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Possible role for molecular chaperones in assembly and repair of photosystem II

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Abstract

Genes of the *HSP70* chaperone family are induced by light. In *Chlamydomonas reinhardtii*, the induction of HSP70 (70 kDa heat shock protein) chaperones by light results in a partial protection of photosystem II against damage by photoinhibitory conditions. Underexpression of a chloroplast-localized HSP70 protein caused an increased sensitivity of photosystem II to light. Overexpression of this protein had a protective effect. Fluorescence measurements and studies of the turnover of photosystem II core components suggest that this HSP70 might function in both the protection and the regeneration of photosystem II. This concept is supported by fractionation studies in which the plastid HSP70 was found associated with chloroplast membranes. Because the light-induced elevation of HSP70 levels provides protection for photosystem II, we examined whether the chloroplast is involved in

Key words: chloroplast–nucleus signalling, HSP70 induction, plastid factor, PS II protection, tetrapyrroles.

Abbreviations used: HSP70, 70 kDa heat shock protein; PROTO, protoporphyrin IX; MgPROTO, Mg-protoporphyrin; MgPROTOMe, methyl ester of MgPROTO; PS, photosystem.

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this regulation and found that mutants defective in plastid-localized chlorophyll synthesis, i.e. the insertion of Mg^{2+} into protoporphyrin IX are impaired in the induction of *HSP70* by light. Exogenous addition of Mg-protoporphyrin in the dark induced the genes. The combined results support a model in which chlorophyll precursors are essential in the signalling from chloroplast to nucleus that regulates the chaperone genes.

Chaperones of the 70 kDa heat shock protein (HSP70) family might facilitate the folding of newly synthesized proteins as well as the correct refolding of polypeptides that, owing to the application of external stress, have assumed incorrect structures [1]. To fulfil this function, HSP70 chaperones are found in all major compartments of a eukaryotic cell. In addition, the chloroplast harbours several chaperones, of which two are associated with the envelope and are possibly involved in the import of proteins from the cytosol to the stroma [2,3].

In the unicellular green alga *Chlamydomonas reinhardtii*, a plant model organism, we observed an induction of three nuclear *HSP70* genes by light: *HSP70A* (encoding a cytosolic/nuclear protein), *HSP70B* (encoding a chaperone of the chloroplast stroma) and *HSP70C* (most probably encoding a mitochondrial chaperone) [4–6]. Induction of these genes by very low light intensities suggested that a specific signalling pathway mediates the light activation of these genes rather than the well-characterized mechanisms activated upon the application of heat stress, for example [7]. This was corroborated by the analysis of promoter deletion constructs that revealed the existence of a sequence element by which light induction is mediated [8]. Induction of *HSP70* genes by light has also been demonstrated recently in higher plants [9].

A reason for the elevated expression of the *HSP70* genes in response to light was speculated to be an enhanced protection of cells against the damaging effect of high light intensities. This led us to analyse the functional state of photosystem (PS) II, the primary target for light-inflicted damage in plants. Fluorescence induction measurements were used to assay the functional state of PS II. Indeed, incubation of dark-adapted cells with low light intensities, a condition known to result in induction of the *HSP70* genes, before treatment with high light intensities improved the recovery of PS II from photoinhibitory damage when compared with non-induced cells. This improved recovery was correlated with a small but significant increase in the levels of the plastid chaperone HSP70B [10]. Subsequent studies with strains that either overexpressed or underexpressed the plastid HSP70 protein revealed strikingly different consequences on PS II upon photoinhibition. Thus the PS II activity of a strain with an approx. 2-fold higher level of HSP70B exhibited not only faster recovery but also a smaller degree of high-light-inflicted damage than a wild-type strain. In contrast, PS II of the underexpressing strain was damaged more severely by the same light treatment than PS II in wild-type cells and in addition exhibited only a very slow recovery [10].

Because severe photoinhibitory conditions are known to lead to a degradation of PS II reaction-centre polypeptides [11], we used Western blot techniques to assay the effect of different HSP70B levels in photoinhibited cells at the levels of reaction-centre protein D1 and the core antenna polypeptide CP43. Photoinhibition in the wild-type resulted in a drastic decrease in D1 levels followed by a gradual increase in this protein during a subsequent recovery phase in low light intensities. The behaviour of CP43 was similar, although less pronounced. In contrast with wild-type cells, upon photoinhibition the HSP70B overexpressor showed only a minor reduction in both D1 and CP43 protein levels. In the underexpressing strain, both D1 and CP43 virtually disappeared upon photoinhibition and showed no recovery at low light intensities within 540 min [10].

