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Review

Plant thioredoxins: the multiplicity conundrum

U. Baumann * and J. Juttner

University of Adelaide, Waite Agricultural Research Institute, Department of Plant Science, Waite Road, Glen Osmond, South Australia 5064 (Australia), Fax + 6 1 8 8303 7102, e-mail: ubaumann@waite.adelaide.edu.au

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Abstract. Thioredoxins are small proteins distinguished by the presence of a conserved dicysteine active site. In oxidized thioredoxin, the two cysteines form a disulfide bond that is targeted by the enzyme thioredoxin reductase. Together with an electron donor, thioredoxin and thioredoxin reductase form the 'thioredoxin system' that is present in all organisms. Thioredoxins participate in dithiol/disulfide exchange reactions with a large range of cellular substrates. Higher plants possess a very complex thioredoxin profile consisting of at least two different thioredoxin systems that contain distinct, multigenic thioredoxin classes which have different intracellular localizations. In this review we summarise the current state of knowledge regarding the function of plant thioredoxins representing all systems and classes.

Key words. Thioredoxin; chloroplast enzyme; redox regulation; NADPH thioredoxin reductase; ferredoxin thioredoxin reductase; multigene family; disulfide; seed germination.

Introduction

Thioredoxins are small (approximately 12-kDa) proteins that appear to be ubiquitous in all organisms. Within the predominantly reductive environment of a cell, thioredoxins are involved in a range of biochemical processes. These include the regulation of enzymes [1, 2], the modulation of transcription factors [3, 4], as hydrogen donors [1] and in oxidative protection [5]. Most, but not all, roles depend upon the capacity of thioredoxins to effectively reduce disulfide bonds in target proteins.

Here we address the genomic organization, stucture and biological activities of thioredoxin systems in plants with an emphasis on the most recent developments. Other proteins that contain a thioredoxin motif or possess thioredoxin folds such as glutaredoxin are not discussed.

All thioredoxins share a conserved active-site motif, Cys-X-Pro-Cys (where X is generally Gly but can also be Pro or Ala), and conserved amino acids at structurally important positions [6]. In the oxidized state of the thioredoxin protein, the cysteines of the active centre form a redox-active disulfide bridge. The disulfide can be reduced to a dithiol by electrons from NADPH or ferredoxin via the enzyme thioredoxin reductase [1]. Reduced thioredoxins have redox potentials (–270 mV for *Escherichia coli* thioredoxin) similar to other cellular reductants such as glutathione [7]. The low redox potential enables thioredoxins to reduces disulfide bonds in target proteins or to transfer reducing equivalents to substrates such as methionine sulfoxide residues [7].

Plants possess two thioredoxin systems that can be distinguished by the electron donor and the enzyme that catalyzes thioredoxin reduction.

The ferredoxin/ferredoxin-thioredoxin reductase/ thioredoxin (FTR/Trx) system

The FTR/Trx system, which is composed of ferredoxin, ferredoxin-thioredoxin reductase (FTR) and two nuclear-

* Corresponding author.

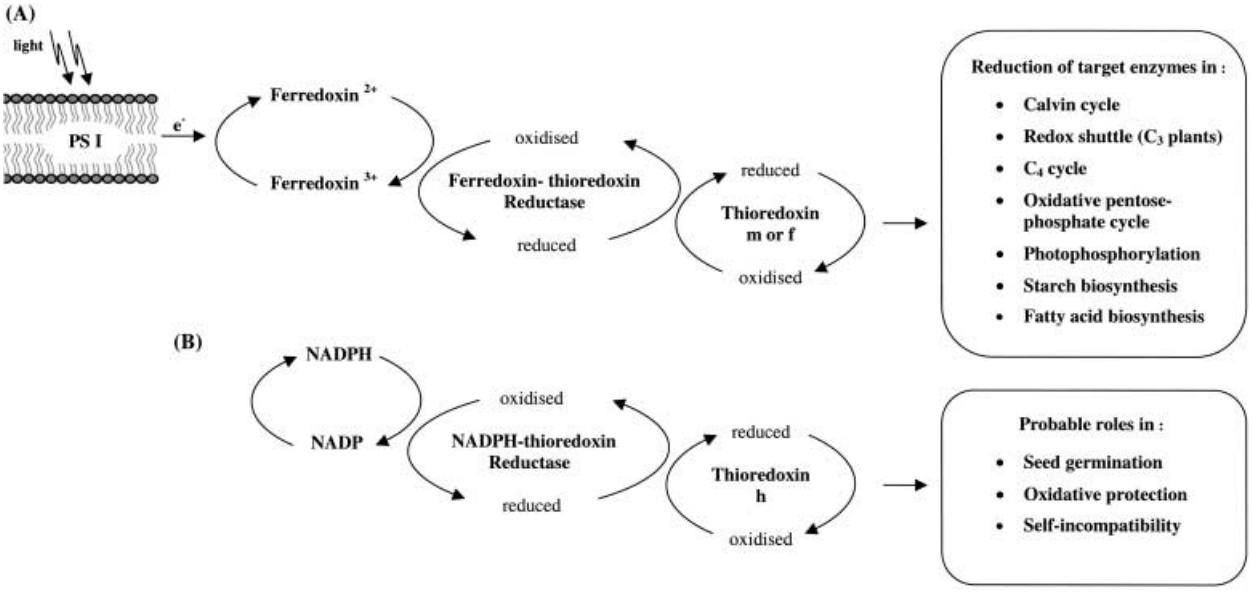

Figure 1. Schematic representation of the two thioredoxin systems found in plants. (*A*). Light-induced regulation of chloroplastic enzymes through the ferredoxin-thioredoxin reductase system. In the light, photosystem I of the photosynthetic electron transfer chain reduces ferredoxin. Ferredoxin can then serve as an electron donor to ferredoxin-thioredoxin reductase, which reduces thioredoxins *m* and *f*. The thioredoxins reduce the disulfide bonds of several target enzymes, thereby modifying their activity. (*B*) The NTR/Trx system. The reduction of plant thioredoxins *h* is catalyzed by NADPH-thioredoxin reductase using NADPH as a source of reducing equivalents. When the thioredoxin cysteine disulfide is reduced to a dithiol, the thioredoxin is able to reduce disulfides in target proteins.

encoded thioredoxins (Trx), *m* and *f* (fig. 1A), is found only in photosynthetic eukaryotes and cyanobacteria. In plants, the system is located in the chloroplast and regulates the redox state of enzymes involved in carbon metabolism and further biochemical processes [8, 9]. The thioredoxins have been designated *m* and *f* in reference to the enzymes with which they were initially found to interact: NADP-malate dehydrogenase (NADP-MDH) and fructose-1,6-bisphosphatase (FBPase), respectively [9–12]. The reducing power for chloroplastic thioredoxins is derived from the electron transport system of illuminated thylakoid membranes (fig. 1A). Electrons are transferred from photosystem I to the iron-sulfur protein FTR via ferredoxin. Subsequently, FTR reduces the thioredoxins which in turn change the redox status of their target proteins, thereby conferring a strict, light-sensitive control of both the assimilatory and dissimilatory pathways.

The NADPH/NADP-thioredoxin reductase/thioredoxin (NTR/Trx) system

In contrast to the chloroplastic thioredoxin system, the NTR/Trx system has been identified in all organisms with the exception of cyanobacteria. In this system, NADPH is the source of reducing power for the NADPH-thioredoxin reductase (NTR)-catalyzed reduction of thioredoxin [2] (fig. 1B). In plants, thioredoxins regulated by this system are commonly termed either thioredoxins *h* or cytosolic thioredoxins in reference to their initial discovery in heterotrophic tissue and predominant subcellular localization.

Plant thioredoxins are a diverse multigene family

Current knowledge supports the view that plants possess the greatest complement of thioredoxins found in all organisms. Multiple thioredoxins have been isolated from algae [13, 14] and several species of flowering plant [15, 16]. Elucidation of the total number of thioredoxins in a given species, their tissue specificity and subcellular location have been the focus of several subsequent studies. Until recently, the most comprehensive picture to emerge has been in soybean where protein and organelle fractionation techniques have been used in conjunction with a range of enzymatic assays to identify no less than six distinct thioredoxin proteins in both leaves [17] and seeds [18]. The multiplicity of thioredoxin proteins as detected by biochemical methods is substantiated by the identification of multiple thioredoxin genes [19]. With the rapid development of whole-genome sequencing and EST sequence databases, determining the total thioredoxin gene complement of several organisms has finally become possible. In the case of *Arabidopsis thaliana,* no less than 13 distinct thioredoxin genes have been identified (fig. 2). Six genes encode non-chloroplastic *h*-type

Figure 2. Comparative alignment of *Arabidopsis* thioredoxin sequences representing all classes of plant thioredoxins and *E. coli* thioredoxin. Sequences and accession numbers are: *Arabidopsis thaliana h1* (Z14084), *h6* (located on BAC clone AC012562), *f1* (AF144386), *m1* (AF095749, *x* (AF095753); *E. coli* (AE000344). Structurally important amino acids are in blue while the active center is in bold. Amino acids representing the predicted N-terminal signal sequence of thioredoxins *f*, *m* and *x* are in italics. The conserved C-terminal cysteine of thioredoxin *f* is in red and the two-amino-acid insertion of thioredoxin *x* is in green.

thioredoxins, five of which have been described in detail previously [19], while the sixth represents the *Arabidopsis* homologue of a conserved group recently reported by us [20]. The other genes comprise two thioredoxins *f*, four thioredoxins *m* and a novel prokaryotic-type thioredoxin called thioredoxin *x* [21]. By contrast, the genomes of *E. coli*, yeast and humans contain two, three and three thioredoxin genes, respectively. The number and diversity of thioredoxins in *Arabidopsis* is probably representative of all higher plants. Database searches performed during the preparation of this review revealed a similar number of all thioredoxins, including thioredoxin *x,* in EST libraries of other plant species such as tomato and soybean.

