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Forum Review

Redox Regulation of Chloroplast Transcription

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ABSTRACT

Chloroplasts are the important plant cell organelles where photosynthesis takes place. Throughout this process, reaction center proteins are degraded and subsequently replenished by redox-responsive gene expression. In addition to well defined posttranscriptional mechanisms at the RNA and protein level, the transcription of chloroplast DNA into RNA precursors has been a focal point of studies in this area. Evidence has become available for a central role of a redox-responsive protein kinase named plastid transcription kinase (PTK) because of its association with the chloroplast transcription complex. The recent cloning of the PTK gene has resulted in a full-length cDNA for a protein related to the catalytic α subunit of nucleocytoplasmic casein kinase (CK2), yet with an additional chloroplast transit peptide. The corresponding protein, termed cpCK2α, was shown to be associated with the major organellar RNA polymerase, PEP-A. Both authentic PTK and recombinant cpCK2α have comparable general properties *in vitro*, and both are subject to regulation by the redox-reactive reagent glutathione. Based on the physical and functional equivalence, it is anticipated that the cloned protein can help clarify the functional role of the transcription kinase *in vivo*, including the identification of interaction partners at the interface between photosynthetic redox signaling and gene expression. *Antioxid. Redox Signal.* 5, 79–87.

INTRODUCTION

CHLOROPLASTS are unique plant cell organelles, well known as the sites of photosynthesis (14). They also harbor a full gene expression machinery (74), which is distinct from that of the nucleocytoplasmic compartment and is specifically adapted to changes in photosynthetic redox state (2, 6).

The signaling mechanisms that connect photosynthetic electron flow with gene expression responses include reversible changes in phosphorylation and thiol (SH) group redox state of proteins that are functionally involved. Proteins that are subject to posttranslational modifications have been identified as constituents of either the photosynthetic or gene expression machinery, and it is conceivable that at least some of them may play an interconnecting role between both multimeric complexes (46). Although we are still far from a complete understanding of the underlying mechanisms, it seems clear already that signal transduction proteins act at more than one level of plastid gene expression. SH-group redox regulation was initially demonstrated to control chloroplast translation initiation in the case of the green alga *Chlamydomonas reinhardtii* (17). The upstream signal is thought to be transmitted via the ferredoxin–thioredoxin system (69) to a redox-responsive oligomeric protein complex capable of binding to the 5´-untranslated region of psbA mRNA (13, 17, 83). Since then, several other posttranscriptional mechanisms in chloroplast gene expression, including translation elongation (88), RNA degradation (43, 66), and RNA splicing (20), were demonstrated also to be targets for redox regulation.

This multitude of redox-regulatory components at posttranscriptional levels has prompted research to investigate the possibility of whether similar connecting mechanisms may exist also at the level of transcription, *i.e.,* the initial steps of chloroplast gene expression resulting in *de novo* production of RNA (precursor) chains. In the remaining sections of this review, various aspects of the available evidence will be discussed and current experimental strategies addressed.

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REDOX-RESPONSIVE TRANSCRIPTIONAL REGULATION

Strong arguments for the possible existence of redox-regulated transcription in chloroplasts came from reports on such mechanisms in bacteria (*e.g.,* 18, 89) and in the nuclei of eukaryotic cells (1, 54, 70, 76). One aspect that has received particular attention is the presence of redox-regulatory systems of the two-component type, such as *RegA/RegB* (75). Two-component systems in general are widespread in prokaryotes and have been identified also in eukaryotic organisms, including plants (72). Considerations guided by the endosymbiont hypothesis have led to the suggestion (2, 3) that chloroplast transcription may be controlled by a mechanism involving a redox sensor and response regulator(s) analogous to a prototype bacterial two-component system. In its typical form, the sensor would have protein kinase activity, using ATP for autophosphorylation at a histidine residue on its so-called transmitter domain. This phosphoryl group would then be transferred to an aspartic acid residue on the receiver domain of the response regulator, which often is a sequence-specific transcriptional regulator.

