Light-dependent Formation of the Photosynthetic Proton Gradient Regulates Translation Elongation in Chloroplasts*

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Upon transfer of lysed chloroplasts from darkness to light, the accumulation of membrane and stromal chloroplast proteins is strictly regulated at the level of translation elongation. In darkness, translation elongation is retarded even in the presence of exogenously added ATP and dithiothreitol. In the light, addition of the electron transport inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyl urea inhibits translation elongation even in the presence of ATP. This inhibition can be overcome by addition of artificial electron donors in the presence of light, but not in darkness. Electron flow between photosystem II and I induced by far red light of 730 nm is sufficient for the activation of translation elongation. This activation can also be obtained by electron donors to photosystem I, which transport protons into the thylakoid lumen. Release of the proton gradient by uncouplers prevents the light-dependent activation of translation elongation. Also, the induction of translation activation is switched off rapidly upon transfer from light to darkness. Hence, we propose that the formation of a photosynthetic proton gradient across the thylakoid membrane activates translation elongation in chloroplasts.

Protein expression in plastids of algae and higher plants is regulated by light during the light-dependent development of chloroplasts as well as during the light-dark transitions of fully differentiated chloroplasts. A great proportion of the regulatory mechanisms were found to be mediated at a posttranscriptional level, as the mRNA levels of many of the concerned genes were little or not affected (1–4). Most of the posttranscriptional changes of protein expression were explained by a regulation of translation initiation; the 5'-untranslated region of the psbA gene encoding the reaction center protein D1 was shown to direct light-dependent accumulation of a reporter gene in transformed tobacco plants (5), and proteins binding to the 5'-regions of several chloroplast genes were observed in *Chlamydomonas reinhardtii* (6). Light-dependent binding of proteins to the 5'-untranslated region of the psbA gene was shown to be responsible for the light-dependent translation initiation in *Chlamydomonas* (7) and in spinach (8).

Other examples of light-regulated protein expression were explained by translation elongation control; the mRNA encoding the large subunit of ribulose-1,5-bisphosphate carboxylase was not translated in the dark but remained bound to polysomes in Amaranthus (9). Accumulation of D1 translation intermediates in the dark was observed in pea chloroplasts (10, 11) and in spinach (12).

Different explanations were given of how light regulated translation. Several groups attributed reduced protein expression in the dark to the reduced level of ATP in the chloroplast in the absence of photosynthetic activity (13, 14). Conversely, reduced translation initiation of the psbA gene has been explained by the increased level of ADP in the dark (15). Binding of an initiation complex to the psbA mRNA was proposed to be regulated by reduced thioredoxin, which is generated in photosynthetic electron transport (16). Redox factors were also suggested to control chloroplast protein expression in general (17) and translation elongation of the D1 protein (14).

Here, we present a new effect of light on the general efficiency of translation elongation in barley chloroplasts, which is superimposed on a regulation of translation by redox factors or ATP. Our results suggest that the light stimulation of translation elongation is dependent on the formation of a proton gradient across the thylakoid membrane, which arises from photosynthetic electron transport. The effect is manifested rapidly in the light and is also rapidly suppressed after transfer to darkness.

**EXPERIMENTAL PROCEDURES**

*Plant Growth and Plastid Isolation—Barley (Hordeum vulgare L. var. Steffi) seeds were planted in moist vermiculite and grown for 7 days at 23 °C in a light/dark cycle of 12 h each. In the light phase of the seventh day, the upper half of the primary leaves was cut, and plastids were isolated as described (18).*

*In Organello Translation and Protein Detection—Isolated chloroplasts were pulse labeled for 2.5 min as described (18) with [35S]methionine and in the presence of 0.5 mM ATP, if not otherwise stated. Additional reagents were added as indicated in the figure legends.*

Chloroplasts were lysed osmotically in the translation reactions because of the absence of sorbitol. Dark reactions were performed in a dim green safe light (<10 nE/m² s), and light reactions were performed in a white light of 50 μE/m² s. Far red light was applied to the samples with a Volpi Intralux 150H cold light source provided with an interference filter of 730 nm, providing a light intensity of about 5 μE/m² s to the reactions. Reactions were terminated by freezing the samples in liquid nitrogen. Samples were separated into stroma and membrane fractions by centrifugation (3000 × g, 3 min). The stromal fraction was centrifuged again (20,000 × g, 5 min), and the membranes were washed in a buffer containing 10 mM Tris-HCl, pH 6.8, 10 mM magnesium acetate, and 20 mM potassium acetate. Membrane-bound polysomes were isolated as described in Ref. 19. Proteins were prepared for SDS-polyacrylamide gel electrophoresis as described (19).