It is known that the recovery of PS II after photoinhibition occurs both by the functional reactivation of PS II centres, not requiring synthesis of its components *de novo*, and by the replacement of D1 protein and other damaged PS II core proteins, which requires chloroplast protein synthesis [12]. To distinguish between the two processes, chloramphenicol, an inhibitor of organellar translation, was added at the end of the photoinhibition period. Under these conditions the D1 protein of the wild-type strain gradually disappeared within 180 min of photoinhibition. In contrast, the overexpressor showed only a minor decrease during this time period. When PS II activity was assayed in the overexpressor after photoinhibition, a transient decrease followed by a rapid but partial recovery in activity was observed in the presence of chloramphenicol. Under these conditions the wild type did not recover PS II activity [10].

These results suggest an interaction of the plastid HSP70 chaperone with proteins in the thylakoid membrane. This was assayed by fractionation of isolated chloroplasts. In these studies we demonstrated that 5–25% of total HSP70B protein is associated with the membrane fraction.

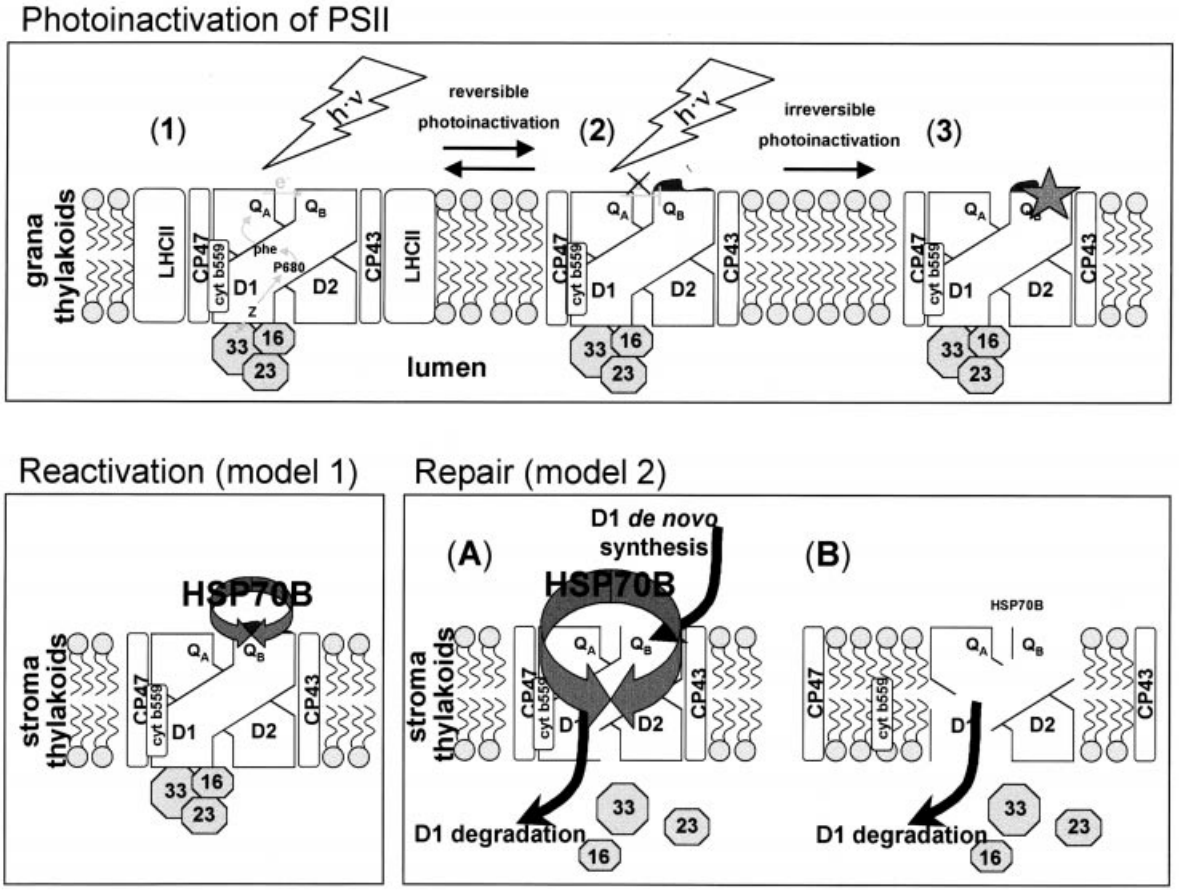
The current view of events that might follow treatment at high light intensities is shown in Figure 1 (upper panel). On treatment at high light intensities, a state transition occurs, resulting in a migration of the light-harvesting chlorophyll-*a*/*b*-binding proteins of PS II to stroma thylakoids. In addition, PS II might be reversibly inactivated, leading to a block of electron flow from Q_A to Q_B and to a conformational change at the Q_B-binding site of D1. Continued exposure to high light intensities leads to the irreversible photoinactivation of PS II and the tagging of D1 for degradation.