Thioredoxin *m* and *f* genes are highly expressed in green tissue and encode the only thioredoxin proteins that have been isolated from chloroplasts [11, 22]. However, their expression may not be restricted to photosynthetic tissue. Chromatographically separated thioredoxins from soybean seed showed a virtually identical protein profile to that of soybean leaf, including two thioredoxins that have been assigned to the chloroplast [18]. Correspondingly, the transcripts of thioredoxin *m* genes have been detected in the seed and root of *Arabidopsis* [21]. Thioredoxin *f* genes are present in EST libraries of tomato seed (GenBank clone accession numbers AW036169, AW036163). The transcription of thioredoxin*s m* and *f* in non-photosynthetic tissue raises the question of whether additional functions are performed by these proteins. While the chloroplastic-type thioredoxins present in seed are suggested to be stored as precursor molecules [18], their presence in root remains to be explained.

As has been observed for thioredoxins *m*, *h*-type thioredoxins are also expressed in a range of tissues and at different developmental stages. Plant thioredoxin *h* mRNAs have the highest expression levels in rapidly growing cells [19, 23] and from EST data appear to be present in all plant tissues. Different members of the thioredoxin *h* class have been purified from the endoplasmic reticulum [24] and the plasma membrane [25], and located in the nucleus by in situ hybridization [26].

In addition, thioredoxin *h* proteins have been located in the sieve tubes of several plants despite not having a signal peptide [27, 28].

As the quantity of data regarding thioredoxin number, expression pattern and intracellular distribution has increased, it has become apparent that the existing system of thioredoxin classification, particularly with regard to *h*-type thioredoxins, is oversimplified.

Thioredoxin structure

Plant thioredoxins share most of the amino acid residues determined as being essential for the maintenance of protein structure and catalysis in *E. coli* thioredoxin [6]. The alignment of all available plant thioredoxins has also identified conserved residues, additional to those of known structural importance, that appear to be specific to each thioredoxin class (fig. 2) [29].

Thioredoxins *m* and *f* from plants are most easily differentiated from *h* class thioredoxins by the presence of an N-terminal transit peptide of varying size [30, 31]. Additionally, *f*-type thioredoxins differ from thioredoxins *m* by being slightly longer, due to further amino acids at their N terminus, and by the presence of a third conserved cysteine in the C-terminal part of the proteins, a feature shared with animal thioredoxins [32]. The newly identified thioredoxin *x* also appears to encode a signal peptide although the corresponding protein has yet to be located in any specific organelle. Thioredoxin *x* can be distinguished from *m* and *f* types by several sequence differences including a two-residue insertion in the C terminus of the protein [21] (fig. 2). A further distinguishing feature of thioredoxins *m* and *x* is that they share greater sequence identity with thioredoxins from prokaryotes than with other plant thioredoxins. Phylogenetic analyses have provided strong evidence to advocate a prokaryotic origin for *m* and *x* thioredoxins and a eukaryotic origin for thioredoxins *h* and *f* [21, 33].

Thioredoxins share a similar three-dimensional structure. Nuclear magnetic resonance spectroscopy of *E. coli* [34] and *Chlamydomonas* [35] thioredoxins and X-ray crystallography of human [36] and spinach [37] thioredoxins has demonstrated a conserved arrangement of five β strands surrounded by four α helices, a structure com-

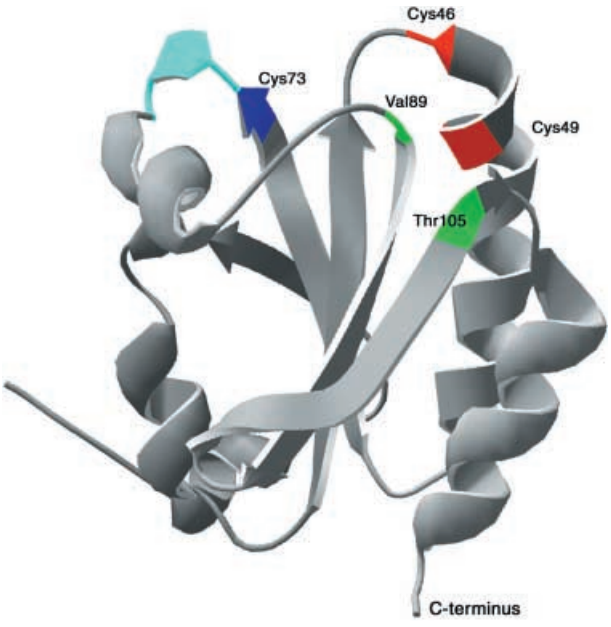

Figure 3. Three-dimensional structure of spinach thioredoxin *f*. The positions of the active-site cysteines within the structure are highlighted in red. The third conserved cysteine of thioredoxins *f* is indicated in dark blue. Regions targeted in mutational studies and mentioned in the text are: amino acids 74–77 (in light blue); Thr^{105}, Val^{89} (in green).

monly referred to as the thioredoxin fold yet not restricted to thioredoxins [38] (fig. 3). Within the protein, the active-site amino acids are located in a protrusion at the end of the second β sheet and the beginning of the second α helix [6]. The N-terminal active-site cysteine is the attacking nucleophile in disulfide reduction and is the more exposed of the active-site cysteines [39]. Reduction of the active-center disulfide bridge is accompanied by localized structural changes including an increase in distance between the two sulfur molecules and rotation of the side chain of the N-terminal cysteine [34, 40].

Thioredoxins *m* and *f* from spinach are the only higher plant thioredoxin proteins for which a crystal structure has been described [37]. The tertiary structure of thioredoxin *m* is more similar to that of *E. coli* than thioredoxin *f.* The most significant difference between thioredoxins *f* and *m* is found in the surface topography around the active site. The active site of thioredoxin *f* is surrounded by positive charges which may be important in the interaction with target proteins [41]. Also exposed on the surface is the conserved C-terminal cysteine which is located 9.7 Å from the N-terminal active-site cysteine. Mutagenesis studies suggest a role for this C-terminal cysteine in interactions with target enzymes [42].

Thioredoxin evolution

The recent availability of a large number of thioredoxin sequences from plants and other organisms has facilitated investigations into the evolutionary history of the different classes of plant thioredoxins. Phylogenetic relationships (fig. 4) were generated from protein sequences of photosynthetic organisms used previously [21, 29] and sequences of all published plant thioredoxins *h*. In addition, sequences of thioredoxins representing a distinct subclass of thioredoxins *h* [20] were included.

Phylogenetic analyses and structural comparisons of thioredoxins from several organisms have clearly demonstrated that higher plant thioredoxins *m* are of prokaryotic origin [29, 32, 37], a finding supported by several lines of evidence. First, of the four thioredoxins identified in the genome of the cyanobacterium *Synechocystis* sp PCC6803, one [open reading frame (ORF) Slr0623] is found on the branch containing all eukaryote thioredoxins *m* [21] including several red algae species. In red algae, thioredoxins *m* are encoded by the chloroplast genome [44] in contrast to the green alga, *Chlamydomonas*, where thioredoxin *m* is nuclear encoded as in higher plants. Given that *Synechocystis* is considered to represent a modern relative of the progenitor of plant chloroplasts, all plant and algal thioredoxins *m* have conceivably evolved from an ancestral thioredoxin similar to Slr0623.

Interestingly, the recently discovered prokaryote-like thioredoxin *x* of *Arabidopsis* has been shown to form a separate group with a different gene from *Synechocystis* sp PCC6803 (Slr1139), suggesting a distinct origin for this plant thioredoxin [21].

Thioredoxins of the *f* class are likely to be of eukaryotic origin as they share highest sequence identity with thioredoxins from eukaryotic organisms and contain an intron at a position conserved between thioredoxins *h* from plants and animal thioredoxins [33].