Protein kinases are associated with thylakoid preparations (23, 63, 86), but none of those that have been characterized seems to qualify as a histidine-specific two-component enzyme. With the exception of a single reported tyrosine kinase activity (84), all thylakoid kinases so far characterized were shown to be serine/threonine-specific enzymes. It should be noted, however, that serine/threonine kinases can act in concert with, or even substitute for, the histidine kinase moiety of prototype two-component systems (87). For instance, the plant photoreceptor phytochrome has significant sequence similarity to the bacterial histidine kinase region, but reveals serine/threonine kinase activity (15). It thus seems premature to exclude the possibility that one of the known thylakoid-associated kinases might have a role as a chloroplast sensor kinase, although none of the DNA binding proteins from higher plant chloroplasts has been identified as a (redox) response regulator.

CHLOROPLAST RNA SYNTHESIS— A TARGET FOR REDOX REGULATION

That light (as compared with dark-grown conditions) can have a profound influence on the accumulation and synthesis of RNA molecules in isolated plastids has been shown in a number of reports from different laboratories (for reviews, see, *e.g.,* 46, 50). Furthermore, isolated transcription complexes from maize chloroplasts were shown to differ in their specific activity from those of etioplasts (5). Evidence was presented that blue light is capable of activating synthesis of a nuclear-encoded transcription factor named AAG-box binding factor (AGF) (39), which then binds to a light-reponsive *cis*-regulatory element upstream of the *psbD-psbC* operon on barley chloroplast DNA (32, 40, 67, 78, 82). Despite these and other data pointing to the involvement of photoreceptors such as cryptochrome (blue/UV light) or phytochrome (red/far-red light), the majority of (white) light effects on plastid gene expression is consistent with a mechanism operating through changes in photosynthetic electron flow, *i.e.,* redox regulation (46).

This question was addressed by Pearson *et al.* (56), who were able to show that illuminated lettuce chloroplasts differed from those kept in darkness with regard to their capacity for RNA synthesis. Furthermore, the rate of synthesis was specifically affected in the light, but not in the dark, by inhibitors of electron flow through photosystem II (PS II) [3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB)] or of the Rieske iron-sulfur protein of the cytochrome b_6f complex (bromanil). In these experiments, however, RNA synthesis was only partially inhibited by actinomycin D, suggesting that only a fraction of the newly synthesized material represented template-dependent RNA synthesis (transcription). In addition, [^{3}H]-NAD was used as the primary radioactive label, which—upon conversion to 5′-AMP and adenosine—was then incorporated into RNA. It is thus conceivable that the observed incorporation was, to a large part, due to (template-independent) terminal addition of NAD-derived residues to preexisting RNA chains.

Using *in organello run-on* transcription in the presence of [α-^{32}]UTP (19, 51), evidence was subsequently provided for the control of specific plastid gene transcription by photosynthetic electron flow during light quality adaptation (59, 60). The mustard seedlings that served as a source for chloroplast isolation were grown under two spectrally different light regimes known to favor either photosystem I (PSI) or PSII function, *i.e.,* resulting in an imbalance of electron flow between the two photosystems (27). Furthermore, the isolated chloroplasts were preincubated in the absence or presence of either DCMU or DBMIB, followed by *in organello run-on* transcription, and the formation of newly synthesized transcripts was then assessed using gene-specific hybridization probes.

Following this strategy, Pfannschmidt *et al.* (59) detected lower transcriptional activity in plastids from seedlings grown under red "PSI" light (primarily absorbed by PSI) than in those grown under yellow "PSII" (preferentially PSII-sensitizing) light by using a *psaAB* probe for the genes that encode the two major reaction center proteins of PSI. Interestingly, the negative effect of PSI-sensitizing light could be mimicked by DCMU and, conversely, the positive effect of the PSII light was partially mimicked by DBMIB. It was concluded that *psaAB* transcription is down-regulated when the plastoquinone pool is oxidized (PSI light or DCMU), whereas it is enhanced when this pool is reduced (PSII light or DBMIB) (59). Using the same red/yellow illumination scheme, effects in an opposite direction compared with those for *psaAB* were detected for *in organello run-on* transcription of *psbA*, *i.e.,* the chloroplast gene for the D1 reaction center protein of PSII (60). Other genes for photosynthesis-related proteins showed only small differences in their transcriptional rates (*rbcL*; gene for the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase) or none at all (*petA*; cytochrome f gene). Together, these data established a role of differential transcription as a compensating mechanism that helps counteract imbalances in energy distribution between the two photosystems (state transitions) and that is possibly involved in adaptations to different light-quality environments.

