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PLANT THIOREDOXIN SYSTEMS REVISITED

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■ **Abstract** Thioredoxins, the ubiquitous small proteins with a redox active disulfide bridge, are important regulatory elements in plant metabolism. Initially recognized as regulatory proteins in the reversible light activation of key photosynthetic enzymes, they have subsequently been found in the cytoplasm and in mitochondria. The various plant thioredoxins are different in structure and function. Depending on their intracellular location they are reduced enzymatically by an NADP-dependent or by a ferredoxin (light)-dependent reductase and transmit the regulatory signal to selected target enzymes through disulfide/dithiol interchange reactions. In this review we summarize recent developments that have provided new insights into the structures of several components and into the mechanism of action of the thioredoxin systems in plants.

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INTRODUCTION

Thioredoxins are small proteins with a redox active disulfide bridge present in the characteristic active site sequence-Trp-**Cys**-Gly-Pro-**Cys**-. They have a molecular mass of approximately 12 kDa and are universally distributed in animal, plant, and bacterial cells. In their reduced form they constitute very efficient protein disulfide oxido-reductases. Initially described as hydrogen carriers in ribonucleotide reduction in *Escherichia coli*, they have been found to serve as electron donors in a variety of cellular redox reactions (65). In oxygenic photosynthetic cells thioredoxins were recognized as important regulatory proteins in carbon assimilation (16, 22).

Only two types of thioredoxins have been found in bacteria and animal tissues, but plant tissues contain multiple forms: two in chloroplasts, one in the cytoplasm, and one in mitochondria. Although all thioredoxins are of comparable size and appear to have very similar redox properties, they fulfill specific functions. This specificity is due to structural complementarity, which allows specific interaction between the different thioredoxins and their respective target proteins.

Depending on their intracellular location, thioredoxins are reduced by a different electron donor system. Thioredoxins in nonphotosynthetic tissue and in the cytosol of photosynthetic cells are reduced with electrons from NADPH via the NADP/thioredoxin system, whereas the chloroplast thioredoxins of plants and eukaryotic algae and the thioredoxins of oxygenic photosynthetic prokaryotes are reduced via the ferredoxin/thioredoxin system with elecrons provided by photosynthetic electron transport.

In this review we describe recent developments, including new structural information, that provide a better understanding of the structure and function of thioredoxin systems in plants and present some information on new developments and on proteins with structural or functional similarity to thioredoxins. Different aspects of the thioredoxin systems in plants have been discussed in several recent publications (7, 17, 18, 20, 21, 44, 49, 49a, 73, 95a, 128, 135a, 136, 140, 166a). For detailed information on earlier developments we refer the reader to former reviews in this series (16, 65).

CYTOPLASMIC AND MITOCHONDRIAL NADP-DEPENDENT THIOREDOXIN SYSTEMS

Plant cells, like bacterial and animal cells, contain a cytosolic thioredoxin system that is dependent on NADPH for its reduction (48, 151). In this cytosolic system,

the reducing power of NADPH is transferred to thioredoxin via a flavoprotein—the NADPH:thioredoxin reductase (NTR).[1]

Genomic Organization, Structure, and Functions of NADPH:Thioredoxin Reductase

A cDNA coding for this enzyme has been isolated from *Arabidopsis thaliana*. The deduced primary structure of the protein displays significant homology to *E. coli* NTR (75). Southern blot experiments indicate that at least two different genes coding for NTR are present in *Arabidopsis*, with the total number of genes being low. Following the isolation of cDNAs coding for NTR, the crystal structure of the recombinant protein has been elucidated. The enzyme is a homodimer with subunits of about 35 kDa. Each of the subunits possesses two subdomains, a central NADPH binding domain, and a domain constituted by the N and C termini, which binds FAD. In addition, each subunit contains a redox active disulfide in a **C**AT**C** motif (35). Much as in the *E. coli* enzyme, the redox active site is facing the isoalloxazin ring of the flavin bringing the redox entities in close contact, thereby facilitating electron transfer from FAD to the disulfide. Comparison of the crystal structures of the *E. coli* and *Arabidopsis* proteins suggests that in order to accommodate a thioredoxin molecule, the reductase must undergo a structural change, likely to occur at a hinge situated at the junction between the FAD and NADPH subdomains. Plant NTR appears to be much more closely related to the prokaryotic type enzyme (prototype *E. coli*) than to the mammalian enzyme that contains an N-terminal extension as well as an essential selenocysteine (53). Like many other flavoproteins, the *Arabidopsis* NTR also possesses diaphorase activity and it is able to reduce quinone and nitrocompounds (11, 101).

Genomic Organization, Structure of the Thioredoxin *h* Component

The cytosolic thioredoxin has been termed thioredoxin *h*, as heterotrophic, since it was originally found in nonphotosynthetic tissues (79, 151). A number of thioredoxin *h* cDNA sequences have been isolated from both nonphotosynthetic and photosynthetic tissues, most notably in *Arabidopsis* (125). All deduced sequences share a rather high degree of homology, except that the canonical active-site motif (W**C**GP**C**) is modified to W**C**PP**C** in several members. At leastfive genes coding

[1] Abbreviations: DTT, dithiothreitol; FBPase, fructose 1,6-bisphosphatase; Fd, ferredoxin; FTR, ferredoxin:thioredoxin reductase; GSH, reduced glutathione; G6PDH, glucose 6-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NADP-MDH, NADP-dependent malate dehydrogenase; NTR, NADPH:thioredoxin reductase; PRK, phosphoribulokinase; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; SBPase, sedoheptulose 1,7-bisphosphatase; Trx, thioredoxin.

for thioredoxin *h* are present in *Arabidopsis*. Primary structure analyses indicate that all these proteins are closely related from an evolutionary standpoint (73). Thioredoxin *h* sequences are slightly longer than the one of *E. coli*—the prototype prokaryotic thioredoxin—mostly owing to N- and/or C-terminal extensions. So far, the NMR solution structure of oxidized thioredoxin *h* from the photosynthetic green alga *Chlamydomonas reinhardtii* is the only one available (102). In agreement with primary structure analyses, the model indicates that plant thioredoxin *h* is much closer to human thioredoxin than to the *E. coli* type. Nevertheless, all thioredoxins share a common fold with a succession of the following secondary structural elements: $\beta 1$, $\alpha 1$, $\beta 2$, $\alpha 2$, $\beta 3$, $\alpha 3$, $\beta 4$, $\beta 5$, $\alpha 4$.

The thioredoxin *h* structure presents only one major difference compared to the models of other thioredoxins, namely an elongated $\alpha 1$ helix. The isolated protein has two specific properties that allow its identification: first, the presence of a conserved Trp residue (Trp13 in *Chlamydomonas*) that imparts peculiar UV spectral characteristics; and second, a marked instability when subjected to high temperature and slightly alkaline pH (150). Analysis of the intron positions suggests that there is a common ancestor for plant and green algal thioredoxin *h* genes (130).

Expression and Targets of Thioredoxin *h*

Although this area is not as well documented by in vitro studies as the one dealing with chloroplast thioredoxins, a number of functions have been proposed for the cytosolic thioredoxin system. It has been suggested to act as a reducing system participating in the mobilization of protein reserves during seed germination, and these reactions may possibly be linked to the reduction of storage proteins and enzyme inhibitors and also mediated by a redox-dependent protease, thiocalsin (8, 82). It has also been proposed that thioredoxin *h* may play a role in sulfate assimilation and in conferring resistance to hydrogen peroxide, similar to what occurs in yeast or mammalian cells since some of the *Arabidopsis* isoforms complement yeast mutants in these two processes (105).