The *h* class represents the largest and most divergent group of thioredoxins found in plants. Thioredoxins *h* of higher plants and *Chlamydomonas* are likely to have evolved from a common ancestor since the position of two introns is conserved across these species [33]. Duplication and divergence of thioredoxin *h* genes appears to be an ancient event as multiple *h*-type thioredoxins seem to be present in all higher plants and thioredoxin h sequences typically display high levels of interspecific sequence identity [19]. A high degree of interspecific sequence identity is particularly evident within the *h* subgroup that we have identified (fig. 4), where thioredoxin homologues from evolutionarily distant species display greater sequence homology to one another than to other thioredoxins *h* from the same or more closely related species. With the exception of *Arabidopsis*, the full thioredoxin *h* complement of plants is still unknown. As other thioredoxin *h* sequences are reported, we expect that the *h* class will be subdivided further.

Thioredoxin reductases

In all organisms, the action of thioredoxins is modulated by dimeric enzymes called thioredoxin reductases. As their name suggests, thioredoxin reductases are able to reduce oxidized thioredoxin; however, this is probably not their exclusive function, as they have been shown to interact with other cellular substrates [45, 46]. The sequencing of thioredoxin reductase genes and crystallization of thioredoxin reductase proteins from several species [47–49] has revealed that two broad classes exist in nature: The FTR that uses ferredoxin as an electron source and NTR which uses NADPH as the source of reducing equivalents.

NTRs of plants are homodimeric flavoproteins that belong to a family of pyridine nucleotide disulfide oxidoreductase enzymes [48]. The protein subunits have a molecular weight of 35 kDa and contain an FAD- and NADPH-binding domain as well as a conserved redox-active disulfide [50]. Electrons are transferred from NADPH to FAD and then to the disulfide. The tertiary structure of NTR from *A. thaliana* has been determined and has revealed that the plant NTR enzyme is more closely related to prokaryotic thioredoxin reductases than to NTR from higher eukaryotes such as mammals [49, 51]. Within the protein, the active disulfide site faces the isoalloxazin ring of the flavin (FAD) thus allowing the transfer of electrons from FAD to the disulfide bridge [52]. As determined for *E. coli* [49], binding of thioredoxin by plant NTRs also requires a structural change in the NTR molecule. The NADPH domain rotates away from the flavin to accommodate the binding and subsequent reduction of thioredoxin [53].

NTRs display varying levels of affinity for thioredoxins from different sources [50]. Interestingly, several thioredoxins from divergent species have been shown to be better substrates for a particular NTR than some thioredoxins derived from the same or more closely related organisms [19, 50]. Given that plants contain several distinct NTR proteins [54], there is likely to be a level of specificity in the interaction between NTRs and thioredoxins.

FTR of higher plants is a nuclear-encoded heterodimer, comprised of two subunits, a variable subunit and a larger catalytic subunit that is conserved between species and contains a [4Fe–4S] centre and the reductive disulfide [55, 56]. The conserved subunit sits on top of the variable subunit forming a thin molecule which resembles a concave disc [41]. The reductive disulfide and the [4Fe–4S] cluster are located at the center of this disc. Dai et al. [57] suggested that both the electron donor (ferredoxin) and the electron acceptor (thioredoxin) simultaneously dock on opposite sides of the FTR molecule. Thioredoxin forms a mixed disulfide bond with the regulatory cysteine of FTR. This bond is resolved after an electron from a second ferredoxin has been received and the reduced thioredoxin is released.

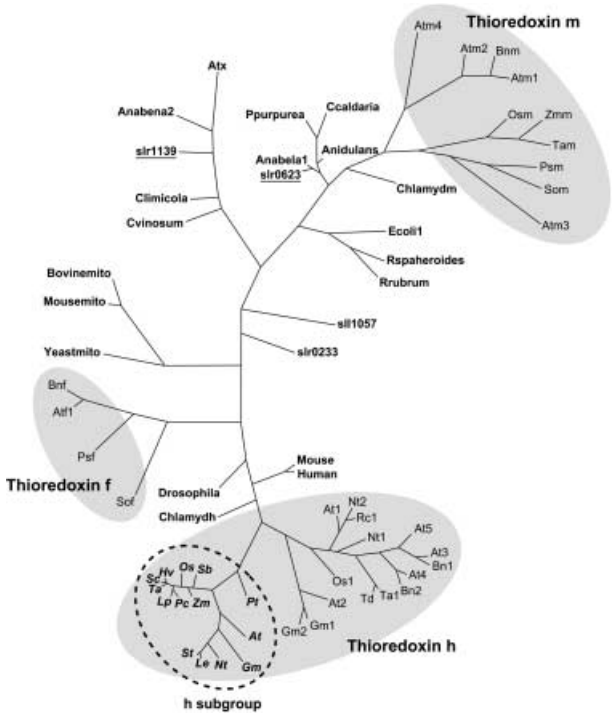

Figure 4. An unrooted consensus tree of thioredoxin protein sequences from photosynthetic organisms and selected non-photosynthetic prokaryotes and eukaryotes. Phylogenetic trees were generated using PAUP [43] and the consensus tree selected using the 50% majority rule method. Branch lengths are proportional to phylogenetic distance. Initials of species in bold italics represent members of the thioredoxin *h* subgroup mentioned in the text. The two *Synechocystis* open reading frames discussed are underlined. *Plant thioredoxins*: *Arabidopsis thaliana* (thioredoxins *h*: At1 = Z14084, At2 = Z35475, At3 = Z35474, At4 = Z35473, At5 = Z35476, ***At*** = AC012562; *m*: Atm1 = AF095749, Atm2 = AF095750, Atm3 = AF095751, Atm4 = AF095752; *f*: Atf1 = AF144385; *x*: Atx = AF095753); *Brassica napus* (*h*: Bn1 = U59379, Bn2 = U59380; *m*: Bnm = U76831, *f*: Bnf = AF018174); *Glycine max* (*h*: Gm1, Gm2 = [ref. 125]; *m*: ***Gm*** = AW101975); *Hodeum vulgare* (*h*: ***Hv*** = AF435815); *Lolium perenne* (*h*: ***Lp***= AF159387); Lycopersicon esculentum (*h*: ***Le*** = AW092362); *Nicotina tabacum* (*h*: Nt1 = X58527, Nt2 = Z11803, ***Nt*** = AF435818); *Oryza sativa* (*h*: Os1 = D265547, ***Os*** = AF435817; *m*: Osm = AJ005841); *Phalaris coerulescens* (*h*: ***Pc*** = AF159388); *Pinus taeda* (*h*: ***Pt*** = AW037917); *Pisum sativum* (*m*: Psm = X76269; *f*: Psf = U35830); *Ricinus communis* (*h*: Rc1 = Z70677); *Secale cereale* (*h*: ***Sc*** = AF159386); *Solanum tuberosum* (*h*: ***St*** = BG888653); *Sorghum bicolor* (*h*: ***Sb*** = BG048153); *Spinacea oleracea* (*m*: Som = X51463; *f*: Sof = X14959); *Triticum aestivum* (*h*: Ta1 = X69915, ***Ta*** = AF438359; *m*: Tam = AJ005840); *Triticum durum* (*h*: Td = AJ001903); *Zea mays* (*h*: ***Zm*** = AF435816; *m*: Zmm = L40957). *Green algae*: *Chlamydomonas reinhardtii* (*h*: Chlamydh = X78822; *m*: Chlamydm = X62335). *Red algae*: *Cyanidium caldaria* (*m*: Ccaldaria = Z21723); *Porphyra purpurea* (*m*: Ppurpurea = U38804). *Cyanobacteria*: *Anabena* sp. (Anabena1 = M14736, Anabena2 = M22997); *Anacystis nidulans* (*Anidulans* = J04475); *Synechocystis* sp. PCC6803 open reading frames sll1057, slr0233, slr0623, slr1139). *Photosynthetic bacteria: Chlorobium limicola* (Climicola = P10472); *Chromatium vinosum* (Cvinosum = P09857); *Rhodobacter sphaeroides* (Rsphaeroides = M33806); *Rhodospirillum rubrum* (Rrubrum = P10473). *Non-photosynthetic eukaryotes*: *Mus musculus* (Mouse = NM011660; mitochondrial, Mousemito = NM019913); *Bos taurus* (mitochondrial, Bovinemito = D87741); *Saccharomyces cerevisiae* (mitochondrial, Yeastmito = NC001135); *Drosophila melanogaster* (Drosophila = AF143404); *Homo sapiens* (Human = X77584). *Non photosynthetic prokaryotes*: *Escherichia coli* (Ecoli1 = P00274).

Chloroplastic thioredoxins

The FTR/Trx system represents one mechanism by which light regulates the activity of various chloroplastic proteins [9] and thereby a range of biochemical processes (fig. 1 A). Through the interaction with key enzymes, the FTR/Trx system acts like a switch between anabolic and catabolic pathways of the chloroplast, thus preventing futile cycling [9, 29, 41].