Like light-quality changes, those in light intensity can also have a significant effect on chloroplast transcription rates. It was shown by Baena-González *et al.* (9) that chloroplasts from mustard seedlings grown under high light are transcriptionally more active than those from seedlings grown under moderate (growth light) irradiance. High-light conditions (affecting photosynthetic electron flow, turnover, and resynthesis of reaction center proteins) are well known to result in enhanced photoinhibition, which is associated with distinct changes in chloroplast redox state (7, 37, 38). From the data provided by Baena-González *et al.* (9), it is evident that this change in redox balance results in a global, rather than differential, activation of chloroplast transcription. Not only were the genes for photosynthetic (reaction center) proteins found to be high-light-activated, but effects of a comparable magnitude were also noticed for nonphotosynthetic genes, including those for organellar tRNAs and rRNAs. This is in contrast to the situation described for chloroplast transcription during state transition and light-quality adaptation (59, 60), suggesting the interesting possibility of multiple (alternative) regulatory mechanisms at this level of plastid gene expression. It thus seems appropriate to review briefly the current picture of the basal and regulatory components that comprise the chloroplast transcription machinery.

COMPLEXITY AND CONTROL OF THE PLASTID TRANSCRIPTION APPARATUS

Like in bacteria and eukaryotic nuclei, RNA synthesis in chloroplasts is a multistep enzymatic process controlled by a number of regulatory cues. Well established features include the involvement of multiple RNA polymerase forms and transcription factors and—on top of this scenery—a variety of specific control mechanisms acting through protein modifications have been identified (33, 45, 49, 50, 71).

Two principal classes of chloroplast DNA-dependent RNA polymerases can be distinguished (49). One of them, named nuclear-encoded polymerase (NEP) because of its nuclear origin (30), is a single-subunit enzyme (42) resembling those of T3/T7 bacteriophages and mitochondria. The other class is named plastid-encoded polymerase (PEP) because of the organellar coding site of its multiple core subunits. It resembles the bacterial type of multisubunit RNA polymerase (33). The latter enzyme was found to exist in two different subforms, PEP-A and PEP-B, each of which has distinct biochemical characteristics and is differentially expressed in plastids (57, 58). The PEP-B enzyme closely resembles bacterial RNA polymerase in its subunit structure and its sensitivity against the transcription inhibitor rifampicin, and it seems to be the predominate PEP form in etioplasts and perhaps other nongreen plastid types as well. The PEP-A enzyme is almost twice the size of the PEP-B form, contains at least 10 additional polypeptides, and is resistant to rifampicin. The latter form is the major RNA polymerase in chloroplasts and is responsible for the (redox-) regulated transcription of photosynthetic genes (45, 46).

It was shown that PEP-A could be at least partially reconverted into a rifampicin-sensitive (B-type) enzyme *in vitro*. Furthermore, both PEP-A and PEP-B were found to contain core subunits that, based on N-terminal sequencing and mass spectrometry, represent products of the same plastid *rpo* genes, suggesting the possibility of reversible interconversion of the two PEP forms *in vivo* (61). These findings point to the involvement of regulatory factors that might be recruited during chloroplast development, and that could be responsible for selective transcriptional responses to changes in photosynthetic redox state.