From these results we postulate that HSP70B might protect PS II against photoinhibitory damage by two distinct mechanisms (Figure 1, lower panels). In one (model 1) it might recognize the altered conformation at the Q_B-binding site of D1 and prevent its tagging for degradation. In the absence of degradation, the D1 protein might revert to a functional state. In the other mechanism, HSP70B might bind to PS II and stabilize the damaged complex until co-ordinated D1 degradation and synthesis *de novo* can occur (model 2A). All components except D1 can be recycled. Insufficient HSP70B levels might lead to rapid D1 degradation and consequently to a destabilization

Figure 1

Photoinactivation of PS II

Upper panel: (1) The functional PS II complex present in the stacked (grana) thylakoids with the D1/D2 heterodimer, the oxygen-evolving complex consisting of OEE1, OEE2 and OEE3, cytochrome *b*-559, the inner antennae CP43 and CP47 and the light-harvesting complex (LHCII). Smaller components are omitted. The reaction centre chlorophyll *a* (P680), the primary donor TyrZ (Z), the primary acceptor phaeophytin (phe) and the binding site for plastoquinone (Q_B) are on the D1 protein; the secondary acceptor (Q_A) is bound to the D2 protein. The electron flow is indicated by the arrows. (2, 3) Changes that occur within PS II upon illumination with high light intensities as discussed in the text. The conformational change induced at the Q_B-binding site of D1 is shown in red, a blue star illustrates tagging of D1 for degradation. Lower panel: models for the action of HSP70B leading to the protection of PS II from photodamage. HSP70B (shown in green) might act at two levels presented in models 1 and 2 as discussed in the text.