Many of the thioredoxin-regulated enzymes are involved in carbon metabolism. Three of them, FBPase, sedoheptulose-1,7-bisphosphatase (SBPase) and phosphoribulokinase (PRK) belong to the Calvin cycle (table 1).

NADP-MDH is a key enzyme of carbon assimilation in C4 plants and participates in exporting reducing equivalents from the chloroplast in C3 plants [69]. Rubisco activase regulates the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase, the enzyme that initiates photosynthetic carbon assimilation in C3 plants, whereas glucose-6-phosphate dehydrogenase (G6PDH) is involved in the oxidative pentose-phosphate pathway. The CF_1 γ subunit contains the catalytic sites of the ATP synthase which provides ATP for the chloroplast. All enzymes with the exception of G6PDH are activated by reduced thioredoxin.

For many target enzymes, the regulatory cysteines that form the redox-active disulfide bridge have been identified by site-directed mutagenesis. In addition, structural data from X-ray crystallography have verified the position of the disulfide bonds in FBPase [59] and NADP-MDH [67]. A comparison of the amino acid sequences revealed no cysteine-containing consensus motif in thioredoxin-regulated enzymes (table1). Nor are the regulatory cysteines necessarily part of the active site of the enzymes. Presently, PRK is the only example of a thioredoxin-regulated enzyme with regulatory cysteines at its active site.

Comparisons of the position of regulatory disulfides in the protein sequences of redox-regulated enzymes and their non-redox-regulated counterparts has provided insight into the evolution of redox regulation. These comparisons have revealed that the regulatory cysteines are either positioned in a sequence block which has diverged between the two isoforms, but is flanked by conserved areas, as in for example FBPase and G6PDH, or are located on extensions which are present in the redox-regulated but absent in the redox-independent isozymes, examples being NADP-MDH, Rubisco activase and GAPDH [68, 70].

Interaction with target proteins

Since this topic has been reviewed recently [29, 58, 70], only certain interacting proteins are discussed in detail.

FBPase

In chloroplastic FBPases, three cysteines are located in a solvent-exposed structure of about 14–19 amino acids, called the 170's loop [71], which is missing in the cytoplasmic isoforms. Mutations of these three cysteines resulted in partial or total loss of activation [72–74]. Recent crystallography of the oxidised form of the pea FB-

Table 1. Characteristics of chloroplast enzymes modulated by thioredoxins. The cysteines of the regulatory site are in bold. n.d., not determined

Target enzyme	Structure	Biochemical pathway	E_m	Activator	Regulatory site	Plant	References
Fructose-1,6-bisphosphatase	homotetramer	Calvin cycle	–305 mV	Trx *f*, also changes in pH and $[Mg^{2+}]$	E**C**X_{19}**C**IVNVCQ 153 173	pea	58, 59
Sedoheptulose-1,7-bisphosphatase	homodimer	Calvin cycle	–300 mV	Trx *f*	S**C**GGTA**C**V 52 57	wheat	60
Phosphoribulokinase	homodimer	Calvin cycle	–295 mV	Trx *m/f*	G**C**X_{38}**C**L 16 55	spinach	61–63
CF_1-ATP synthase γ subunit	subunit	photophosphorylation	–280 mV	Trx *f*	I**C**DINGN**C**V 198 204	pea	58, 64
Glucose-6-phosphate dehydrogenase	homotetramer	oxidative pentose-phosphate pathway	n.d.	inactivated by Trx *m*	**C**RIDKRED**C** 149 157	potato	65
NADP-malate dehydrogenase	homodimer	C3 plants: redox shuttle C4 plants: carbon assimilation	–330 mV	Trx *f/m*	E**C**FGVF**C**T 24 29 K**C**X_{11}**C**I 365 377	sorghum	66, 67
Rubisco-activase (long form)	monomer	Calvin cycle (indirectly)	n.d.	Trx *f*	G**C**X_{18}**C**V 392 411	*Arabidopsis*	68

Pase revealed a disulfide bond between the Cys^{153} and Cys^{173}, whereas the third cysteine is present as a free sulfhydryl located on the buried side of the following helix [59]. The Cys^{153} (155 in spinach) has been identified as the one forming the transient mixed disulfide bond with Cys^{46} of thioredoxin *f* [75].

The presence of a highly negative potential in the 170's loop is of considerable interest as it may explain the preferential interaction of FBPase with thioredoxin *f*. The distribution of positive charges around the active site of thioredoxin *f* differs from that of thioredoxin *m* and may be the most significant difference between the two thioredoxins. Furthermore when 17 amino acids (from L^{154} to E^{170}) of the 170's loop of the pea enzyme were removed, thioredoxin *f* binding was prevented and thioredoxin modulation lost, indicating that the region is essential for the interaction between the two proteins [76]. However, the replacement of the 170's loop of the pea enzyme by that of wheat, thereby reducing the number of net negative charges from seven to three, did not seem to affect significantly the interaction with thioredoxin, although its activity was reduced. More detailed analyses will be necessary to determine the role of each charged residue in the binding with thioredoxin [76].

NADP-MDH

Among the redox-regulated enzymes of the chloroplast, NADP-MDH is the most extensively studied and has a complex activation mechanism [for recent reviews see refs 77, 78]. The activity of this homodimeric enzyme is strictly redox regulated and the enzyme is inactive in the dark. Results obtained by chemical modification and site-directed mutagenesis of the cysteine residues together with the recently published three-dimensional structures of sorghum [67] and *Flaveria* [79] have shown that five of the eight conserved cysteines present in NADP-MDH are involved in the reductive activation of the enzyme. Two N-terminal cysteines (Cys^{24} and Cys^{29}, sorghum numbering) form a disulfide bridge as do two C-terminal cysteines (Cys^{365} and Cys^{377}), while an internally located cysteine (Cys^{207}) is proposed to form a transient disulfide with one of the N-terminal cysteines during the activation process [80, 81]. The removal of the N-terminal disulfide yielded a dithiol-dependant enzyme which was much more rapidly activated by thioredoxin than the wild-type protein [82]. Substitution of the C-terminal cysteines did not affect the activation kinetics but the activation process was no longer inhibited by NADP and the mutant enzyme remained thioredoxin dependent for full activity. However, in the oxidized state, the mutant enzyme was weakly active and showed a tenfold-increased K_m for oxalacetate [83] indicating that the opening of the disulfide bridge had improved access to the active site. Combining N-terminal and C-terminal mutations resulted in a permanently active, thioredoxin-independent enzyme [83, 84].

The model of activation that is currently discussed assumes that the C-terminal disulfide is responsible for obstructing the active site. In the oxidised configuration, the C-terminal end of the enzyme reaches into the active-site and acts as an internal inhibitor through the interaction of the last two residues (Glu^{388} and Val^{389} in sorghum) with the active site residues and the positively charged $NADP^+$. Upon reduction of the C-terminal disulfide, the C terminus is released, allowing the substrate access to the active site. The role of the N-terminal disulfide is less well understood. Given its location at the dimer interface, it has been proposed to rigidify the structure in an unfavorable conformation. Its reduction would loosen the interaction between the subunits, providing the enzyme with the necessary flexibility for catalytic activity.

ATP synthase

The structural basis for thiol regulation of the chloroplastic ATP synthase is a sequence motif of nine amino acids including the two regulatory cysteines in the γ subunit of CF_1, the hydrophilic, membrane-attached moiety of the synthase. The identity of the regulatory cysteines was confirmed by mutagenesis [85, 86] and by inserting the nine-amino-acid motif into the ATP synthase of *Synechocystis*, thereby conferring thioredoxin sensitivity to the recombinant enzyme [87, 88]. The chloroplastic ATP synthase is a latent enzyme which is activated by the proton gradient ($\Delta\mu_{H^+}$) upon illumination of the thylakoid membrane. The regulatory segment of the γ subunit is thought to become accessible to thiol modulation due to a proton-gradient-induced conformational change and activation of the ATP synthase. Reduction of the disulfide lowers the $\Delta\mu_{H^+}$ threshold for the activation of the enzyme, hence allowing ATP synthesis at lower proton gradients. Recently, the structure of spinach chloroplast ATP synthase was determined to 3.2-Å resolution [89]. Unfortunately, the structure of the γ subunit could not be clearly resolved because of the threefold crystallographic symmetry in the complex.