CHLOROPLAST TRANSCRIPTION FACTORS AND REDOX CONTROL

How can we envisage the transduction of a photosynthetic redox signal to the chloroplast transcription apparatus? One possibility, for which experimental evidence has become available, is the modification of regulatory proteins that act as transcription factors. This is exemplified by the reversible phosphorylation/dephosphorylation of organellar proteins that resemble bacterial sigma factors in conferring promoter selection and transcription initiation specificity on the catalytic core RNA polymerase (28, 31, 36, 48). For instance, three different proteins named sigma-like factors (SLFs) were purified from mustard chloroplasts (81). Similar-sized factors were also shown to be present in etioplasts, yet with enzymatic properties distinct from those of their chloroplast counterparts (79). Furthermore, *in vitro* phosphorylation treatment resulted in conversion of chloroplast into "etioplast-type" factors and, conversely, phosphatase treatment of the latter led to partial recovery of "chloroplast-type" properties (80). Together, these *in vitro* data suggested that plastid transcription might be regulated by the phosphorylation state of the SLFs (and perhaps other regulatory proteins) *in vivo*.

Further support for this idea came from the purification of the chloroplast protein kinase that was shown to be responsible for sigma factor phosphorylation. It was characterized as a serine-specific enzyme associated with chloroplast RNA polymerase PEP-A. For this reason, and because the kinase activity was found to determine the extent of faithful transcription from the *psbA* promoter in a homologous plastid *in vitro* system, the enzyme was named plastid transcription kinase (PTK) (10). As might be expected for a regulatory component, PTK was only loosely associated with the polymerase and could be partially separated as an active "free" kinase complex, which contained the catalytic component as well as two as yet unidentified interacting proteins. Furthermore, both the "free" and the polymerase-bound PTK activities were found to be dependent on phosphorylation state, suggesting the possible existence of at least one other kinase as an upstream component of a signaling cascade, in which PTK would then act as the downstream transmitter that directly controls chloroplast transcription *in vivo* (10).

Additional clues that may help clarify the role of PTK in chloroplast transcriptional regulation came from findings that this kinase phosphorylates not only plastid sigma factors, but also several other polypeptides that are part of the PEP-A complex (9, 11, 61). Equally significant, the kinase activity *in vitro* responded to both phosphorylation and SH-group redox

state in an antagonistic manner. The active enzyme, which was phosphorylated only to a low extent or not at all, was inhibited by the redox reagent glutathione (GSH); conversely, the inactive (phosphorylated) PTK form was shown to be reactivated in the presence of GSH (11). Based on this *in vitro* behavior, PTK might be a target for both phosphorylation and redox control also *in vivo* in the intact chloroplast context, possibly representing a key component in a signal transduction pathway that connects photosynthetic electron flow to chloroplast gene expression at the transcriptional level (21, 46, 47).

It has recently been shown that *in vitro* PTK efficiently phosphorylates the associated PEP-A RNA polymerase in an isolated transcription complex from seedlings grown under moderate light intensity (growth-light), but not from those grown under high-light conditions (9). Although only a single PEP-A subunit was readily detected as a preferred substrate for the endogenous PTK under these conditions, several other polypeptides were found to be phosphorylated by adding a heterologous casein kinase 2 (CK2). Some of these "second-site" substrates were more efficiently labeled in "high-light" than "growth-light" complexes and vice versa, suggesting that polymerase subunits can have differential phosphorylation state at the time of isolation depending on the previous light conditions. Based on these experimental data, a hypothetical scheme depicting possible *in vivo* mechanisms involved in transcriptional redox regulation can be inferred (Fig. 1).

In addition to PTK, several other accessory factors have been purified as part of the PEP-A RNA polymerase complex. N-terminal sequencing and/or mass spectrometry has identified proteins that are sequence-related to iron-superoxide dismutase (Fe-SOD), annexins, and RNA-binding proteins (61) (K. Ogrzewalla, M. Piotrowski and G. Link, unpublished observations) (Table 1). Any of these components found in highly purified chloroplast transcription complexes could have a role in signaling processes that connect transcription to posttranscriptional processes, photosynthetic