*Chemicals—[35S]Methionine (Rediue) was purchased from Amer sham & Buchler (Braunschweig, FRG). Ascorbate, carbonyl cyanide-m-chlorophenylhydrazon (CCCP) 1, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DCMU, 2,6-dichlorophenolindophenol; DQH2, reduced duroquinone; PMS, phenazine methosulfate; PQ, plastocyanin; PQH2, reduced plastocyanin; PS I/II, photosystem I/II.*

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urea (DCMU), duroquinone, gramicidin, lincomycin, and phenazine methosulfate (PMS) were from Sigma (Munich); dithiothreitol was purchased from Biomol (Hamburg), 2,6-dichlorophenolindophenol (DCPIP) from Merck (Darmstadt), and ATP and GTP from Boehringer Mannheim. Plastoquinone-1 was a gift from W. Oettmeier (Bochum) and was reduced with NaBH₄ and purified by extraction with ether. Duroquinone was reduced with NaBH₄ according to Ref. 20. Hydrophobic reagents were dissolved in ethanol; the final concentration of ethanol in the translation reactions and in the control reactions performed in parallel was kept below 2%.

**RESULTS**

*Light Regulates Translation Elongation in Chloroplasts—* The regulation of translation elongation was examined by radiolabeling of lysed barley chloroplasts with [³⁵S]methionine in the presence of the translation initiation inhibitor lincomycin. When membrane-bound polysomes were isolated from chloroplasts pulse labeled for 2.5 to 15 min, radiolabel incorporation into defined polysome-bound translation intermediates was high after 2.5 min in the light but low in darkness. Thereafter, radiolabel accumulation in translation intermediates decreased between 2.5 and 15 min in the light, whereas in darkness radiolabel increased for 7.5 min and only decreased thereafter. This indicated that translation elongation was increased in the light by increased ribosome run off (Fig. 1).

**DCMU Inhibits Light Stimulation of Translation Elongation in Chloroplasts—** In lysed chloroplasts, radiolabeling of soluble and membrane proteins was strongly enhanced in the presence of light (Fig. 2; light, lanes 3 and 5; dark, lane 1). The main translation product found in the membrane fraction of 7-day-old barley chloroplasts is the 33-kDa precursor of the D1 protein (pD1) (Fig. 2A). During a 10-min chase with nonradioactive methionine, many protein bands with a lower molecular weight disappeared, whereas pD1 and mature D1 accumulate (Fig. 2A, lanes 7–10). These lower molecular weight protein bands can be immunoprecipitated with a N-terminal D1 antibody, indicating that they correspond to translation intermediates of the D1 protein. However, the light stimulation of translation elongation was not specific for the D1 protein, as light also increased radiolabeling of higher molecular weight proteins in the membrane and in the stromal fraction (Fig. 2B). In the stroma, many bands immunoprecipitated in the presence of an antibody against the large subunit of the ribulose-1,5-bisphosphate carboxylase (LSU) and seem to be translation intermediates, as they disappear during a 10-min chase (Fig. 2B, lanes 7–10). Hereby, a large proportion of the translation products in the stroma is shown to correspond to LSU, whereas proteins not elongated during the chase may correspond to other stromal proteins.

The light-dependent stimulation of translation elongation was strongly inhibited by addition of the electron transport inhibitor DCMU (Fig. 2, lanes 3 and 4). DCMU showed no effect on translation in darkness, indicating that DCMU did not inhibit translation elongation in general (Fig. 2, lanes 1 and 2). The inhibitory effect of DCMU in the light was also observed in
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The presence of exogenously added ATP (Fig. 2, lanes 5 and 6). Hence, the reduced translation efficiency in the light was not caused by DCMU-dependent inhibition of ATP synthesis but by inhibition of the photosynthetic electron transport.

The influence of light on translation elongation was observed in all experiments for membrane and stromal proteins. Nevertheless, in the following figures only radiolabeling of membrane proteins will be shown to focus the data presented.

**Electron Donors Overcome the DCMU-dependent Inhibition of Translation Elongation**—To test which part of the electron transport chain is responsible for light stimulation of translation elongation, we restored electron transport in the presence of DCMU by addition of selected electron donors. As DCMU inhibits reduction of plastoquinone (PQ) by photosystem II (PS II), we restored the pool of reduced plastoquinone, and hereby photosynthetic electron transport, by addition of reduced duroquinone (DQH$_2$) or plastoquinone-1 (PQH$_2$) (20–22). Both substances were able to release the inhibition of translation by DCMU in the light but did not stimulate translation in darkness (Fig. 3, A and B). These data indicated that light is required in addition to an electron donor component to overcome the inhibition of electron transport at the site of PQ reduction. Thus, we concluded that the pool of reduced plastoquinone is not the signal for light-dependent activation of translation elongation.