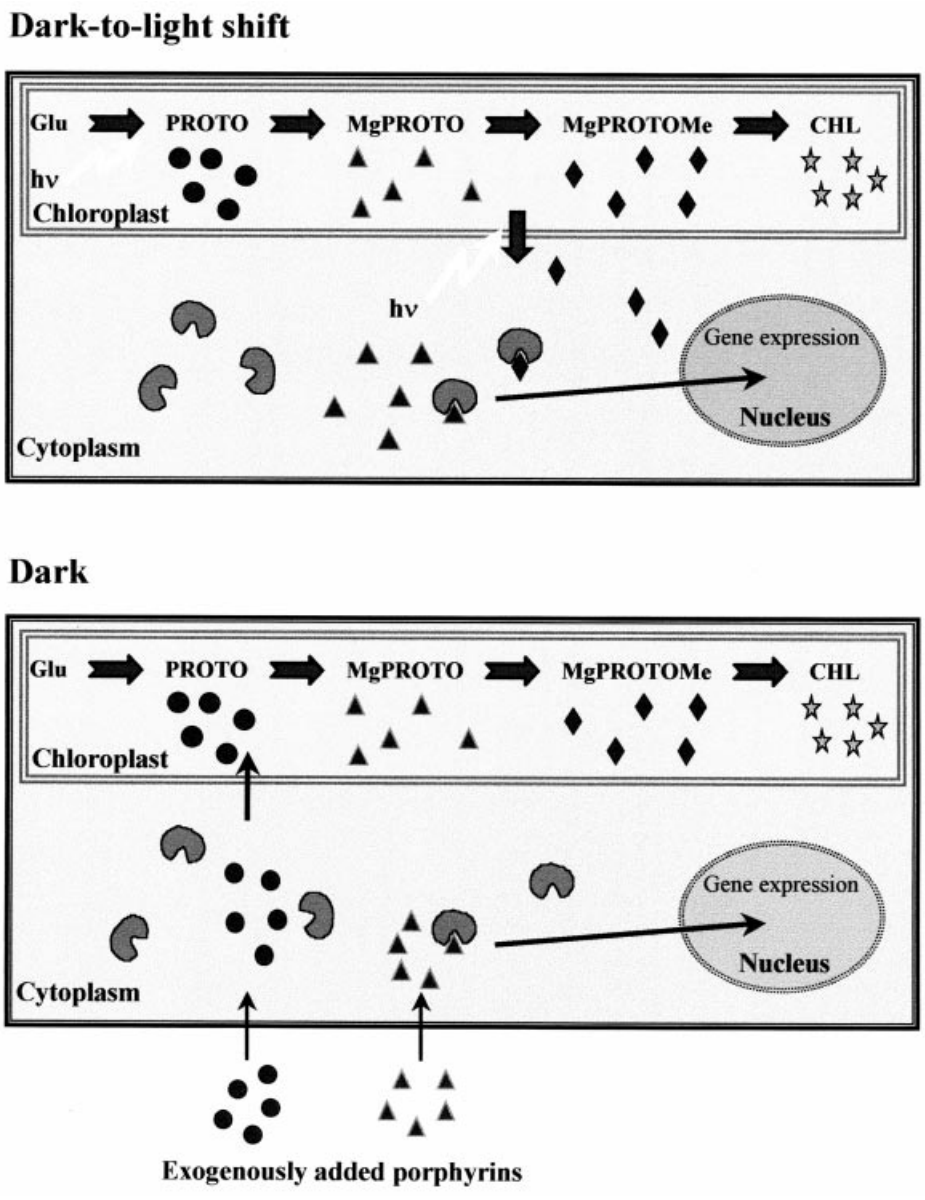


of PS II (model 2B). PS II then falls apart and its components are subject to degradation. Reconstitution requires the synthesis of D1 *de novo* and the reassembly of all subunits. However, because HSP70B might have several other functions within the chloroplasts, such as protein import, we cannot at present exclude the possibility that the PS II protection/repair described above is medi-

Figure 2

Model for the induction of nuclear genes by light mediated by MgPROTO and/or MgPROTOMe

The pathway for chlorophyll synthesis, starting with glutamate, and the steps postulated to be light controlled are indicated. Upper panel: the postulated situation upon treatment of dark-adapted cells with light (*hν*). Various intermediates of chlorophyll (CHL) accumulate and MgPROTO/MgPROTOMe become accessible to the cytoplasm. The downstream signalling pathway is activated by interaction with a cytosolic or nuclear factor, resulting in the activation of the *HSP70* genes. Lower panel: cells incubated in the dark do not accumulate the various chlorophyll precursors. Exogenously added PROTO is taken up by the chloroplasts and converted into MgPROTO/MgPROTOMe. These compounds are retained by the chloroplast. Exogenous addition of MgPROTO results in a direct activation of signalling pathways located in cytoplasm/nucleus and in the induction of nuclear *HSP70* genes. The various porphyrins and their locations are indicated.



ated by an as yet unknown factor whose biogenesis depends on HSP70B function.

Because the induction of *HSP70B* by light seems to be important in the functional maintenance of PS II during light stress, the analysis of the mechanism for this induction was of prime interest. An involvement of the chloroplast in this regulation was suggested by the finding that mutants in the plastid-localized biosynthetic pathway for chlorophyll showed a defect in the induction of the *HSP70* genes by light. Thus, in mutants defective in Mg-chelatase (the first enzyme specific for chlorophyll synthesis), the *HSP70* genes were no longer inducible by light; however, the induction by heat shock in these mutants was intact [13]. This result suggested a function for chlorophyll precursors as a plastid signal regulating the expression of a subset of nuclear genes. A role of these compounds in communication from plastid to nucleus has been suggested previously [14,15].

A regulatory role for certain tetrapyrroles was substantiated by experiments in which we demonstrated that the exogenous feeding of Mg-protoporphyrin (MgPROTO) or its methyl ester (MgPROTOMe) in the dark results in an induction of the *HSP70* genes; indicating that these compounds could substitute for the light signal [13]. Specificity for these two porphyrins as inducers was deduced from the observation that neither the MgPROTO precursor protoporphyrin IX (PROTO) nor an intermediate further downstream (protochlorophyllide) had inducing potential.

On the basis of these results we propose that the signal chain for the induction of the *HSP70* genes is activated by light within the chloroplast or its envelope (Figure 2, upper panel). After this activation, MgPROTO and/or MgPROTOMe might become accessible to the cytoplasm or the nucleus [16].