Several recent reports indicate that in cyanobacteria and plants thioredoxin is involved in the detoxification of H_2O_2 via the enzyme thioredoxin peroxidase, or peroxiredoxin. In cyanobacteria, the reduction of H_2O_2, alkyl hydroperoxides and *t*-butyl hydroperoxide was shown to be significantly increased by dithiol reagents in vitro or by light in vivo, which suggests that in cyanobacteria the activity of thioredoxin peroxidase is coupled to the photosynthetic electron transport system (155, 168). In the cytosol, peroxiredoxin might be a target of thioredoxin *h*, since this enzyme has been isolated as an in vivo target of plant thioredoxin *h* in yeast by stabilizing a mixed disulfide intermediate (158). Structural and functional equivalents of the yeast and mammalian peroxiredoxin are present in plant cells. Peroxiredoxins of the two-Cys type, believed to function as antioxidants, have also been characterized in plants such as *Hordeum* and *Spinacia* (5).

Although no 3-D structure of plant peroxiredoxins is available yet, the crystal structure of the human enzyme has been solved. Figure 1 (see color section) shows a structural composite of the thioredoxin cytosolic system comprised of NADPH, the *Arabidopsis thaliana* NTR monomer, *Chlamydomonas reinhardtii* thioredoxin (Trx) *h*, and human peroxiredoxin.

Thioredoxin *h* is also a potent regulator of membrane-bound, receptor-like kinases in plants (13). Expression studies (essentially northern and western blots) indicate that thioredoxin *h* proteins are present in many plant organs and that their expression is developmentally regulated. Transcription of the gene in *Chlamydomonas* was found to be under the control of light and a circadian rhythm (87). Furthermore, it is strongly activated after addition of heavy metals, such as Cd^{2+}, to algal cells (86).

Thioredoxin *h* as a Messenger in Plants?

Several experiments suggest that thioredoxin *h* could act as a messenger protein in plants. First, thioredoxin *h* was found to be one of the major proteins in the phloem sap of rice (72). Furthermore, it is detected not only in the phloem sap of monocots such as rice and wheat, but also in dicots such as *Ricinus* and *Cucurbita* (138). Since mature phloem cells are enucleated, the site of mRNA synthesis was located to companion cells, and transcripts coding for thioredoxin *h* were transferred to the sieve tubes (132). In addition, microinjection of recombinant thioredoxin *h* demonstrated that the protein itself has the capacity to mediate its own cell-to-cell transport through plasmodesmata (71). It thus appears that thioredoxin *h* is a mobile element in plants and could act as a message carrier, presumably by regulating membrane receptors, as described for the membrane-bound, receptor-like kinase of *Brassica* (13).

Thioredoxin Systems in Mitochondria

It is now well established that animal cells contain the full complement for a functional thioredoxin system (selenium-containing NTR and thioredoxin) in mitochondria (100, 111). Early reports indicated that plant mitochondria also contain at least one thioredoxin (12, 92), but no cDNA sequence has been unambiguously shown to code for a transit peptide that targets the mitochondrial compartment. Similarly, no plant mitochondrial NTR sequence has yet been isolated. Although more solid evidence for the presence of a functional thioredoxin system in plant mitochondria is still lacking, these thioredoxins can be reduced in vitro by lipoic acid generated via the 2-oxoacid dehydrogenase complex, as in animal mitochondria (24). A possible role for plant mitochondrial thioredoxins could be to activate/stabilize the 2-oxoacid dehydrogenase complex present in this organelle (23). Another possible role for mitochondrial thioredoxin could be the redox regulation of the cyanide-resistant alternative oxidase in the plant electron transport chain (124, 157a).

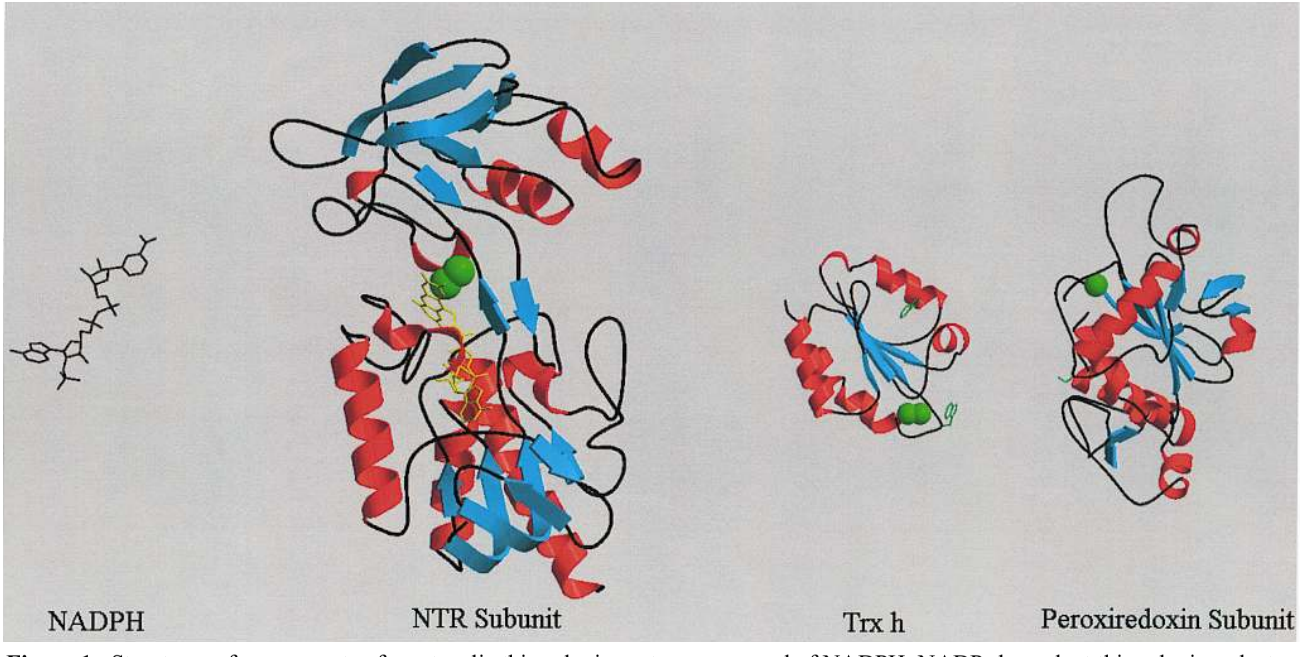

Figure 1 Structures of components of a cytosolic thioredoxin system composed of NADPH, NADP-dependent thioredoxin reductase (NTR) thioredoxin *h* (Trx h) and peroxiredoxin as target protein. The sulfur atoms of the active sites are shown as green balls and the FAD of the NTR, is represented in yellow.

THE CHLOROPLASTIC, FERREDOXIN-DEPENDENT THIOREDOXIN SYSTEM

In chloroplasts and cyanobacterial cells thioredoxins are reduced by way of light. Light-driven photosynthetic electron transport produces reduced ferredoxin (Fd), which serves as electron donor for ferredoxin:thioredoxin reductase (FTR). This enzyme in turn reduces thioredoxins, which interact with target proteins to reduce and activate biosynthetic, or deactivate catabolic pathways. This system of enzyme regulation is the ferredoxin/thioredoxin system (16, 17). Significant progress has been made in understanding this system with respect to function and especially to structure, inasmuch as the crystal structure of each component protein member is now known (Figure 2; see color section).