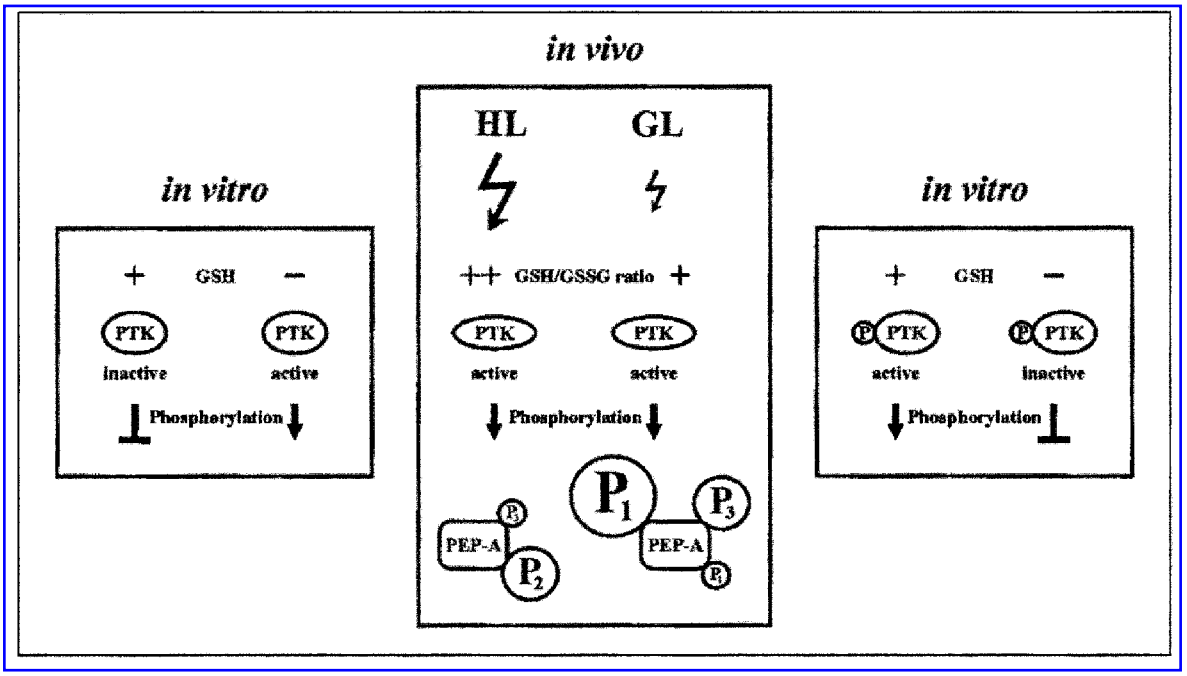

FIG. 1. Current view of redox regulation of chloroplast transcription. The focus of this scheme is on the role of the PTK, which is known to be under both SH-group redox control and phosphorylation control *in vitro* (10, 11). As is indicated in the outer panels, these two mechanisms act antagonistically, *i.e.,* the nonphosphorylated kinase is inhibited by GSH (**left**), whereas the phosphorylated form is activated (**right**). Recent work (9) provides evidence for differential transcription activity and PEP-A RNA polymerase phosphorylation following growth of mustard seedlings under high [high-light (HL)] as compared with moderate light intensity [growth-light (GL)]. Based on these data, a scheme predicting possible *in vivo* mechanisms involved in transcriptional redox regulation is shown in the central panel. As a consequence of the higher GSH/oxidized glutathione (GSSG) ratio in HL compared with GL chloroplasts, the latter would be expected to contain a more active PTK. This results in efficient PEP-A phosphorylation at a site, named P_1, which is responsible for the observed lower transcription activity under GL conditions (9). Although in HL chloroplasts (to a lesser extent) PTK seems to be active as well, it reveals a different substrate preference. P_2, rather than P_1, is now the preferred phosphorylation site. Unlike P_1, this site is also accessible (though less effectively) in GL chloroplasts, and the converse is true for a third site, named P_3, which is more accessible in GL than HL conditions. The phosphorylation state at the P_1 site seems to determine the overall activity of the PEP-A transcription complex, although it is conceivable that P_2/P_3 phosphorylation state may be involved in (gene- and stage-) specific regulation of plastid transcription. The details of the underlying mechanisms remain to be identified, but can be envisaged to include differential conformation and interaction partners of PTK under variable redox condititions.