The importance of amino acid residues downstream of the two regulatory cysteines for ATPase activity and interaction with thioredoxins was investigated by Konno et al. [90] using substitution and deletion mutants of the γ subunit. The substitution of three negatively charged amino acids just adjacent to the regulatory cysteines (E^{210} D^{211} E^{212} spinach numbering) with alanines resulted in redox insensitivity, without changing the ATPase activity of the reconstituted chimaeric F_1 complex used in the experiment. The deletion of these three amino acids, however, led to inactivation of the complex in the presence of dithiothreitol (DTT). Thioredoxins *m* and *f* both accelerated this inactivation. The ATPase activity of this mutant in the reduced state was similar to that of the wild-type complex in its oxidized form and vice versa, indicating that the deletion of the charged amino acids had an in-

verse effect on the redox regulation of the γ subunit. All other mutants were neither significantly activated nor inactivated by reduction, but showed increased ATPase activity in both the oxidized and the reduced state.

G6PDH

Of the cytosolic and chloroplastic isoforms of G6PDH, only the chloroplastic ones are redox regulated by thioredoxin [65]. Congruent with its role in a dissimilatory pathway, chloroplastic G6PDH is inactivated when reduced by thioredoxin, preventing futile cycling between carbohydrate synthesis and catabolism. Mutations of all six cysteines of the potato enzyme revealed that Cys^{149} and Cys^{157} are likely to form the regulatory disulfide bridge [65] as these mutants were insensitive to redox regulation and their K_ms for glucose-6-phosphate were increased by about 20-fold. Homology modeling based on the *Leuconostoc* enzyme suggested that the two regulatory cysteines are located on an exposed loop on the surface of the protein [65] and thus easily accessible to thioredoxin.

Recently, a second plastidic isoform of G6PDH, P2, has been isolated from potato [91]. A comparison of the P2 protein sequence with that of the P1 isoform of potato revealed that the regulatory cysteines were present at the same positions. The recombinant P2 isoform was susceptible to redox regulation, however, in contrast to P1, which was completely inactivated, P2 activity decreased to about 50% in the presence of saturating concentrations of reduced thioredoxin. The physiological need for two G6PDH isoforms in the chloroplast is not yet fully understood.

G6PDH is an excellent example of evolutionary convergence. The G6PDH enzymes of cyanobacteria, although also redox regulated [92, 93] are distinct from their chloroplastic counterparts [94]. Not only are the regulatory cysteines at different positions from the chloroplastic enzymes, but the enzymes seem to have a different evolutionary origin as suggested in a recent study [94]. The chloroplastic enzymes possibly evolved from a copy of cytoplasmic G6PDHs recruited into the chloroplast. Subsequently, the redox regulation had to be reinvented for the chloroplastic isoform, resulting in an enzyme with a regulatory disulfide bridge at a different position. However, given that the introduction of the loop (^{149}CRIDKRENC^{157}, potato) containing the regulatory cysteines of chloroplastic G6PDH into the cytosolic isoform did not result in a redox-sensitive enzyme [65], a closer analysis to identify all the necessary structural components for the thioredoxin-G6PDH interaction is required.

Potential target proteins

Glyceraldehyde 3-phosphate dehydrogenase

Chloroplastic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a heterotetrameric (2A2B) enzyme of the Calvin cycle. The A and B subunits are similar to each other, except that B subunits have a C-terminal extension (28–30 amino acids) containing two cysteine residues. When purified from darkened leaves, the enzyme occurs as hexadecameric aggregates that exhibit low affinity to the substrate 1,3-bisphosphoglycerate (1,3PGA) [95]. Exposure of the hexadecamer to a reductant, such as DTT, decreases the K_m for the substrate and destabilizes the hexadecameric form. The binding of 1,3PGA to the reduced hexadecamers has been suggested to lead to dissociation and yields fully activated tetramers [96].

Removal of the C-terminal extension of the B subunit resulted in an enzyme which existed only as a tetramer, was insensitive to DTT, showed high affinity for 1,3PGA and exhibited full catalytic activity [95, 97, 98]. A very recent study by Qi et al. [99] provided evidence that the cysteine residues in the C-terminal extension of the B subunit (Cys^{354} and Cys^{363}, pea GAPDH) do form a disulfide bridge, whereas other cysteines present in the A and B subunits do not. However, whether thioredoxin is indeed the reductant of GAPDH is unclear, since when tested in vitro, low concentrations of glutathione or DTT were as effective as thioredoxin *f* [100].

Acetyl CoA carboxylase

Acetyl Coa carboxylase (ACCase) catalyzes the first committed step in de novo fatty acid biosynthesis. With the exception of the grasses, plants contain two isoforms of ACCase, a multifunctional eukaryotic form in the cytosol, and a prokaryotic, multisubunit form in the plastid. In vitro, the activity of the prokaryotic but not the eukaryotic ACCase is influenced by pH, Mg^{2+} concentration and the presence of dithiols [101]. Reduced thioredoxin activated the enzyme more efficiently than DTT alone, and thioredoxin *f* was more effective than thioredoxin *m* and *E. coli* thioredoxin. Of the proteins that make up the ACCase multienzyme, the activity of carboxyltransferase was influenced by DTT whereas that of biotin carboxylase was not [102]. Recently, Kozaki and co-workers [103] succeeded in expressing an active carboxyltransferase in *E. coli.* The activity of the recombinant enzyme was redox dependent, and thioredoxin *f* proved to be a more efficient activator than DTT. It will be interesting to determine which of the 13 cysteines in carboxyltransferase are the regulatory residues.

Enzymes involved in nitrogen metabolism

When the activities of chloroplastic nitrate reductase (NiR), glutamate synthetase and Fd-dependent glutamate synthase (Fd-GOGAT) were tested for regulation by thioredoxin, Fd-GOGAT was found to be significantly stimulated by DTT, but not by reduced glutathione (GSH). Thioredoxin *m* was more efficient than thioredoxin *f* in the activation of the spinach enzymes which showed an increased reaction velocity upon reduction

[104]. Neither NiR nor glutamate synthetase from spinach and soybean exhibited thioredoxin-dependent activation. In contrast to the latter finding, Choi et al. [105] reported that the chloroplastic glutamate synthetase isoform from *Canvalia lineata* was activated by DTT and to a lesser degree by GSH and β-mercaptoethanol. Furthermore, glutamate synthetase was among the enzymes isolated in a recent trap experiment that used resin-bound mutant thioredoxin *m* ($Cys^{41} \rightarrow Ser$) to capture target proteins. Its isolation indicates that the interaction between thioredoxin and glutamate synthetase needs revisiting [106].

ADP-glucose pyrophosphorylase

Thioredoxin was recently proposed to be involved in the fine regulation of ADP-glucose pyrophosphorylase (AGPPase) in photosynthetic tissues [107]. AGPPase is a heterotetrameric enzyme that catalyzes the first committed step in starch biosynthesis. Reduction of the enzyme leads to an opening of the intermolecular disulfide bridge between the small subunits and a concomitant increase in affinity to 3-phosphoglycerate, the main activator of the enzyme. The regulatory cysteine (Cys^{12}, potato numbering) is located in a region that is highly conserved in chloroplastic AGPPases of both monocots and dicots [108].

LHCII

Investigating the regulation of photosystem II protein phosphorylation, Carlberg and co-workers [109] observed that the addition of thiol-reducing compounds had a strong inhibitory effect on the phosphorylation of LHCII proteins (the chlorophyll a/b-binding proteins of the PSII antenna). Both thioredoxins *f* and *m* inhibited phosphorylation, with thioredoxin *f* being slightly more effective at lower concentrations. Thioredoxin has been proposed to reduce and thereby inactivate the LHCII kinase rather than interacting directly with the LHCII proteins [110].

Peroxiredoxins

Among the chloroplastic proteins captured by immobilized thioredoxin m_{C41S} resin [106] were two different thioredoxin-dependent peroxiredoxins (Prx), a 2-Cys-Prx and a homologue to PrxQ, a novel peroxiredoxin first isolated from *Sedum lineare* [111]. Peroxiredoxins of the 2-Cys-type are posttranslationally imported into the chloroplast [112, 113] where they protect the photosynthetic apparatus from oxidative damage [114]. Biochemical analysis of recombinant 2-Cys-Prx showed that the reduction of H_2O_2 required all components of the yeast thioredoxin system (yeast thioredoxin, yeast NTR and NADPH). The interaction of these peroxiredoxins with the chloroplastic thioredoxin system remains to be verified.

The recently isolated PrxQ protein from *S. lineare* was identified as the first plant member of the Bcp (bacterioferritin co-migratory protein) group of peroxiredoxins. It not only showed highest sequence similarity to proteins of the Bcp group, but was also able to reduce H_2O_2 with the assistance of the NTR/Trx system (but not GSH) of *E. coli*, and complemented the hypersensitivity of an *E. coli bcp* mutant to organic peroxides [111]. Measurement of the peroxidase activity of recombinant PrxQ of *Arabidopsis* in the presence of thioredoxin *m* and *E. coli* NTR demonstrated that thioredoxin *m* can indeed reduce PrxQ. No activity was observed in the presence of mutant thioredoxin m_{C41S} [106].

Specificity or redundancy?