Ferredoxin

Fd, the soluble, stromal [2Fe-2S] protein, is a negatively charged electron donor that interacts quite strongly with its electron acceptor proteins to form electrostatically stabilized complexes. It was demonstrated that spinach ferredoxin and FTR form a high-affinity, 1:1 complex (61). Negatively charged residues involved in this complex formation have been located in spinach and *Chlamydomonas reinhardtii* ferredoxin (37, 77). A crystal structure is now available for spinach ferredoxin at 1.7 Å resolution (10). Although there are positively charged residues in the putative ferredoxin interaction domain of spinach FTR, no experiments have yet shown their involvement in protein-protein interaction.

Ferredoxin:Thioredoxin Reductase

FTR is the central enzyme of the ferredoxin/thioredoxin system; it converts a photosynthetic "electron signal" received from reduced ferredoxin to a "thiol signal" that is transmitted to thioredoxin. Unlike cytoplasmic NTR, this chloroplast stromal enzyme is a yellowish-brown [4Fe-4S] protein, composed of two dissimilar subunits, designated catalytic and variable. The catalytic subunit of the enzyme from different photosynthetic organisms has a constant size of about 13 kDa, whereas the variable subunit ranges between 8 and 13 kDa (41, 69).

Gene and Protein Sequences In plants both subunits of FTR are nucleus-encoded. Two slightly different cDNAs have been sequenced for both subunits of spinach FTR, suggesting the presence of two genes (46, 50). In *Porphyra purpurea*, a red alga (121), and in *Guillardia theta*, a cryptomonad (40), a single gene for the catalytic subunit was found in the chloroplast genome.

The variable subunits show pronounced sequence diversity, with only 46% to 60% identity within the eukaryotes and 33% to 40% between eukaryote and prokaryotes. The most striking difference is the extended N terminus, present in all three known eukaryotic subunits, but absent in the prokaryotic ones. In spinach

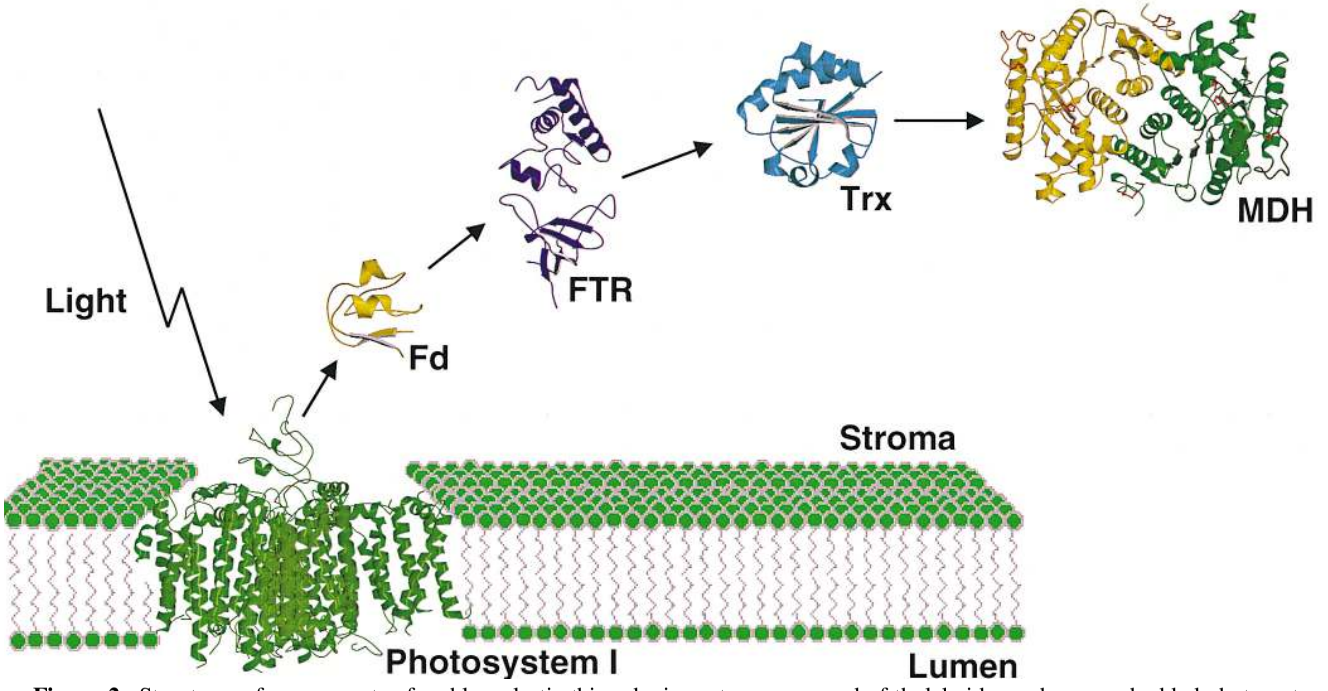

Figure 2 Structures of components of a chloroplastic thioredoxin system composed of thylakoid membrane embedded photosystem I, ferredoxin (Fd), ferredoxin:thioredoxin reductase (FTR), thioredoxin (Trx) and NADP-dependent malate dehydrogenase (MDH) as target protein. Modified figure reprinted with permission from (78). Copyright 1999 American Chemical Society.

FTR, this N-terminal extension was found to be unstable; it was degraded to discrete shorter peptides (157) that exhibit no difference in functional properties.

By contrast, the catalytic subunits have a highly conserved primary structure. Among the strictly conserved residues are seven Cys, six of which are organized into two CPC and one CHC motifs. These six Cys are the functionally essential residues that constitute the redox active disulfide bridge and ligate the Fe-S cluster. Cluster ligation does not follow known consensus motifs, but shows a new arrangement with the following fingerprint: **C**PCX$_{16}$**CP****C**X$_8$**C**HC (cluster ligands are in bold). In spinach FTR Cys54 and Cys84 form the active-site disulfide. Cys54 is accessible to solvent, whereas Cys84 is protected. The four remaining cysteines, Cys52, Cys71, Cys73, and Cys82, are ligands to the iron center. This arrangement places the redox-active disulfide bridge adjacent to the [4Fe-4S] cluster (30).

The genomes of two archaebacteria, *Archaeoglobus fulgidus* (81) and *Methanobacterium thermoautotrophicum* (146), both contain a gene coding for a protein with a striking resemblance to the catalytic subunit of FTR. Although the overall identities between the archaebacterial proteins and the photosynthetic FTR are rather low (25%–35%), the residues found to be essential for the functioning of FTR, the CXC motifs, are conserved. However, no functions are known for these proteins in the archaea. Nonetheless, the striking structural similarities suggest that the catalytic subunit of photosynthetic FTR might be derived from an ancient precursor protein whose function has been adapted during evolution.

The genes coding for the two subunits of FTR from spinach (50) and *Synechocystis* (142) have been introduced into expression vectors. These constructs yielded soluble, heterodimeric recombinant enzymes containing the correctly inserted Fe-S cluster, demonstrated by spectral properties and enzyme activity.

Crystal Structure Recombinant *Synechocystis* FTR has recently been crystallized and its structure solved (34, 35a). The variable subunit has an open β-barrel structure and the catalytic subunit is essentially α-helical. The two subunits, with the catalytic subunit sitting on top of the variable subunit, form an unusually thin molecule, with the shape of a concave disc measuring only 10 Å across its center. In this center are located the functionally important structures, the cubane [4Fe-4S] cluster and, in close proximity, the redox active disulfide bridge. The Fe-S cluster is accessible from one side of the concave disk, the ferredoxin docking side, whereas the disulfide bridge is accessible from the opposite side, the thioredoxin docking side. This allows simultaneous docking of the electron donor and electron acceptor proteins—a prerequisite for the proposed reaction-mechanism.