Although reduction of plastocyanin by reduced PQ can take place in the dark (22), the amount of oxidized plastocyanin most likely limited electron flow in the dark, whereas in the light constant electron flow was possible due to PS I action. Hence, after inhibition of electron transport by DCMU, translation elongation could be reinduced by restoring electron transport after PS II because of the action of DQH$_2$ or PQH$_2$ as electron donors.

**Electron Flow between PS II and PS I Is Sufficient for Stimulation of Translation Elongation**—To further localize the activation of translation elongation to a specific site of the electron transport chain, we directly reduced PS I by addition of reduced DCPIP (24). Reduced DCPIP was able to overcome the translation inhibition by DCMU in the light to the same extent as DQH$_2$ and PQH$_2$ (Fig. 3C). Also, PMS, which mediates cyclic electron flow around PS I (24), was able to overcome the inhibition of DCMU in the light (Fig. 3D). These data indicated that PS I activity alone was sufficient for light stimulation of translation. However, PMS and DCPIP are both known to form a proton gradient during electron transport by PS I (24, 25). We therefore investigated the formation of a photosynthetic proton gradient by electron transport between PS II and PS I by illuminating translation reactions with monochromatic light of 730 nm, which excites PS I but not PS II (26). Clearly, a stimulation of translation elongation in far red light occurred only in the presence of an electron donor (DQH$_2$) (Fig. 4, lane 4 compared with lanes 1–3). This demonstrated that electron transport between PS II and PS I was sufficient for induction of translation, whereas activation of PS I alone was not. To further see whether the redox state of ferredoxin, thioredoxin, or NADPH, which are reduced by PS I during photosynthesis, could trigger induction of translation, the isolated redox compounds were added to the reactions in their reduced state. However, none of these substances was able to stimulate translation elongation in the dark (data not shown).

**Release of the Photosynthetic Proton Gradient Prevents Light-stimulated Translation Elongation**—Our data presented above suggested that the light-dependent signal for activation of translation elongation was not triggered by electron transport or a single redox component of the chain but by the formation of a proton gradient across the thylakoid membrane. We therefore investigated the rate of translation elongation during the release of the photosynthetic proton gradient by addition of uncouplers of photophosphorylation in the light and in presence of exogenously added ATP. Addition of the uncouplers Nigericin and CCCP clearly reduced translation elongation to the level obtained in darkness (Fig. 5, open bars).
pore-forming protonophore gramicidin, however, which inhibits photophosphorylation in thylakoid membranes already at a concentration of 0.1 mM (27), did not block light-dependent stimulation of translation elongation even in a concentration of 10 μM (Fig. 5). Obviously, gramicidin only decreases the photosynthetic proton gradient to a level not high enough for ATP synthesis but does not abolish it even in high concentration (27). We therefore completely abolished the proton gradient by addition of gramicidin in combination with NH₄Cl (27). Hereby, translation elongation in the light was reduced to the level obtained in darkness. Hence, light-dependent formation of a weak proton gradient is sufficient to activate translation elongation. In parallel, we tested the influence of uncouplers on electron transport by measuring NADP reduction in the light (Fig. 5, filled bars). Data clearly revealed that electron transport was not (nigericin, gramicidin, gramicidin plus NH₄Cl) or only slightly affected (CCCP) by the uncouplers, indicating that electron transport components are not involved in regulation of translation elongation.

Light Induction of Translation Elongation Is Rapidly Switched off upon Transfer of Chloroplasts to Darkness—The photosynthetic proton gradient is rapidly released upon transfer of cells from light to darkness (29). However, a proton gradient-induced signal could sustain a high rate of translation elongation for some time in darkness, if it was sufficiently long-lived. We therefore preilluminated plastids for 1 min on ice to stabilize the putative light signal and compared the activation of translation with preillumination at 25 °C. After preillumination, [35S]methionine and lincomycin were added, and translation reactions were performed in darkness or light. Preillumination had no stimulating effect on translation in the dark, regardless of the temperature in which the preillumination was performed (Fig. 6). This indicated that the putative proton gradient-induced signal was not long-lived but was lost upon transfer of chloroplasts to darkness. We conclude that the light-induced formation of the photosynthetic proton gradient serves a dual function for induction of translation elongation. First, a short-lived signal may be induced to increase the rate of translation elongation; second, ATP formation is induced after full development of the proton gradient to sustain the translation elongation process in general.

DISCUSSION

Regulation of Translation Elongation by Light—Most studies on posttranscriptional regulation of gene expression in chloroplasts have concentrated on gene-specific regulation mechanisms (3–5, 7–11, 14–16). Here, we describe a light-dependent mechanism that is not gene specific but enhances radiolabel
accumulation in at least two proteins localized in the chloroplast stroma and membrane fraction. Light seems to directly activate radiolabeling by a factor involved in the enzymatic process of chain elongation, e.g. a ribosomal component, one of the chloroplast elongation factors, or chloroplast tRNA-acyl synthases. Alternatively, light-dependent structural changes at the level of the mRNA or during folding of the nascent protein chain could indirectly affect the rate of chain elongation.