The physiological need for two different thioredoxins in the chloroplast was initially explained by their selective interaction with target proteins under the conditions tested. Despite their similar tertiary structure [37] and midpoint oxidation-reduction potential (E_m of thioredoxin *m* is -300 mV and of thioredoxin *f* is -290 mV [115]), early studies suggested that thioredoxin *f* was more effective in the activation of FBPase, SBPase and PRK, whereas thioredoxin *m* preferentially reduced NADP-MDH and G6PDH [9–12]. More recent results, however, question the strict separation of interactions. Regarding the reduction of NADP-MDH, thioredoxin *f* appeared to be more efficient than thioredoxin *m* [116]. In contrast to prior results with spinach thioredoxin *m* [117], López Jaramillo et al. [118] showed that thioredoxin *m* of pea was capable of activating FBPase, though not very efficiently. Recently, Geck and Hartmann [119] examined the activation of PRK and found that thioredoxin *m* is twofold more effective than thioredoxin *f*, whereas thioredoxin *f* appeared slightly more efficient in the reduction of the CF_1-γ subunit of the chloroplastic ATPase [64, 90]. Of the two isoforms of G6PDH isolated from potato, P1 appeared to be specifically and completely inactivated by thioredoxin *m*, whereas the P2 isoform responded equally to both thioredoxins [91].

Site-directed mutagenesis studies have provided some insight into the structural features of the thioredoxin *f* and *m* proteins that are relevant for the specificity of protein-protein interaction. The importance of the third cysteine specific for thioredoxin *f* (Cys^{73}, spinach; see fig. 3) was demonstrated by changing the cysteine to serine or alanine. Both mutants showed impaired interaction with FBPase and NADP-MDH, resulting in reduced activation of the enzymes [42].

When amino acids in the region 74–77 of spinach thioredoxin *f* (see fig. 3) were deleted or substituted to those presented in thioredoxin *m* at the equivalent position, a decrease in activity with FBPase and an increase with NADP-MDH was observed [116]. The same mutants

showed weakened interaction with PRK, which was, however, compensated for by an increased activation rate, so that the overall efficiency was either unchanged or reduced by about twofold [119]. Similarly, the thioredoxin *f* mutant T105I and the double mutant V89I/T105I (these amino acids are located at a putative hydrophobic contact surface) showed reduced affinity for FBPase, in the double mutant down to about 1/50 of the wild type [119], whereas both mutants activated PRK more efficiently than the wild-type protein [119]. Again, the amino acid changes created proteins which had amino acids substituted for those present in thioredoxin *m*.

The contribution of the charged amino acids in the interactions between thioredoxins and their target proteins was investigated by generating several thioredoxin *m* mutants. Replacing positively charged amino acids with negatively charged ones led to a decrease in FBPase activation as well as reduced affinity to the enzyme [120] as did the introduction of a negative charge directly upsteam of the active site [121].

Considering the diversity of the proteins with which thioredoxins interact, further analyses will be required to fully elucidate the specificity between the thioredoxins and their target molecules.

Mitochondrial thioredoxins

The mitochondria of plants and animals contain a distinct thioredoxin system comprising an NADPH-dependant thioredoxin reductase and specific thioredoxins [54, 122–124]. Evidence for this system in plants comes from the isolation of mitochondrial thioredoxin proteins [125], and the identification of a mitochondrial-specific protein utilizing NADPH and plant thioredoxins as substrates [54]. However, unlike other organisms, the genes encoding plant mitochondrial NTRs and thioredoxins have not been identified.

In animals, thioredoxins of mitochondria have been shown to be involved in the regulation of mitochondrial 2-oxoacid dehydrogenase complexes [126, 127] and thioredoxin-dependant peroxidases [128, 129], functions that could also apply to mitochondrial thioredoxins in plants. In addition, plant mitochondria contain biochemical pathways and enzymes not found in the mitochondria of other organisms, for example the alternative oxidase, some of which have been proffered as possible regulatory targets for mitochondrial thioredoxins [130].

Cytosolic thioredoxins

Thioredoxins in seed germination

Germination of cereal seeds involves the reduction of seed storage proteins [131, 132]. Evidence for the involvement of thioredoxins in seed germination is based largely on the observation that thioredoxins from plant and bacterial sources have the biochemical capacity to reduce seed storage proteins and inactivate amylolytic enzyme inhibitors as determined by in vitro studies [133]. For example, wheat and *E. coli* thioredoxins were found to reduce and inactivate α-amylase and trypsin inhibitor proteins from several plant species [134, 135]. Thioredoxin also reduced a seed-specific serine protease, thiocalsin, that following reduction was activated by calcium [136]. Reduced thiocalsin was able to cleave thioredoxin-reduced gliadins and glutenins. More direct evidence for thioredoxin-mediated regulation of seed enzymes has come from a transformation experiment in which barley transgenic lines, overexpressing thioredoxin *h* in seed, displayed a fourfold increase in the activity of pullanase (limit dextrinase), an enzyme that cleaves α-1, 6 linkages in starch [137].

Examination of the level of thioredoxin protein in the endosperm of wheat revealed a progressive reduction during germination and that this process was enhanced by gibberellic acid (GA_3) [131]. In contrast to the endosperm, the level of thioredoxin protein present in wheat aleurone and scutellum remained unchanged during germination [26]. Moreover, GA_3 did not have an effect on the level of thioredoxin in these localities, indicating the presence of multiple differentially regulated thioredoxins in seed. The thioredoxins isolated by Serrato and coworkers [26] were predominantly localized in the nucleus of scutellum and aleurone cells and at a lower level in endosperm. The thioredoxin proteins of both studies are possibly the same, and the GA_3-associated disappearance of thioredoxin in the endosperm described by Lozano et al. [131] may be due to the action of GA_3-regulated proteases.

The presence of thioredoxin in the nucleus of aleurone cells is an interesting discovery reminiscent of observations in animal cells where thioredoxins have been shown to interact with transcriptional factors [138].

Thioredoxin *h* is not apparently the only native protein with a capacity to reduce seed proteins in vitro. Kobrehel et al. [134] reported that the α-amylase inhibitors DSG-1 (from durum wheat) and CM-1 (from bread wheat) as well as a corn kernel trypsin inhibitor and two purothionin proteins (α-1 and β from durum wheat) were all more effectively reduced by *E. coli* glutaredoxin, a redox-active protein similar to thioredoxin, than by the *E. coli* thioredoxin system. Furthermore, Kobrehel et al. [133] found that wheat gliadins and glutenins can also be reduced by *E. coli* glutaredoxin, albeit less effectively than by thioredoxin. Since the publication of this work, glutaredoxins have been cloned from the seeds of rice [139] and cotyledons of castor bean [140] and are represented in EST seed libraries from wheat and maize. Consequently, future research into the redox-regulated

processes of germination will need to consider the possible involvement of multiple cellular reductants.

Thioredoxin *h* in the companion cell-sieve element complex

Thioredoxins *h* represent a common [28], and often predominant [27], component of the large number of proteins present in the phloem sap of plants [141, 142]. Glutaredoxin has also been identified in the sieve tube exudate of four dicotyledonous species [28, 140], suggesting that redox systems are an integral component of functional sieve tube complexes.

Mature sieve tubes are enucleate and contain no cellular organelles yet require a functional plasma membrane [143]. Consequently, sieve tubes are thought to be supported by proteins synthesized in neighboring companion cells and transferred via plasmodesmata [28]. Microinjection studies with mesophyll cells from rice [144], *Cucurbita* and *Ricinus* [145] have revealed that recombinant thioredoxin *h* and glutaredoxin have the capacity to mediate their own transport through plasmodesmata. To determine the structural motifs required for cell-cell transport, Ishiwitari et al. [144] generated two thioredoxin *h* mutants incapable of plasmodesmatal movement. In both cases, mutations involved the modification of charged residues predicted to project from the protein surface, implying that surface charge topology is important for the binding and/or transport of thioredoxins through plasmodesmata.

Within sieve tubes, numerous roles have been proposed for thioredoxins including oxidative protection and maintenance of sieve tube proteins, differentiation of vascular tissue and as a messenger molecule; however, as yet, no evidence has emerged to support any of these proposals.

Thioredoxins in self-incompatibility

A role for thioredoxins in the self-incompatibility (SI) response of plants was first reported when two *h*-like thioredoxin proteins (THL-1 and THL-2) were found to interact with the kinase domain of the SI locus receptor kinase (SRK), the female component of the *Brassica* SI system [146]. Mutational studies have demonstrated that the interaction is dependent upon a functional thioredoxin active site and the presence of a conserved cysteine in the N terminus of the SRK [147]. Recently, SI in *Brassica oleracea* was shown to involve the phosphorylation of SRK, and a protein present in stigma extract is able to inhibit SRK phosphorylation [148]. Exposure of SRK and stigma extract to the SI protein component of incompatible pollen results in phosphorylation, suggesting that the inhibitor protein prevents spontaneous activation of the SI signaling pathway. Thioredoxin has been proposed as a candidate for the stigma inhibitor, since THL-1 and *Spirulina* thioredoxin were found to inhibit SRK phosphorylation in vitro [148]. Furthermore, the depletion of thioredoxins from stigma extract was reported to abolish the inhibitory effect of SRK phosphorylation. However, an interesting finding arising from this research has been the observation that a functional, reduced active site is required for thioredoxin-mediated inhibition of SRK phosphorylation, yet SRK inhibition appears to involve direct binding of the inhibitor. How thioredoxin might form an inhibitory complex with SRK through the active site remains as yet unexplained.