The docking sites are also well adapted for their function: The ferredoxin docking site contains four positively charged residues for specific interaction with negatively charged ferredoxin, whereas the thioredoxin docking site contains mainly hydrophobic residues, making it compatible for interaction with different thioredoxins.

Surprisingly, the shape of the variable subunit shows striking similarities to PsaE, the ferredoxin-binding protein of photosystem 1, with the SH3 domain and with GroES, although there are no sequence similarities with either protein (34).

Chloroplast Thioredoxins

Chloroplasts of plants and algae contain two different types of thioredoxins, an *f*- and an *m*-type. They have different phylogenetic origins (58) and different target-enzyme specificities. Thioredoxin *f* was originally described as the activator protein for spinach stromal fructose 1,6-bisphosphatase (FBPase) and thioredoxin *m* for NADP-malate dehydrogenase (NADP-MDH) (16, 17, 73). Although a clear distinction between the two Trx types has not always been obtained in experiments under in vitro conditions, these two types can clearly be distinguished by their primary structures.

Structure of Thioredoxin m This thioredoxin, present in oxygenic prokaryotes, algae, monocots, and dicots, strongly resembles the thioredoxin from prokaryotes, both heterotrophic and anoxygenic photosynthetic. In plants and green algae this Trx is nuclear-encoded, but in red algae its gene was found in the chloroplast genome (121, 123), which supports its bacterial endosymbiotic origin. Functionally and structurally, it strongly resembles bacterial thioredoxins, which can be used interchangeably. Thioredoxins of the *m*-type are, therefore, considered bacterial-type thioredoxins. A comparison of the primary structures of all *m*-type thioredoxins available in the databases shows that they are clearly related. However, there is less sequence similarity than for the *f*-type proteins. Recombinant *m*-type thioredoxins have been reported from *Chlamydomonas* (84), spinach (139), and pea (90). For the *Chlamydomonas* protein, an NMR analysis showed that its secondary structure and general protein folding is similar to its *E. coli* counterpart (84). For the recombinant spinach thioredoxin *m*, the crystal structure has been solved in the oxidized and reduced state at 2.1 and 2.3 Å resolution, respectively (27). As already found for *Chlamydomonas* thioredoxin *m*, its structure is very similar to that of *E. coli* thioredoxin (80), with nearly identical secondary structure. The surface around the active-site Cys residues largely resembles its *E. coli* counterpart (Figure 3, center and bottom panels; see color section). This corroborates biochemical evidence that the proteins are functionally interchangeable. There is little difference in conformation of the active site between oxidized and reduced thioredoxin *m*; however, some slight structural changes in the main chain conformation of the active site render the solvent-exposed Cys (Cys37) more accessible upon reduction (G Capitani, Z Markovic-Housley, G del Val, M Morris, JN Jansonius, P Schürmann, Crystal structures of two functionally different thioredoxins in spinach chloroplasts. In preparation).

Structure of Thioredoxin f There are fewer sequences known for this Trx, which is considered to be restricted to photosynthetic eukaryotic organisms. Thioredoxin *f* is nuclear-encoded and apparently derived from the eukaryotic host during

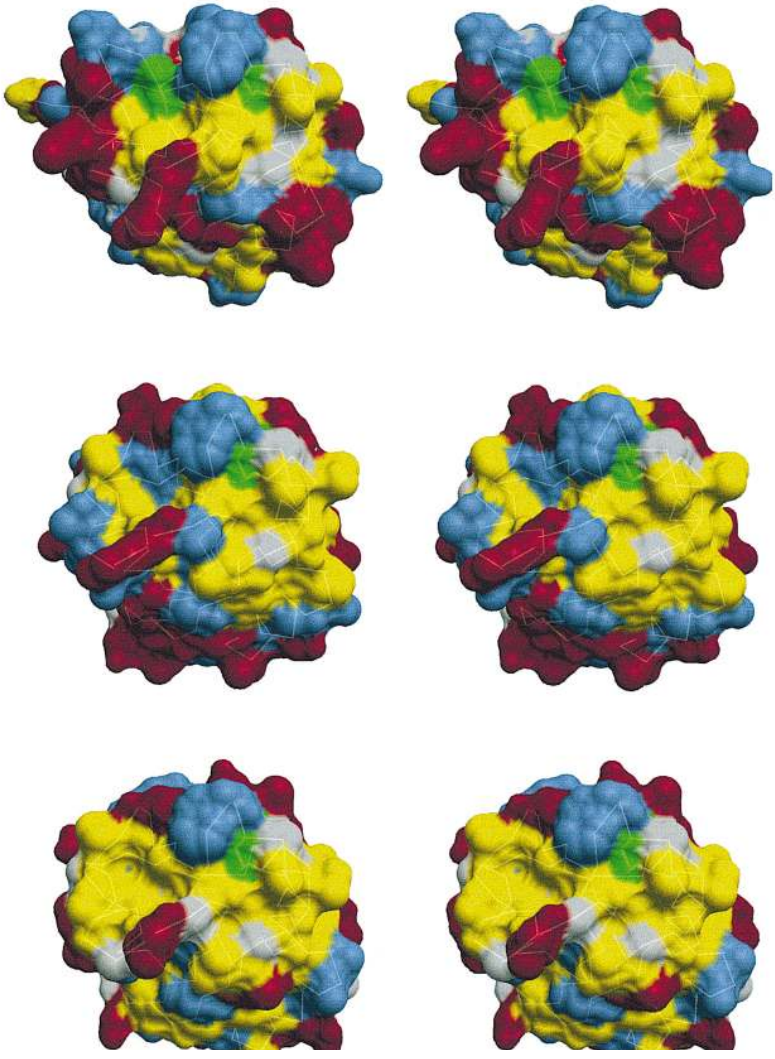

Figure 3 Surface stereo views of spinach chloroplast thioredoxin *f (top)*, thioredoxin *m (center)* and thioredoxin from *E. coli (bottom)*. The colors represent the following residue types: green–Cys; red–charged (+ or -); blue–polar; yellow–apolar; gray–backbone.

evolution. *f*-Type thioredoxins are highly conserved, with 75% to 90% residue identity in the mature protein from dicots and with 60% to 67% residue identity between dicots and monocots (140). Owing to additional amino acids at the N terminus, they are slightly longer than other Trx types. Interestingly, the C-terminal part of the sequence resembles classical animal thioredoxin in containing a third, strictly conserved Cys (Cys73 in spinach). The crystal structure of two different forms of recombinant thioredoxin *f* has been solved (G Capitani, Z Markovic-Housley, G del Val, M Morris, JN Jansonius, P Schürmann, Crystal structures of two functionally different thioredoxins in spinach chloroplasts. In preparation), a long form closely resembling the in vivo form (1) and an N terminally truncated short form (38). Both structures are essentially identical aside from the N terminus, which contains an additional α-helix in the long form. Although the overall structure of thioredoxin *f* does not differ markedly from a typical thioredoxin, its surface topography is distinct from that of other Trxs (27) (Figure 3). Positive charges surrounding the active site may be instrumental in orienting thioredoxin *f* correctly with its target proteins. Hydrophobic residues, also prominent in this area, may be more important in the nonspecific interaction with FTR that reduces various thioredoxins. A striking difference is the presence of the third Cys exposed on the surface (Cys73 in spinach), 9.7 Å away from the accessible Cys of the active-site dithiol (Cys46). As mentioned, this third Cys is conserved in all *f*-type thioredoxins. The structural analysis also shows that the active-site Cys closest to the N terminus (Cys46 in spinach) is exposed, whereas its partner (Cys49 in spinach) is buried, confirming experiments which indicated that Cys46 is the attacking nucleophile in the reduction of the target protein's disulfide (14).