TABLE 1. ASSIGNMENT OF PEP-A POLYPEPTIDES BY MASS SPECTROMETRY

Size (kDa)	*Tentative protein*	*GenBank Identifier (gi)*
140	β″ core subunit	5725463
110/107	β core subunit	563343
75/72	β′ core subunit	5457428
38*	α core subunit	7388101
38*	Plastid transcription kinase PTK (cpCK2α)	17977867
36	RNA-binding protein	2765081
29	Annexin-like protein	3785997
26	Fe-superoxide dismutase	15237281

RNA polymerase was purified from chloroplasts of 5-day-old mustard (*Sinapis alba*) seedlings. Polypeptides were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by MALDI and/or ESI-QTOF mass spectrometry and database searches. Proteins defined by gi 3785997, 2765081, and 15237281 were from *A. thaliana*; all others were from *S. alba.*

*The cpCK2α polypeptide comigrates with the α core subunit and is present in substoichiometric amounts (61) (K. Ogrzewalla, M. Piotrowski, and G. Link, unpublished observations).

electron flow, or both. For instance, RNA-binding redox-responsive proteins are well known factors in posttranscriptional regulation of chloroplast gene expression (17, 43, 44), and it has been established in other systems that proteins acting at the posttranscriptional level can have an additional regulatory effect also on transcription. Plant annexins are phospholipid-binding and often membrane-"anchoring" proteins (16), and one member of this class was recently shown to counteract oxidative stress upon transformation of an *oxyR*-deficient *E. coli* strain (26).

Reactive oxygen species, including hydrogen peroxide, are an inevitable consequence of photosynthetic electron flow within the context of the living plant cell, and effective scavenging mechanisms have evolved (8, 12, 22, 53, 85). Hydrogen peroxide has been found to affect chloroplast *in organello* transcription (T. Pfannschmidt, C. Stansen, and G. Link, unpublished observations), suggesting a functional role also for the polymerase-associated Fe-SOD, which may act both as a scavenger in the detoxification of reactive oxygen species and as a sensor for redox regulation of transcription. An increasing number of dual-function chloroplast proteins have recently been identified (21, 65), and both the Fe-SOD and the fructokinase associated with the PEP-A transcription complex may fall within this category. In this respect, it is notable that the flavoprotein ferredoxin-$NADP^+$ reductase is released from the thylakoids into the stroma under oxidative stress conditions, with an associated change from an NADPH-producing to an NADPH-consuming enzyme form (55). In view of these data, and other known paradigms (24, 64), it is tempting to speculate that the PTK may have distinct functions depending on its "free" or polymerase-bound state, and it will be important to define the interaction partners for this redox-regulatory chloroplast protein. In order to make progress in this direction, one of the immediate goals to achieve has been to clone and overexpress the catalytic kinase polypeptide. This recent work will be briefly discussed below (K. Ogrzewalla, Ph.D. thesis, University of Bochum, 2001; K. Ogrzewalla, M. Piotrowski, S. Reinbothe, and G. Link, manuscript in preparation).

CLONING OF THE cDNA FOR A PUTATIVE CHLOROPLAST CK2 KINASE

The PTK transcription kinase from mustard chloroplasts was purified and biochemically characterized (10, 11), resulting in its classification into the so-called CMGC group of protein kinases (73). This group includes mostly nucleocytosolic members, many of which represent terminal components of signaling chains that phosphorylate (nuclear) transcription factors (62). A well known member is CK2, which can act as a transcriptional regulator in both animal and yeast (25), as well as in plant systems (41).

CK2-type kinase activity is known to exist also in chloroplasts (34). Identified phosphoacceptors include the photosynthetic proteins CP29 (77) and the β subunit of the ATP synthase (35). Taking into account the biochemical similarity of the PTK with (nucleocytoplasmic) CK2 kinases (10, 11) has enabled homology-based strategies for cloning of the gene for the catalytic PTK component, and allowed the study of the recombinant protein in comparison with the authentic chloroplast enzyme.

