase, or may be required for activation of the LHC II kinase. Further studies on chloroplast thylakoid redox-controlled kinase activity is presented elsewhere (18). To our knowledge, no previous study of the LHC II kinase has examined the possible role of acid-labile, histidine phosphoproteins. The data on mitochondrial acid-labile phosphoproteins (Fig. 3) complements studies on acid-stable redox-regulated phosphoproteins (16), one of

which is implicated from sequencing as a component of respiratory complex I (Struglics, Davies and Allen, unpublished).

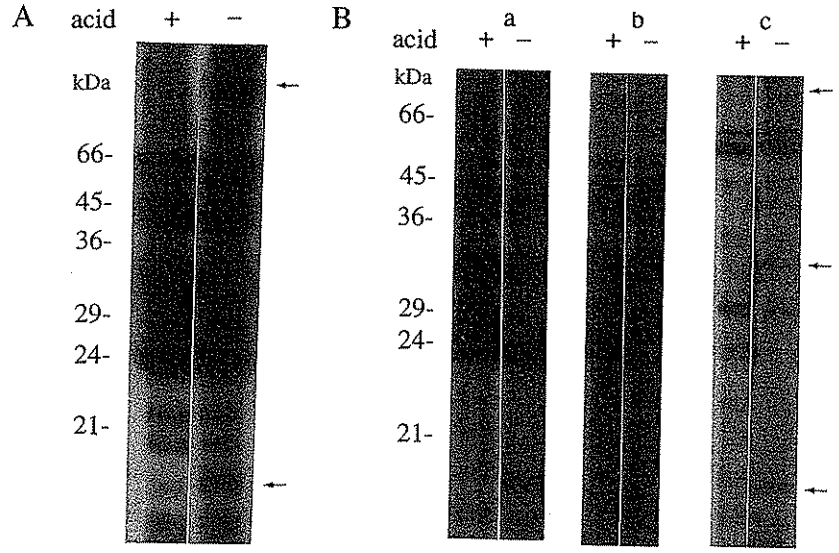

Fig. 2. **Acid-labile phosphoproteins in chloroplasts.** Panel A shows an autoradiograph of [^{32}P]P_i -phosphorylated intact chloroplasts. The 3 major acid-labile phosphoproteins of approximate molecular weights, 80, 40 and 20 kDa are highlighted.
Panel B shows an autoradiograph of chloroplasts treated in the same manner as above (a), and subsequently separated into thylakoid (b) and stroma (c) fractions by lysis and short centrifugation of the chloroplasts. The acid-labile phosphoproteins of panel A are found in the soluble fraction and not in the thylakoid one.

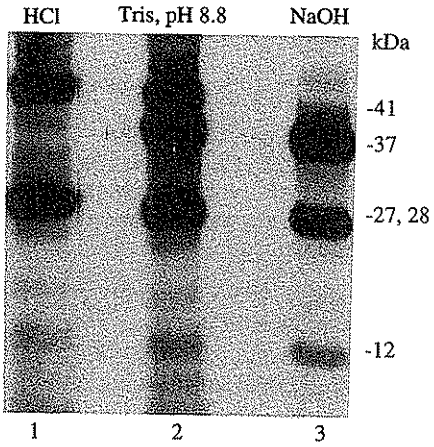

Fig. 3. **Acid and alkali stability of mitochondrial phosphoproteins.** Blots of identical protein lanes were treated for 60 mins in 0,5 M HCl, 0,5 M Tris-HCl (pH 8.8) or 0,5 M NaOH at 65°C, and subsequently autoradiographed. Three phosphoproteins (12, 27 and 28 kDa), which have earlier been shown to undergo redox-regulated phosphorylation (15), have phosphoryl groups which are

stable under both acid (lane 1) and alkali (lane 3) conditions, suggesting phosphorylation of tyrosine residues. The 41 kDa phosphoprotein is the well-known α-subunit of pyruvate dehydrogenase (PDH) whose phosphoryl group is labile when treated with alkali, but stable under acid conditions, which is consistent with the fact that PDH is phosphorylated at serine residues. The 37 kDa phosphoprotein, on the other hand, exhibits an acid-labile but alkali-stable phophoryl group, suggesting that the phosphoryl group is carried by a histidine residue (15).

In bacteria, histidine kinase sensors are often implicated in control of gene expression. One function of the putative sensors discussed here may therefore be to place chloroplast and mitochondrial gene expression under the regulatory control of photosynthetic and respiratory electron transport. Redox conditions, selected either by redox reagents or by site-specific electron donors and electron transport inhibitors, have recently been shown in our laboratory to select proteins for synthesis in isolated chloroplasts and mitochondria (14). The data presented here represent a further test of the hypothesis that chloroplast and mitochondrial genomes have been retained, in evolution, to encode proteins of photosynthesis and respiration whose synthesis must be tightly under the control of the primary redox chemistry in which they function, or on which they have a direct influence (19, 20). Other predictions of this hypothesis are currently being explored.

Acknowledgements

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