Specificity of Thioredoxins Chloroplast thioredoxins show selectivity in their interaction with target enzymes when tested under conditions approaching their in vivo situation. The stromal Calvin cycle enzymes FBPase (51), SBPase (166), PRK (165), as well as Rubisco activase (169) and CF_1 (141), are exclusively or most efficiently activated by thioredoxin *f*. NADP-MDH, although originally reported to specifically interact with thioredoxin *m*, was shown to be even more efficiently activated by thioredoxin *f* (51, 64). G6PDH (glucose 6-phosphate dehydrogenase) of the stromal oxidative pentose phosphate pathway, on the contrary, interacts specifically with thioredoxin *m* (162). These observations suggest that, at least as far as carbohydrate metabolism is concerned, reduced thioredoxin *f* functions primarily in enzyme activation (i.e. enhancing the rate of biosynthesis), whereas reduced thioredoxin *m* acts mainly in enzyme deactivation (i.e. decreasing the rate of degradation).

The specificity of thioredoxin *f* raises the question of which structural features are possibly responsible for it. An answer has been sought using site-directed mutagenesis of thioredoxins. The results confirm the importance of the third Cys (38) and of positive charges on the contact surface of thioredoxin *f* (51, 83, 103), and can essentially be reconciled with the recent structural model of Trx *f* (27).

Mechanism of Thioredoxin Reduction by FTR

There is clear evidence from experiments with isolated chloroplasts (33) and purified protein components (42) that FTR converts the photosynthetic electron transport signal, provided by reduced ferredoxin, to a thiol signal, detected with the fluorescent label mono-bromobimane, which then appears sequentially in thioredoxin and the target enzyme. Based on recent spectroscopic (148, 149) and structural (30, 34) analyses, it has become possible to propose a reaction mechanism of how FTR might achieve this conversion. To obtain the two electrons needed for reduction of the active-site disulfide bridge FTR mediates two consecutive one-electron transfers from reduced ferredoxin and stabilizes the one electron–reduced reaction intermediate. Characterization of the Fe-S cluster in native and chemically modified FTR (148, 149) provided evidence for a cubane $[4Fe\text{-}4S]^{2+}$ cluster in the oxidized enzyme, which cannot be reduced, but oxidized (redox potential for the couple $[4Fe\text{-}4S]^{3+/2+} = +420$ mV). The postulated reaction mechanism features a new biological role for this Fe-S cluster that catalyzes the reduction of a disulfide bond by establishing a transient $[4Fe\text{-}4S]^{3+}$ cluster coordinated by five cysteinates (149), with a cluster Fe atom as the fifth ligand (34, 35a).

Another aspect of the mechanism of thioredoxin-mediated enzyme regulation concerns the redox potentials of the component proteins in the regulatory chain. The oxidation-reduction properties of the different regulatory disulfides influence the activation state of the individual target enzymes. To understand the kinetics of activation, one needs to know the oxidation/reduction midpoint potentials (E_m) of the disulfide/sulfhydryl couples involved. Over the past several years, midpoint potentials of the members of the regulatory chain and several target enzymes have been obtained by cyclic voltammetry (131), the fluorescent probe monobromobimane, and activity measurements (60, 62, 63, 70, 120). Despite some relatively small differences for the E_m values determined by the different groups, there emerges a general agreement as to which members of the regulatory chain have the most positive E_m values and which the most negative. The more negative midpoint potential of the disulfide of spinach FTR ($E_m = -320$ mV, pH 7) allows both thioredoxins (-290 mV for *f*; -300 mV for *m*) to become reduced. Target enzymes involved indirectly or directly in regeneration of the CO_2 acceptor, CF_1 (-280 mV) and PRK (-295 mV), have the most positive potentials. FBPase [-305 mV, pH 7, spinach, P Schürmann, M Hirasawa & DB Knaff, unpublished data, replacing a former erroneous E_m value of -330 mV (63)], and SBPase (-300 mV, pH 7, tomato), which together control the entry into the regenerative phase of the Calvin cycle and into starch synthesis, have a slightly more negative potential than thioredoxin *f*, whereas NADP-MDH has a significantly more negative potential (-330 mV, sorghum) that keeps this latter enzyme oxidized/inactive as long as there is no surplus of reducing equivalents (NADPH). The differences in redox potential suggest a sequential order in the activation of chloroplast enzymes in the light, with the most critical metabolic processes given priority. They further suggest that the redox equilibria reached will result in different degrees of activation.

These equilibria may then be modified by enzyme effectors (47) that would allow for fine-tuning of activity after the target enzymes are switched on by light.

Chloroplast Target Enzymes

The fact that the light-dependent increase in enzyme activity could be mimicked in vitro by sulfhydryl compounds like dithiothreitol (DTT) was traditionally taken as evidence of an involvement of reduced thioredoxin. However, final proof of a redox event involving thioredoxin needs experimental evidence for an absolute requirement and the demonstration of an accessible disulfide bridge on the target structure. The presence of a redox-active disulfide has been confirmed for most of the light-regulated enzymes and its involvement verified by site-directed mutagenesis. For some of the enzymes, three-dimensional models, based either on X-ray crystallography or homology modeling, have provided further insights into possible structural changes occurring due to reduction.

Comparison of primary structures reveals that there is no Cys-containing consensus motif present in most of the light-regulated target enzymes, although in some a **C**XXXX**C** sequence is the responsible element or part of it (Table 1). However, the two active cyst(e)ines can also be separated by many residues. For certain enzymes, particularly those also occurring as cytosolic isoforms (e.g. FBPase, MDH), the regulatory disulfide structures are located on extra loops or extensions, indicating that they were added during adaptation to photosynthetic function. These observations suggest that the adaptation of enzymes to light-mediated redox regulation arose multiple times during evolution.

Reduction of the regulatory disulfides of the target enzymes by thioredoxin proceeds, like thioredoxin reduction, with the formationof a transient heterodisulfide

TABLE 1 Regulatory site sequences of chloroplast target enzymes and their principal activating thioredoxin

Target enzyme	Plant	Regulatory site[a]	Activator
FBPase	Spinach	**Cys**155X$_{18}$**Cys**174Val *Val*Asn*Val*Cys179	Trx *f*
SBPase	Wheat	**Cys**52Gly*GlyThr*Ala**Cys**57	Trx *f*
PRK	Spinach	**Cys**16X$_{38}$**Cys**55	Trx *f*
ATP synthase (γ-subunit of CF$_1$)	Spinach	**Cys**199*Asp*lleAsn *Gly*Lys**Cys**205	Trx *f*
NADP-MDH	Sorghum	**Cys**24Phe*Gly*Val*Phe***Cys**29 **Cys**365X$_{11}$**Cys**377	Trx *f* (*m*)
G6PDH	Potato	**Cys**149*Arg*lle*Asp*Lys *Arg*GluAsp**Cys**157	Trx *m*
Rubisco activase	*Arabidopsis*	**Cys**392X$_{18}$**Cys**411	Trx *f*

[a]The regulatory Cys are in bold and additional conserved residues are in italics.

complex between the two reaction partners. The reactive Cys, which is the solvent-accessible residue closest to the N terminus of thioredoxin, cleaves the target disulfide by nucleophilic attack, thereby forming a covalently linked mixed disulfide. In a rapid second step, the second sulfhydryl, which is inaccessible to solvent, attacks the mixed disulfide to produce oxidized thioredoxin and reduced target enzyme.