If a nuclear gene for (the catalytic α polypeptide of) a plastid-localized CK2 existed, the derived protein should reveal both an N-terminal transit peptide and conserved CK2α elements. By searching the plant databases, an *Arabidopsis* EST was detected that could potentially specify a protein defined by this strategy. By using primers derived from this EST, a PCR product was amplified from a mustard cDNA library and then used as a probe, resulting in the isolation of a full-size cDNA from this library. BLAST searches (4) and multiple alignments of the derived protein with (nucleocytoplasmic) CK2 sequences from both animal and plant species indicated that this mustard cDNA clone contained the coding region for a mature CK2α protein. In addition, it revealed an N-terminal extension indicative of chloroplast transit peptides, *i.e.,* it was rich in serine and threonine residues and contained many positively charged but only few acidic amino acids (68) (Fig. 2).

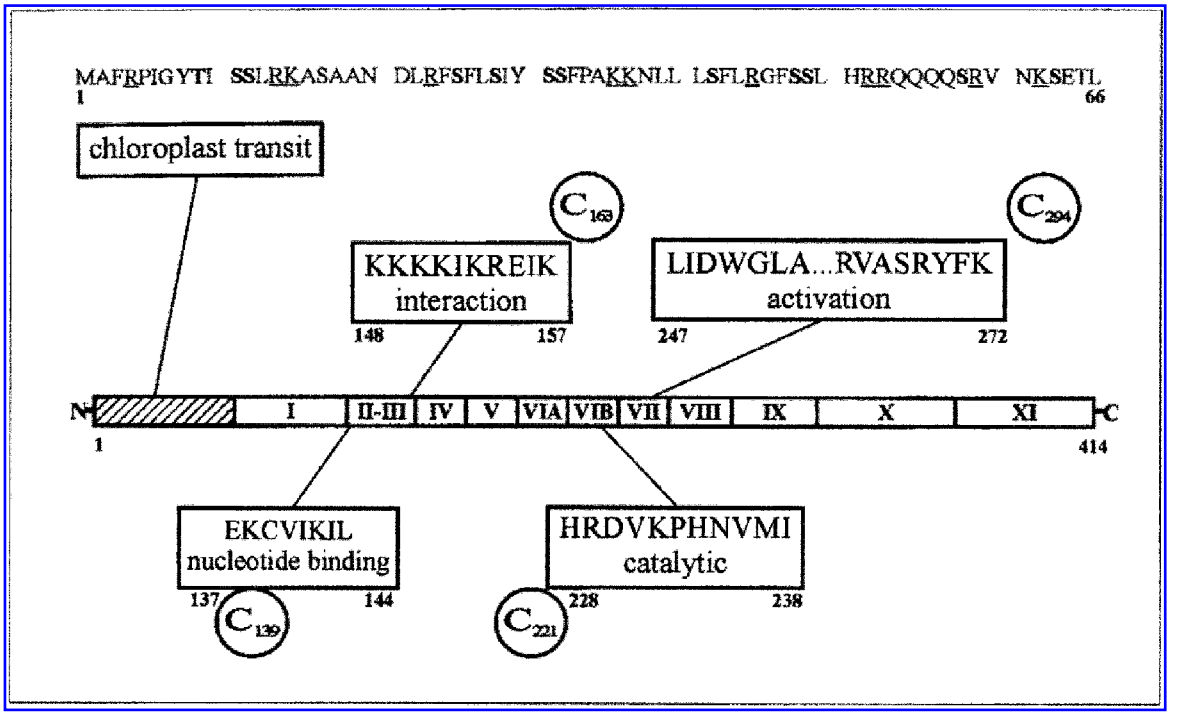

FIG. 2. Sequence characteristics of the cloned cpCK2α polypeptide from mustard. The CK2 domains I–XI (29, 62) are given by roman numerals. The hatched N-terminal region contains the transit peptide, details of which are provided on an extra line. Typical features include the high contents of serine and threonine residues (bold) and of positively charged amino acids (underlined). Indicated regions on the main line have been functionally defined in nucleocytoplasmic CK2α, including the putative catalytic loop, the nucleotide-binding region, as well as regulatory regions known to contain determinants activation and protein–protein interaction (52). The four cysteine residues present in the cpCK2α polypeptide that are potential sites for SH-group redox regulation are marked (C followed by residue number).