Although direct evidence for transport of MgPROTO/MgPROTOMe out of the chloroplast is still lacking, we have obtained clues for a dual role of light in this signalling pathway. (1) Shifting dark-adapted cultures into the light causes a transient increase in the pool levels of PROTO, MgPROTO and MgPROTOMe. The kinetics of this increase precedes the kinetics of accumulation of *HSP70* mRNA [17]. To assay the role of the light-induced increase in precursor pool levels we employed inhibitors of cytoplasmic protein synthesis that had previously been shown to inhibit the induction of the *HSP70* genes by light. These inhibitors also prevent the light-induced increase in porphyrin pool levels [17]. Because the addition of MgPROTO to cell cultures causes induction of the *HSP70* genes in the presence of these inhibitors, we conclude that the target of the inhibitors within the light-signalling route is not the downstream signalling pathway in cytosol or nucleus. Rather, the absence of *HSP70* gene induction seems to be a direct consequence of an inhibition of the light-induced accumulation of chlorophyll precursors. (2) Evidence for a second, light-requiring, step in the induction of the *HSP70* genes came from an analysis of porphyrin pool levels after the feeding of PROTO in the dark. This feeding does not result in an induction of the *HSP70* genes. However, pool measurements revealed that the PROTO fed was converted into MgPROTO and MgPROTOMe, leading to a substantial increase in the pool sizes of these compounds [17]. The lack of induction of the *HSP70* genes observed upon PROTO feeding, even though the MgPROTO/MgPROTOMe pool levels were high, suggests that these compounds are retained by the chloroplasts in the dark and are not released to the cytoplasm (Figure 2, lower panel). We conclude that light, in addition to inducing the accumulation of chlorophyll precursors in the chloroplasts, also is required for a second step in this plastid-to-nucleus signalling pathway, in other words in making these compounds accessible to cytosol or nucleus. It is possible that MgPROTO/MgPROTOMe are sequestered within the chloroplast in the dark and that illumination causes a release of the porphyrins, permitting their diffusion to cytosol or nucleus. As an alternative mechanism we envisage a light-driven transport of MgPROTO/MgPROTOMe from the chloroplast to the cytoplasm (Figure 2, upper panel). There these porphyrins might activate the downstream signalling pathway.

These results illustrate an intimate involvement of the chloroplast in the regulation of HSP70 chaperone synthesis by light. Such a regulatory scheme is plausible when we assume that the chaperone concentration in the chloroplast is a crucial parameter in the protection of PS II against light stress.

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Incorporation of iron–sulphur clusters in membrane-bound proteins

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Abstract

The completely sequenced genome of the cyanobacterium *Synechocystis* PCC 6803 contains several open reading frames, of which the deduced amino acid sequences show similarities to proteins known to be involved in FeS cluster synthesis of nitrogenase (Nif proteins) and other FeS proteins (Isc proteins). In this article, the results of our studies on these proteins are summarized and discussed with respect to their relevance in FeS cluster incorporation in chloroplasts. In cyanobacteria, there appears to exist several pathways for FeS cluster synthesis.

Introduction

Iron–sulphur (FeS) proteins are present in all organisms studied so far. The nature of their redox-active centre has attracted significant interest, and was clarified in the 1960s by the chemical synthesis of FeS clusters in apoproteins [1,2]. However, the chemical synthesis of FeS clusters required millimolar concentrations of iron salts and sulphide, both known to be highly toxic for living cells. Therefore the sources of iron and sulphur *in vivo* had to be different from those used in the *in vitro* reconstitution experiments. The first light on the biological mechanism of FeS cluster synthesis was shed in 1993 when Dean and colleagues discovered the enzymic activity of a protein, NifS [3], an enzyme essential for the synthesis of active nitrogenase Fe protein in *Azotobacter vinelandii* [4]. NifS was shown to be a cysteine desulphurase, which converts free cysteine into alanine. The sulphur from free cysteine becomes bound to a cysteine side chain in the active centre of NifS. In the presence of a reductant this sulphur is released as sulphide. Another protein essential for the synthesis of active nitrogenase Fe protein, NifU, was shown to be able to bind one iron ion at its N-terminal part [5]. When the Fe-binding NifU was then incubated with NifS in the presence of cysteine and a reductant a (relatively instable) FeS cluster is assembled at NifU [6]. This preassembled cluster is thought to be transferred then to the apoprotein of nitrogenase.

Proteins with similarity to NifS and NifU are encoded in the genome of almost all organisms, including the nitrogen-fixing organisms themselves [7]. Biochemical and genetical studies demonstrated that at least *A. vinelandii*, *E. coli*, yeast and the cyanobacterium *Synechocystis* PCC 6803 contain proteins with a function similar to NifS responsible for FeS cluster synthesis in proteins other than nitrogenase. With NifU, the situation is more complicated, since the protein from *A. vinelandii* consists of three domains, and some

Key words: cysteine desulphurase, FeS cluster, nitrogenase, nitrogen synthesis, open reading frame, Synechocystis.
Abbreviations used: ORF, open reading frame; Cm, chloramphenicol.
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