Fructose 1,6-Bisphosphatase Stromal FBPase is one of the first enzymes in which activity was demonstrated to be clearly dependent on reduced thioredoxin *f* (16). Sequencing revealed the presence of an insert in the middle of the primary structure compared to its cytosolic isoform (118). This insert contains three Cys—**Cys**155X_{18} **Cys**174ValValAsnVal**Cys**179 in spinach—two separated by four hydrophobic residues and the third, Cys155, by 18 residues upstream toward the N terminus (Table 1). Cys174 and Cys179, present in a **C**XXXX**C** motif, were thought to constitute the redox active disulfide bridge (93). However, site-directed mutagenesis experiments indicated that all three Cys present in the insert are involved in regulation. The replacement of Cys155 results in a constitutively fully active enzyme, while the replacement of either of the remaining Cys (C174, C179) results in a partially active enzyme that still requires reduction by thioredoxin for full activity. These results suggest that Cys155 is an obligatory part of the regulatory disulfide, whereas the remaining two Cys (C174, C179) act interchangeably in constituting its bonding partner (74, 76, 126). Cys155 also forms the transient heterodisulfide bond during reduction by thioredoxin *f*, which indicates that it must be surface exposed (Y Balmer, P Schürmann, unpublished data). Interestingly, the region situated between Cys155 and Cys174 contains several negative charges (seven Asp and Glu in the pea sequence, with three of them highly conserved). Mutagenesis of this regulatory region suggests that these anionic charges are important for thioredoxin docking, presumably by establishing electrostatic interactions with positively charged, surface residues of thioredoxin *f* (129).

The structure of spinach chloroplast FBPase has been solved by X-ray crystallography at 2.8 Å resolution (159). The model shows that the three regulatory Cys are on a loop extending out of the core structure of the enzyme. Unfortunately, the definition of its crystal structure, probably due to its flexibility, was not sufficient to locate a disulfide bridge. Recent crystallographic analysis of the oxidized recombinant pea enzyme reveals a disulfide bond between Cys153 (Cys155 in spinach) and Cys 173 (Cys174 in spinach), whereas the third Cys in the loop is present as a free sulfhydryl, which is sufficiently close to form an alternate disulfide with Cys153 when Cys173 is altered by site-directed mutagenesis (29a).

Sedoheptulose 1,7-Bisphosphatase A substrate-specific SBPase is found only in oxygenic photosynthetic eukaryotes. It is unique to the Calvin cycle and has no cytosolic counterpart (117). Primary structures for this 76-kDa, homodimeric, nucleus-encoded chloroplast enzyme are known from *Arabidopsis* (164), *Chlamydomonas* (56), spinach (94), and wheat (119). In cyanobacteria, SBPase activity is contributed by a bifunctional FBPase/SBPase enzyme (153, 154).

Eukaryotic SBPase shows considerable overall sequence similarity with stromal FBPase; however, it lacks the regulatory Cys insert. The number of Cys varies, but four are found at strictly conserved positions in the N-terminal domain. The two most N-terminal Cys, in wheat arranged as a **Cys**52GlyGlyThrAla**Cys**57 motif (Table 1), have been shown to be involved in redox regulation. Mutation of these two Cys to Ser resulted in an active, redox-insensitive SBPase (43). As in stromal FBPase, the regulatory Cys residues of SBPase are not part of the catalytic site. Based on structural similarities between the two enzymes, the regulatory disulfide could be located on a flexible loop near the junction between the two SBPase monomers. Oxidation or reduction of the regulatory disulfide may alter the conformation of the homodimer and thereby change the activity of the enzyme (117). Unlike FBPase, this enzyme exhibits an absolute requirement for redox activation (106) and once reduced, its activity is modified further by stromal factors such as pH, $[Mg^{2+}]$, and metabolites (25, 118, 137).

Phosphoribulokinase A redox-regulated PRK is present as a 80-kDa homodimer in photosynthetic eukaryotes (113) and as a homotetramer in cyanobacteria (160). Each subunit contains four Cys residues at conserved positions, two near the N terminus and two near the C terminus. For the spinach enzyme the N-terminal pair, Cys16 and Cys55, were identified by chemical modification (115) and site-directed mutagenesis (98) as the regulatory cysteines forming an intramolecular disulfide bond in the oxidized/inactive enzyme. Both are located in the nucleotide binding domain of the active site where Cys55 may play a facilitative role in catalysis (112, 114) by binding the sugar phosphate substrate (98). This Cys forms the transient heterodisulfide with Cys46 of thioredoxin *f* during reductive activation (15). PRK is the only example of a thioredoxin-linked enzyme with a regulatory Cys as part of its active site. Since one of the reduced Cys residues appears to be involved in substrate binding, the formation of a disulfide bridge very effectively blocks catalytic activity, a situation found also in NADP-MDH.

A crystal structure of the allosterically regulated PRK from *Rhodobacter sphaeroides* is now available (57). Although this enzyme is not redox regulated, the structural data will be useful for modeling.

ATP Synthase The chloroplast ATP synthase complex is composed of the integral thylakoid membrane portion CF_0 and the hydrophilic CF_1, which is composed of five different subunit-types. The γ subunit of the latter contains the structural element, which allows for thiol modulation of the enzyme by Trx *f* (108, 141). The regulatory motif— **Cys**199AspIleAsnGlyLys**Cys**205 in spinach (Table 1)— is present in the subunit from plants (97) and green algae (32), but is absent in the enzyme of cyanobacteria (32, 95, 163) and diatoms (109) or mitochondrial F_1. The disulfide bridge between the two Cys residues seems to be inaccessible in the inactive enzyme in the dark and becomes exposed upon activation by the transmembrane electrochemical proton gradient (35b). No information is available on possible structural changes brought about by reduction of the regulatory disulfide. However, the main purpose of reduction does not seem to be the modulation of

enzyme activity, but rather to permit a higher rate of ATP formation at limiting electrochemical potentials. Regulation linked to thioredoxin also allows the enzyme to be switched off in the dark to avoid wasteful hydrolysis of ATP.

NADP-Dependent Malate Dehydrogenase NADP-MDH is the most extensively studied thioredoxin-regulated enzyme. It appears to have a rather complex regulatory mechanism, involving more than one disulfide bridge, which has recently been described in detail (96, 128). The homodimeric enzyme of 85 kDa differs from its cytoplasmic, NAD-dependent homologue by the presence of N- and C-terminal extensions and by containing eight Cys residues at strictly conserved positions. Two are located in the N-terminal extension in a **Cys**24PheGlyValPhe**Cys**29 motif (C_4 sorghum), and another two, Cys365 and Cys377, separated by 11 residues, are in the C-terminal region (Table 1). The four remaining Cys are located in the core part of the polypeptide. Systematic functional analysis of the Cys residues by chemical modification and site-directed mutagenesis provided evidence that one regulatory disulfide is formed between the two N-terminal Cys. A second regulatory disulfide bridge is present in the C terminus. These two regulatory sites are not functionally equivalent but have distinct effects on activation. Removal of the N-terminal disulfide yields an inactive, oxidized enzyme still in need of thioredoxin activation. However, this activation is almost instantaneous upon addition of reduced thioredoxin, which contrasts with the slow activation seen with the native enzyme containing both regulatory disulfides. Removal of the C-terminal disulfide produces a mutant enzyme with activation properties very similar to wild type, but the activation process is no longer inhibited by NADP. In addition, this mutant enzyme exhibits a slight constitutive activity (i.e. independent of reduction), but with a very high K_m for oxaloacetate. Removal of both N- and C-terminal disulfides results in a thioredoxin-insensitive, permanently active enzyme.