In the course of this study (K. Ogrzewalla, Ph.D. thesis, University of Bochum, 2001; K. Ogrzewalla, M. Piotrowski, S. Reinbothe, and G. Link, manuscript in preparation), further evidence was obtained for the existence of a nuclear-encoded chloroplast protein from mustard (*Sinapis alba* L.) that can be assigned as a CK2α-type protein kinase on the basis of the following criteria. (a) The gene product synthesized *in vitro* by coupled transcription–translation was found to be imported into isolated chloroplasts as a precursor, followed by processing to a size expected for the mature protein. (b) The bacterially overexpressed and purified recombinant protein had biochemical characteristics typical of CK2α. (c) The authentic chloroplast protein was detected as a component of the organellar transcription apparatus both by antibodies raised against the recombinant protein and by mass spectrometry.

The cloned recombinant cpCK2α protein was shown to resemble the authentic chloroplast PTK in several respects. Both enzyme preparations are capable of using ATP as well as GTP as a phosphodonor. They both are inhibited by the polyanion heparin and the group-specific kinase inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (10), and the latter was found also to severely affect *run-on* transcription in isolated chloroplasts (T. Pfannschmidt, K. Ogrzewalla, and G. Link, unpublished observations). Both PTK and recombinant cpCK2α seem to act independently of second messenger molecules, and both are capable of using plastid sigma factor(s) and other RNA polymerase-associated proteins as phosphorylation substrates (10) (K. Ogrzewalla, M. Piotrowski, S. Reinbothe, and G. Link, manuscript in preparation). Finally, both PTK and cpCK2α activities are negatively affected *in vitro* by the presence of GSH, whereas other reducing reagents, such as β-mercaptoethanol and dithiothreitol, seem to have little effect (11, 47) (K. Ogrzewalla, Ph.D. thesis, University of Bochum, 2001). Together, these data suggest that the cloned recombinant protein representing cpCK2α closely mimics the catalytic component of the PTK that is associated with the PEP-A polymerase (Fig. 2).

In view of the close physical and functional similarities between the cloned cpCK2α polypeptide and the authentic PTK kinase moiety of chloroplast RNA polymerase PEP-A, it seems reasonable to suggest that it is this CK2α-type activity that is responsible for phosphorylation and redox control of the PEP-A transcription system (10, 11). As it is available in cloned and overexpressed form, this gene product now lends itself to being used in protein–protein interaction studies, as well as being a target for mutagenesis and functional analyses of chloroplast transcription. Such combined biochemical and molecular-genetics approaches can be expected to provide the necessary power for a full clarification of the redox signaling mechanisms that connect photosynthetic electron transport with gene expression at a transcriptional level. The usage of mutant lines, including over- and underexpressors, *knockouts,* and RNAi lines will be valuable in

dissecting this system further. Functional reconstitution, interaction cloning, and related strategies will help advance our understanding of redox signaling across compartmental boundaries of plant cells as well. It thus seems that the recent identification and cloning of the redox-responsive PTK is only the beginning, rather than the end, of a continuing story.

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ABBREVIATIONS

AGF, AAG-box binding factor; CK2, casein kinase 2; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ESI-QTOF, electrospray ionization quadruple time of flight; Fe-SOD, iron-superoxide dismutase; GSH, reduced glutathione; MALDI, matrix-assisted laser desorption/ionization; NEP, nuclear-encoded phage-type plastid RNA polymerase; PEP, bacterial-type plastid RNA polymerase with core subunits encoded by organellar genes; PEP-A (cp-pol A), form A multisubunit plastid RNA polymerase; PEP-B (cp-pol B), form B multisubunit plastid RNA polymerase; PSI and PSII, photosystem I and II, respectively; PTK, plastid transcription kinase; SH, thio; SLFs, sigma-like factors.

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