Conclusions

In contrast to the situation in animals, few of the putative functions ascribed to thioredoxins in plants have been verified. This is particularly evident within the *h* class, where no single biological function has been unambiguously attributed to a thioredoxin *h*. The difficulties associated with determining plant thioredoxin function are compounded by the fact that plants contain approximately four to five times as many thioredoxins as have been identified in animals. There is currently no inclusive set of genomic and biochemical data of the thioredoxins for one plant species. Determination of the actual cellular location of the protein product of all thioredoxin genes will provide a more logical basis for the analyses of thioredoxin functions.

The temporal and spatial regulation of thioredoxin expression is largely unknown. New technologies such as microarrays could reveal the regulation of thioredoxin expression in different tissues, at specific developmental stages and under different environmental conditions.

The presence of multiple thioredoxins of the same type raises the question of functional redundancy. Gene knockout experiments targeting individual thioredoxins could provide insight into the degree of redundancy within and between thioredoxin classes.

Transformation experiments using a thioredoxin with a mutated second cysteine (which will form a stable mixed disulfide with interacting proteins) may provide a method for clarifying the specificity of existing thioredoxin-target protein interactions in vivo and could, additionally, allow the identification of new targets, particularly with regard to thioredoxins *h*.

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Note added in proof

Recent work has described the first isolation of genes encoding the mitochondrial thioredoxin and NTR from plants [149]. The thioredoxin (AtTrx-o1) and NTR (AtNTRA) were isolated from *Arabidopsis thaliana* and contain typical N-terminal mitochondrial target signals. The subcellular location of both ArTrx-o1 and AtNTRA

was confirmed by Western blot analysis of cellular fractions and by *in-vitro* mitochondrial import experiments.

1 Holmgren A. (1989) Thioredoxin and glutaredoxin systems. J. Biol. Chem. **264:** 13963–13966
2 Arnér E. S. J. and Holmgren A. (2000) Physiological functions of thioredoxin and thioredoxin reductase. Eur. J. Biochem. **267:** 6102–6109
3 Schenk H., Klein M., Erdbrugger W., Droge W. and Schulze-Osthoff K. (1994) Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappa-B and AP-1. Proc. Natl. Acad. Sci. USA **91:** 1672–1676
4 Hirota K., Murata M., Sachi Y., Nakamura H., Takeuchi J., Mori K. et al. (1999) Distinct roles of thioredoxin in the cytoplasm and in the nucleus – a two-step mechanism of redox regulation of transcription factor NF-kappa B. J. Biol. Chem. **274:** 27891–27897
5 Chae H. Z., Robison K., Poole L. B., Church G., Storz G. and Rhee S. G. (1994) Cloning and sequencing of a thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. Proc. Natl. Acad. Sci. USA **91:** 7017–7021
6 Eklund H., Gleason F. K. and Holmgren A. (1991) Structural and functional relations among thioredoxins of different species. Proteins **11:** 13–28
7 Follmann H. and Häberlein I. (1995) Thioredoxins: universal, yet specific thiol-disulfide redox cofactors. Biofactors **5:** 147–156
8 Scheibe R. and Anderson L. E. (1981) Dark modulation of NADP-dependent malate dehydrogenase and glucose-6-phosphate dehydrogenase in the chloroplast. Biochim. Biophys. Acta **636:** 58–64
9 Buchanan B. B. (1991) Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective on its discovery, present status, and future development. Arch. Biochem. Biophys. **288:** 1–9
10 Schürmann P., Wolosiuk R. A., Breazeale V. D. and Buchanan B. B. (1976) Two proteins function in the regulation of photosynthetic CO_2 assimilation in chloroplasts. Nature **263:** 257–258
11 Jacquot J. P., Vidal J. and Gadal P. (1976) Identification of a protein factor involved in dithiothreitol activation of NADP malate dehydrogenase from french bean leaves. FEBS Lett. **71:** 223–227
12 Jacquot J. P., Vidal J., Gadal P. and Schürmann P. (1978) Evidence for the existence of several enzyme-specific thioredoxins in plants. FEBS Lett. **96:** 243–246
13 Crawford N. A., Yee B. C., Hutcheson S. W., Wolosiuk R. A. and Buchanan B. B. (1986) Enzyme regulation in C4 photosynthesis: purification, properties, and activities of thioredoxins from C4 and C3 plants. Arch. Biochem. Biophys. **244:** 1–15
14 Langlotz P., Wagner W. and Follmann F. (1986) Green algae (*Scendesmus obliquus*) contain three thioredoxins of regular size. Z. Naturforsch. **41:** 979–987
15 Berstermann A., Vogt K. and Follmann F. (1983) Plant seeds contain several thioredoxins of regular size. Eur. J. Biochem. **131:** 339–344
16 Vogt K. and Follmann F. (1986) Characterization of three different thioredoxins in wheat. Biochem. Biophys. Acta **873:** 415–418
17 Häberlein I. (1991) Separation of the complete thioredoxin pattern of soybean leaves (*Glycine max*) by high-performance anion exchange chromatography on mono Q. J. Chromatogr. **587:** 109–115
18 Häberlein I., Wolf M., Mohr L. and Follmann H. (1995) Differentiation of six distinct thioredoxins in seeds of the soybean. J. Plant Physiol. **146:** 385–392
19 Rivera-Madrid R., Mestres D., Marinho P., Jacquot J. P., Decottignies P., Miginiac-Maslow M. et al. (1995) Evidence for five divergent thioredoxin h sequences in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **92:** 5620–5624
20 Juttner J., Olde D., Langridge P. and Baumann U. (2000) Cloning and expression of a distinct subclass of plant thioredoxins. Eur. J. Biochem. **267:** 7109–7117
21 Mestres-Ortega D. and Meyer Y. (1999) The *Arabidopsis thaliana* genome encodes at least four thioredoxins m and a new prokaryotic-like thioredoxin. Gene **240:** 307–316
22 Wolosiuk R. A., Crawford N. A., Yee B. C. and Buchanan B. B. (1979) Isolation of three thioredoxins from spinach leaves. J. Biol. Chem. **254:** 1627–1632
23 Marty I., Brugidou C., Chartier Y. and Meyer Y. (1993) Growth-related gene expression in *Nicotiana tabacum* mesophyll protoplasts. Plant J. **4:** 265–278
24 Marcus F., Chamberlain S. H., Chu C., Masiarz F. R., Shin S., Yee B. C. et al. (1991) Plant thioredoxin h: an animal-like thioredoxin occurring in multiple cell compartments. Arch. Biochem. Biophys. **287:** 195–198
25 Shi J. R. and Bhattacharyya M. K. (1996) A novel plasma membrane-bound thioredoxin from soybean. Plant Mol. Biol. **32:** 653–662
26 Serrato A. J., Crespo J. L., Florencio F. J. and Cejudo F. J. (2001) Characterization of two thioredoxin h with predominant localization in the nucleus of aleurone and scutellum cells of germinating wheat seeds. Plant Mol. Biol. **46:** 361–371
27 Ishiwatari Y., Honda C., Kawashima I., Nakamura S., Hirano H., Mori S. et al. (1995) Thioredoxin h is one of the major proteins in rice phloem sap. Planta **195:** 456–463
28 Schobert C., Baker L., Szederkenyi J., Grossmann P., Komor E., Hayashi H. et al. (1998) Identification of immunologically related proteins in sieve-tube exudate collected from monocotyledonous and dicotyledonous plants. Planta **206:** 245–252
29 Jacquot J. P., Lancelin J. M. and Meyer Y. (1997) Tansley review no 94: thioredoxins – structure and function in plant cells. New Phytol. **136:** 543–570
30 Lepiniec L., Hodges M., Gadal P. and Cretin C. (1992) Isolation, characterization and nucleotide sequence of a full-length pea cDNA encoding thioredoxin-f. Plant Mol. Biol. **18:** 1023–1025
31 Wedel N., Clausmeyer S., Herrmann R. G., Gardet-Salvi L. and Schürmann P. (1992) Nucleotide sequence of cDNAs encoding the entire precursor polypeptide for thioredoxin m from spinach chloroplasts. Plant Mol. Biol. **18:** 527–533
32 Hartman H., Syvanen M. and Buchanan B. B. (1990) Contrasting evolutionary histories of chloroplast thioredoxins f and m. Mol. Biol. Evol. **7:** 247–254
33 Sahrawy M., Hecht V., López Jaramillo J., Chueca A., Chartier Y. and Meyer Y. (1996) Intron position as an evolutionary marker of thioredoxins and thioredoxin domains. J. Mol. Evol. **42:** 422–431
34 Jeng M. F., Campbell A. P., Begley T., Holmgren A., Case D. A., Wright P. E. et al. (1994) High-resolution solution structures of oxidized and reduced *Escherichia coli* thioredoxin. Structure **2:** 853–868
35 Mittard V., Blackledge M. J., Stein M., Jacquot J. P., Marion D. and Lancelin J. M. (1997) NMR solution structure of an oxidised thioredoxin h from the eukaryotic green alga *Chlamydomonas reinhardtii*. Eur. J. Biochem. **243:** 374–383
36 Weichsel A., Gasdaska J. R., Powis G. and Montfort W. R. (1996) Crystal structures of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer. Structure **4:** 735–751
37 Capitani G., Markovic-Housley Z., Val G. del, Morris M., Jansonius J. N. and Schürmann P. (2000) Crystal structures of two functionally different thioredoxins in spinach chloroplasts. J. Mol. Biol. **302:** 135–154
38 Martin J. L. (1995) Thioredoxin – a fold for all reasons. Structure **3:** 245–250