A fifth, internal Cys residue might also be involved in these regulatory events by forming a transient disulfide bridge with one of the N-terminal Cys (127). This internal Cys207 (in C_4 sorghum) becomes accessible after removal of the N-terminal disulfide by mutation and can form a heterodisulfide with thioredoxin (54).

Because the crystal structures of C_4 NADP-MDH from *Flaveria* (29) and sorghum (78) have recently been solved, the biochemical results can now be placed in a structural perspective. The models confirm the presence of two surface-exposed, thioredoxin-accessible disulfide bonds. The C-terminal disulfide holds down the C-terminal extension, which bends back over the surface and reaches with its tip into the active site, obstructing access of the C_4 acid substrate (82a). The tip of the C terminus carries two negative charges that interact with bound NADP, but not with NADPH. These interactions retard the release of the C-terminal extension and can explain the inhibition of reductive activation by NADP observed earlier (29). The N-terminal extension appears to be rather flexible, and the structural changes due to reduction of its disulfide bond are less clear. Based on the two structures, two possibilities are offered: The N-terminal disulfide might lock domains and thus maintain an unfavorable conformation (78) or the N-terminal residues might reach over the surface of the molecule toward the adenosine end of

the active site and limit substrate access (29). The mobility of the N-terminal extension would also allow for the proposed formation of a transient disulfide bridge between an N-terminal Cys and the internal Cys207 (127). However, it is suggested that this transient disulfide is more likely formed between the two subunits of the homodimer than within a single subunit (29).

The rather complex regulation of NADP-MDH by redox equilibrium and the NADP/NADPH ratio appears to be important in C_3 and C_4 plants. In C_3 plants, where this stromal enzyme exports reducing equivalents in the form of malate from the chloroplast to the cytosol through the "malate valve" (135), it is essential that reducing equivalents are exported only when they are in excess in the chloroplast. In certain C_4 plants, where the enzyme functions as an essential catalyst of the CO_2 trapping and transport mechanism in mesophyll cells, it is equally important to turn off the C_4 pathway in the dark. The additional requirement of pyruvate, Pi dikinase and phosphoenolpyruvate carboxylase for reversible light activation helps to insure that the C_4 pathway is switched off completely in darkness.

Glucose 6-Phosphate Dehydrogenase G6PDH, catalyzing the first step of the oxidative pentose phosphate cycle, exists in plants in at least two isoforms, one in the cytosol and one in chloroplasts. Only the latter isoform is subject to redox regulation by thioredoxin. As this stromal enzyme is part of a dissimilatory pathway, it is regulated in the opposite way from biosynthetic enzymes, i.e. it is inactive in the light (reduced state) and active in the dark (oxidized state). The chloroplast isoform as well as the enzyme from cyanobacteria contain a number of Cys residues that are not present in the cytosolic counterpart. Mutational analysis of the six Cys of the recombinant potato enzyme, all situated in the N-terminal part of the polypeptide, revealed that Cys149 and Cys157 are engaged in a regulatory disulfide bridge (162). This disulfide, specifically reduced by thioredoxin *m*, is part of a regulatory sequence with no similarity to others. The cyanobacterial enzyme, although redox regulated (3, 31, 52), is quite different, with fewer (two to four) Cys residues at other positions. Two Cys (Cys188, Cys447 in *Synechococcus*) are at conserved positions within the cyanobacterial enzyme and might form the regulatory disulfide (52, 134), although there is no supportive experimental evidence to date.

Homology modeling using the crystal coordinates of the *Leuconostoc* enzyme locates the proposed regulatory cysteines of the chloroplast enzyme on an exposed loop, sufficiently close to permit a disulfide bridge, and freely accessible for interaction with thioredoxin (162). However, this model does not provide any insight into how disulfide reduction brings about the observed 30-fold increase in K_m for the substrate.

Rubisco Activase Until recently, no link between the observed light activation of Rubisco and thioredoxin could be made, the sole effect of light being indirect via a change in the stromal ADP/ATP ratio modulating activase activity (116). In certain plants, such as *Arabidopsis*, the activase polypeptide exists in two forms: short and long. The long form was recently found to be activated by reduced thioredoxin *f* in vitro at physiological ADP/ATP ratios and the regulatory disulfide, consisting

of Cys392 and Cys411, was identified at the C-terminal end of the protein (169). However, in other plants, such as tobacco, where there is apparently no long-form activase, the mode of light-induced regulation (activation) remains to be clarified.

Other Possible Chloroplast Targets Three additional chloroplast enzymes—glyceraldehyde 3-phosphate dehydrogenase (GAPDH), acetyl CoA carboxylase, and Fd-dependent glutamate synthase (GOGAT)—are reported to be activated by reduced thioredoxin; however, no specific regulatory sites have yet been demonstrated. Although GAPDH activity can be stimulated by reduced thioredoxin *f*, no compelling evidence exists to date for an absolute requirement for thioredoxin as reductant. Indeed, recent experiments have shown that the same effects can be achieved by low concentrations of GSH (4). Acetyl CoA carboxylase, which catalyzes the first committed step in de novo fatty acid biosynthesis, appears to be activated by thioredoxin (68, 133). This enzyme exists in two isoforms in most plants, a eukaryotic, multifunctional form in the cytoplasm and a prokaryotic, multisubunit isoform in the chloroplast. The activity of the prokaryotic, pea plastid enzyme is significantly increased by the addition of dithiols, but not monothiols, and thioredoxin *f* proved to be the most efficient activator (133). Varying numbers of conserved Cys residues are present in the primary structure of the enzyme from different plant species, but no regulatory Cys are known. The activity of Fd-dependent GOGAT from spinach and soybean chloroplasts was reported to be significantly stimulated by DTT, but not GSH. Thioredoxin *m* was the most efficient activator of the spinach enzyme, which upon reduction exhibited an increased reaction velocity (89). Finally, the addition of DTT-reduced thioredoxin to *Chlamydomonas reinhardtii* preparations significantly enhanced translation of *psbA* RNA, which encodes the D1 protein of photosystem II. This provides a possible direct link between light and the replacement (via translation) of a reaction center protein known to be subject to photooxidative damage (36).

OTHER CATALYSTS WITH THE THIOREDOXIN FOLD IN PLANTS

Several catalysts contain in their polypeptide structure either a simplification of the "thioredoxin fold," e.g. glutaredoxin, or a thioredoxin module that is often present as an extension of the polypeptide.

E. coli glutaredoxin is prototypical of a simplified thioredoxin molecule, which lacks the two first structural units of thioredoxin, i.e. the $\beta 1$ and $\alpha 1$ modules. It is thus a shorter polypeptide ($\sim$85 residues) with an active site, typically -Cys-Pro-Phe/Tyr-Cys, that is readily reduced by GSH and kept reduced by NADPH and glutathione reductase. *E. coli* glutaredoxin is an excellent hydrogen donor for ribonucleotide reductase. Glutaredoxin has also been isolated from plant tissues (104, 144). It was found to be located in the cytosol and easily reduced by GSH. The isolation of a cDNA and gene from *Oryza sativa* (99, 145) established that

the protein is longer than its bacterial counterpart (∼112 residues) and that there is a low number of gene copies (∼2) in rice. Glutaredoxin was also found to be a major component of the phloem sap, together with its reductant glutathione, which suggests that it could potentially play a role in the redox regulation of sieve tube proteins or membrane-located receptors (152).