It has been observed earlier that inhibitors of photosynthetic electron flow reduce light-dependent protein expression in chloroplasts (13, 14). Light activation of translation was attributed merely to the light-dependent increase in the ATP level, and inhibition of translation by inhibitors of photosynthetic electron flow was explained by inhibition of the light-dependent ATP synthesis. Our experiments, however, show that the presence of ATP is only one of the regulating factors, as addition of exogenous ATP was not sufficient for full activation of translation in the dark or for restoration of translation in the presence of DCMU or uncouplers. Kuroda et al. (14) reported an accumulation of translation intermediates of the D1 protein in the dark, which could not be overcome by ATP alone, and proposed the involvement of a light-dependent reductant component for activation of translation, as dithiothreitol partly released the translational block. However, our experiments were performed in the presence of dithiothreitol and still revealed a light-dependent regulation of translation elongation that is superimposed on possible reduct control mechanisms operating on the level of translation elongation or initiation.

Control mechanisms on initiation level, as proposed for the psbA gene by several groups (5, 7, 8), may still exist beside the regulation of translation elongation described here and may be important for the developmental control of photosystem accumulation, i.e. the onset of translation after prolonged etiolation or energy depletion in darkness. The regulation of D1 accumulation by translation elongation instead of initiation could be necessary to inhibit singlet oxygen formation by immediate binding of “free” chlorophyll to nascent apoprotein sites, even in darkness (28).

Regulation of Translation Elongation by the Proton Gradient—In the light, translation elongation was only activated by reduct substances that allow the formation of a proton gradient, either by release of protons in the thylakoid lumen (PMS, DCPIP) or by allowing proton transport via the cytochrome b/f complex (DQH_2, PQH_2). In contrast, addition of the same substances in the dark, or addition of reduct substances that do not promote proton transport, like thioredoxin and ferredoxin, could not activate translation elongation (Fig. 7A). Furthermore, we show that a lower proton threshold level is required for the induction of translation elongation than for synthesis of ATP. Hence, the photosynthetic proton gradient, which is formed and released within seconds, is used at a very sensitive setting to monitor photosynthetic electron transport and therefore light (29). Such a regulatory mechanism seems useful, as the highly energy-consuming process of translation will be immediately retarded upon light-dark transfer of the plant, before the level of ATP is decreased within the organellar. A further indication that the proton gradient in chloroplasts may be used as a light sensor is its requirement for the light-dependent translocation of several nuclear-encoded proteins across the thylakoid membrane (30). Although it is unknown how the proton gradient signals activation of translation elongation and membrane translocation, these data indicate that the formation of a proton gradient is a prerequisite for the assembly of several chloroplast- and nuclear-encoded proteins in the thylakoid membrane.

**Fig. 7. Working model for regulation of translation elongation by the photosynthetic proton gradient.** In the light, proton gradient formation can be blocked by DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyleurea)-dependent inhibition of electron transport (black line) between H_2O and NADP^+ and can be reactivated by DQ (d duroquinone)-dependent redox of PQ- or phenazine methosulfate-dependent (PMS) electron plus proton transport around PS I (A). Reduced DCPIP (2, 6-dichlorophenolindophenol) serves as a reductant to PS I, and carbonylcyanide-m-chlorophenylhydrazon (CCCP) releases the proton gradient over the thylakoid membrane (A). The translation elongation rate of the psbA mRNA may be regulated by a proton-sensitive thylakoid membrane component (X), binding the chloroplast ribosome (R) and the D1 nascent chain (B). In the light, a high proton concentration in the thylakoid lumen will structurally alter the configuration of factor X. Hereby, the light signal will be transmitted from the thylakoid lumen to the membrane-bound polysomes on the stromal side of the membrane, and the rate of translation elongation will be increased. In darkness, and upon proton release in the light, factor X will be reset and translation elongation will be decreased (B).

Most interestingly, the main stromal component activated by the light-induced proton gradient, LSU, was found to be translated on membrane-bound polysomes (31, 32); this could also be the case for other stromal proteins. Therefore, we suggest the following model for the general activation of translation elongation by a proton gradient (Fig. 7B). The rate of translation elongation is coupled to the pH gradient by a thylakoid membrane component involved in the binding of ribosomes or in the enzymatic control of translation elongation activity. An increase in the luminal pH levels during illumination alters the conformation of a membrane component leading to increased chain elongation. The very rapid switch-off observed in our experiments would favor such a direct mechanism. Also, no indications for the requirement of signal transduction steps like protein phosphorylation have been found in our system.²

² S. K. Mühlbauer and L. A. Eichacker, unpublished observations.
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