Among the plant enzymes that feature an extension comprised of either a thioredoxin or a glutaredoxin module is 5′ adenylsulfate reductase (APS reductase) (9, 55, 143, 151a, 167). In plants, this protein is the equivalent of the bacterial PAPS reductase and is involved in the assimilation of sulfur. Two types of cDNA sequences coding for APS reductase have been isolated that feature either a transit peptide or no transit peptide. APS reductase can be produced as a precursor of ∼460 amino acids with an N-terminal extension (∼70 amino acids) believed to target the protein to the chloroplast, a central domain equivalent to bacterial PAPS reductase, and a C-terminal domain of about 140 amino acids with a thioredoxin-like sequence and a glutaredoxin activity when isolated. Other cDNA clones do not show the putative transit sequence, which indicates that there may be several subcellular localizations for this enzyme. However, the thioredoxin-like module is consistently found in all analyzed clones. In bacteria, PAPS reductase is dependent on the addition of reduced thioredoxin for in vitro activity. In contrast to the bacterial enzyme, plant APS reductase is active independently of exogenous thioredoxin, presumably because of the presence of the thioredoxin module in its own polypeptide.

When plants such as *Solanum tuberosum* are subjected to water stress, they produce a variety of stress-related proteins among which is one stromal protein, CDSP 32, for *c*hloroplastic *d*rought-induced *s*tress *p*rotein (122). This nuclear-encoded, 32-kDa protein is comprised of two thioredoxin-like domains, with only the C-terminal domain containing a **C**GP**C** active site. CDSP 32 is proposed to play a role in the preservation of the thiol/disulfide redox potential of chloroplast proteins during water deficit.

A new protein of the thioredoxin family called nucleoredoxin (NRX) has recently been isolated from maize (85). This protein is located in the nucleus and contains three consecutive repeats of the thioredoxin domain. The first and third modules contain the active site W**C**PP**C**, which suggests a potential for catalyzing redox reactions, and the C-terminal part of the protein can form a Zn finger. The presence of this protein in developing kernels indicates that it could regulate the activity of transcription factors, presumably by altering their redox state.

NEW FUNCTIONS FOR THIOREDOXINS IN PLANTS

This topic has been extensively discussed in recent reviews (49, 73), so only the most recent developments are reported here. Thioredoxin has recently been implicated in the organization of the photosynthetic apparatus. A *Rhodobacter sphaeroides* thioredoxin minus mutant has been constructed and shown to be impaired in the formation of its photosynthetic apparatus (110). In this photosynthetic

bacterium, thioredoxin appears to exert a dual role, activating aminolevulinic acid synthetase and regulating *puf* (an operon encoding pigment-binding proteins of the LHC and bacterial reaction center complexes) expression at the transcriptional level. In plants, phosphorylation of certain PSII proteins (D1, D2, and LHCII), although dependent on the redox state of plastoquinone, is also dependent on the presence of thiol-containing compounds (D1 and D2 phosphorylation is stimulated by reduced compounds and LHCII phosphorylation by oxidized compounds) (28). It is thus proposed that a second loop of redox regulation of thylakoid protein phosphorylation is under control of the ferredoxin/thioredoxin system.

ADP-glucose pyrophosphorylase, catalyzing the first committed step of starch biosynthesis, can be reduced by thioredoxins, thereby increasing its affinity for 3-phosphoglycerate, the principal activator (6). The reduction opens an intermolecular disulfide bridge between the two small subunits of the heterotetrameric enzyme, which probably imparts better access to 3-phosphoglycerate. It is proposed that in photosynthetic tissue the ferredoxin/thioredoxin system could be involved in this covalent mechanism of regulation of starch biosynthesis (6).

Three other enzymatic systems are also thioredoxin dependent in plants. The first one is enolase of *Arabidopsis* and ice plant, which is activated by oxidation, although not in other species such as tomato or maize (2). The second is carboxyarabinitol-1-phosphate (CA1P) phosphatase, an enzyme involved in the hydrolysis of CA1P, which is a naturally occurring nocturnal inhibitor of Rubisco. CA1P phosphatase is activated by either reduced thioredoxin or GSH (59). A third protein that could be redox regulated is CP12, a small nuclear-encoded protein capable of binding both PRK and NADP-GAPDH through conserved cysteine residues (161).

Finally, thioredoxin-like molecules have been found on extracellular pollen and detected in self-incompatibility reactions (88, 156). A thioredoxin activity linked to the C terminus of the S gene product seems to be responsible for self-incompatibility. In addition, thioredoxin *h*-like proteins have been shown to interact with the kinase domain of membrane-bound receptor-like protein kinases, which suggests a role in the self-incompatibility signal cascade (13).

TECHNOLOGICAL DEVELOPMENTS

This area has also been covered in earlier recent reviews (49, 73), and therefore only the latest developments are described. It was recently reported that thioredoxin has the capacity to decrease the allergenicity of wheat and milk proteins (19, 39). These findings may have considerable impact on human nutrition, since allergens have many undesirable effects.

Many expression systems have been devised for thioredoxin, which is readily produced with extremely high yields in bacteria such as *E. coli*. The high stability and solubility of thioredoxin has prompted several groups to use it as a stabilizer/solubilizer of peptides/proteins that are normally produced in inclusion

bodies (45, 66, 67, 107, 147). With these expression systems, the protein to be solubilized is fused to an *E. coli* thioredoxin sequence, and, if necessary, to a poly His-tag. The resulting fusion protein can, in turn, be isolated by chromatography on a Ni^{2+}-containing affinity matrix and the thioredoxin module cleaved, if necessary, by a protease. The large number of successful reports attests to the power and efficiency of this technique.

A very elegant method to map protein-protein interactions by using thioredoxin has been reported by Lu et al (91). Briefly, the thioredoxin sequence is inserted into flagellin, a major structural component of the *E. coli* flagellum system. Small peptides can then be introduced into the loop of thioredoxin that contains the active site, a location known to be extremely permissive for peptide insertion. Bacteria engineered in this way will display the peptides in their pili, and this property can be used to build peptide libraries that allow, in turn, the identification of proteins binding with a high affinity. This method is thus an alternative to the well-developed yeast two-hybrid system.

CONCLUDING REMARKS

During the past several years, the use of recombinant technology has greatly contributed to advancements in the understanding of the thioredoxin systems. The availability of recombinant proteins has enabled crystallographers to solve the structures of all the members and even of a few target enzymes. Most of the regulatory sites have been located and the functions of their Cys elucidated by site directed mutagenesis. This same technique has also been employed to probe the protein-protein interaction surfaces for residues responsible for the specificity of these interactions. Not only has the structural field greatly advanced, the mechanism of thioredoxin reduction in chloroplasts has also been intensively studied. This has led to a comprehensive model involving a new role for a Fe-S protein in the reduction of a disulfide bridge, as well as to knowledge of the redox potentials of the regulatory chain and target enzymes.

A number of new functions for thioredoxin have recently been described in mammalian cells; these mostly relate to redox regulation of transcription factors, possibly mediated through the translocation of some components of these systems to the nucleus. It will be interesting to observe whether such developments are reported in plant systems in the future.

It is also interesting to comment on how differently the thioredoxin systems have evolved in plants compared with mammalian cells. In plants, evolution has brought an incredible diversification of the thioredoxin sequences in relation to the existence of another intracellular compartment, the chloroplast. Mammalian cells, on the other hand, have acquired a new NTR catalyst containing a selenocysteine at the active site required for catalytic activity. This modification has not yet been reported in plants or bacteria and seems to be specific to animal cells.

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