39 Holmgren A. (1995) Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. Structure **3:** 239–243

40 Qin J., Clore G. M. and Gronenborn A. M. (1994) The high-resolution three-dimensional solution structures of the oxidized and reduced states of human thioredoxin. Structure **2:** 503–522

41 Dai S. D., Schwendtmayer C., Johansson K., Ramaswamy S., Schürmann P. and Eklund H. (2000) How does light regulate chloroplast enzymes? Structure-function studies of the ferredoxin/thioredoxin system. Q. Rev. Biophys. **33:** 67–108

42 Val G. del, Maurer F., Stutz E. and Schürmann P. (1999) Modification of the reactivity of spinach chloroplast thioredoxin f by site-directed mutagenesis. Plant Sci. **149:** 183–190

43 Swafford D. L. (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4, Sinauer Associates, Sunderland, Mass.

44 Reynolds A. E., Chesnick J. M., Woolford J. and Cattolico R. A. (1994) Chloroplast encoded thioredoxin genes in the red algae *Porphyra yezoensis* and *Griffithsia pacifica*: evolutionary implications. Plant Mol. Biol. **25:** 13–21

45 Andersson M., Holmgren A. and Spyrou G. (1996) Nk-lysin, a disulfide-containing effector peptide of T-lymphocytes, is reduced and inactivated by human thioredoxin reductase: implication for a protective mechanism against Nk-lysin cytotoxicity. J. Biol. Chem. **271:** 10116–10120

46 May J. M., Mendiratta S., Hill K. E. and Burk R. F. (1997) Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. J. Biol. Chem. **272:** 22607–22610

47 Waksman G., Krishna T. S., Williams C. H. Jr and Kuriyan J. (1994) Crystal structure of *Escherichia coli* thioredoxin reductase refined at 2 Å resolution: implications for a large conformational change during catalysis. J. Mol. Biol. **236:** 800–816

48 Williams C. H. Jr (1995) Mechanism and structure of thioredoxin reductase from *Escherichia coli*. FASEB J. **9:** 1267–1276

49 Dai S., Saarinen M., Ramaswamy S., Meyer Y., Jacquot J. P. and Eklund H. (1996) Crystal structure of *Arabidopsis thaliana* NADPH dependent thioredoxin reductase at 2.5 Å resolution. J. Mol. Biol. **264:** 1044–1057

50 Jacquot J. P., Rivera-Madrid R., Marinho P., Kollarova M., Le Marechal P., Miginiac-Maslow M. et al. (1994) *Arabidopsis thaliana* NADPH thioredoxin reductase: cDNA characterization and expression of the recombinant protein in *Escherichia coli*. J. Mol. Biol. **235:** 1357–1363

51 Williams C. H., Arscott L. D., Muller S., Lennon B. W., Ludwig M. L., Wang P. F. et al. (2000) Thioredoxin reductase – two modes of catalysis have evolved. Eur. J. Biochem. **267:** 6110–6117

52 Wang P. F., Veine D. M., Ahn S. H. and Williams C. H. Jr (1996) A stable mixed disulfide between thioredoxin reductase and its substrate, thioredoxin: preparation and characterization. Biochemistry **35:** 4812–4819

53 Lennon B. W., Williams C. H. Jr and Ludwig M. L. (2000) Twists in catalysis: alternating conformations of *Escherichia coli* thioredoxin reductase. Science **289:** 1190–1194

54 Banze M. and Follmann H. (2000) Organelle-specific NADPH thioredoxin reductase in plant mitochondria. J. Plant Physiol. **156:** 126–129

55 Droux M., Jacquot J. P., Miginac-Maslow M., Gadal P., Huet J. C., Crawford N. A. et al. (1987) Ferredoxin-thioredoxin reductase, an iron-sulfur enzyme linking light to enzyme regulation in oxygenic photosynthesis: purification and properties of the enzyme from C3, C4, and cyanobacterial species. Arch. Biochem. Biophys. **252:** 426–439

56 Huppe H. C., Lamotte F de., Jacquot J. P. and Buchanan B. B. (1990) The ferredoxin-thioredoxin system of a green alga, *Chlamydomonas reinhardtii*: identification and characterization of thioredoxins and ferredoxin-thioredoxin reductase components. Planta **186:** 341–351

57 Dai S., Schwendtmayer C., Schürmann P., Ramaswamy S. and Eklund H. (2000) Redox signaling in chloroplasts: cleavage of disulfides by an iron-sulfur cluster. Science **287:** 655–658

58 Schürmann P. and Jacquot J. P. (2000) Plant thioredoxin systems revisited. Annu. Rev. Plant Physiol. Plant Mol. Biol. **51:** 371–400

59 Chiadmi M., Navaza A., Migniac Maslow M., Jacquot J.-P. and Cherfils J. (1999) Redox signalling in the chloroplast: structure of oxidized pea fructose-1,6-bisphosphate phosphatase. EMBO J. **18:** 6809–6815

60 Dunford R. P., Durrant M. C., Catley M. A. and Dyer T. A. (1998) Location of the redox-active cysteines in chloroplast sedoheptulose-1,7-bisphosphatase indicates that its allosteric regulation is similar but not identical to that of fructose-1,6-bisphosphatase. Photosyn. Res. **58:** 221–230

61 Hirasawa M., Brandes H. K., Hartman F. C. and Knaff D. B. (1998) Oxidation-reduction properties of the regulatory site of spinach phosphoribulokinase. Arch. Biochem. Biophys. **350:** 127–131

62 Porter M. A., Stringer C. D. and Hartman F. C. (1988) Characterization of the regulatory thioredoxin site of phosphoribulokinase. J. Biol. Chem. **263:** 123–129

63 Brandes H. K., Hartman F. C., Lu T. Y. S. and Larimer F. W. (1996) Efficient expression of the gene for spinach phosphoribulokinase in *Pichia pastoris* and utilization of the recombinant enzyme to explore the role of regulatory cysteinyl residues by site-directed mutagenesis. J. Biol. Chem. **271:** 6490–6496

64 Schwarz O., Schürmann P. and Strotmann H. (1997) Kinetics and thioredoxin specificity of thiol modulation of the chloroplast H^+-ATPase. J. Biol. Chem. **272:** 16924–16927

65 Wenderoth I., Scheibe R. and Schaewen A. von (1997) Identification of the cysteine residues involved in redox modification of plant plastidic glucose-6-phosphate dehydrogenase. J. Biol. Chem. **272:** 26985–26990

66 Hirasawa M., Ruelland E., Schepens I., Issakidis-Bourguet E., Miginiac-Maslow M. and Knaff D. B. (2000) Oxidation-reduction properties of the regulatory disulfides of sorghum chloroplast nicotinamide adenine dinucleotide phosphate-malate dehydrogenase. Biochemistry **39:** 3344–3350

67 Johansson K., Ramaswamy S., Saarinen M., Lemaire-Chamley M., Issakidis-Bourguet E., Miginiac-Maslow M. et al. (1999) Structural basis for light activation of a chloroplast enzyme: the structure of sorghum NADP-malate dehydrogenase in its oxidized form. Biochemistry **38:** 4319–4326

68 Zhang N. and Portis A. R. (1999) Mechanism of light regulation of rubisco: a specific role for the larger rubisco activase isoform involving reductive activation by thioredoxin-f. Proc. Natl. Acad. Sci. USA **96:** 9438–9443

69 Scheibe R. (1991) Redox modulation of chloroplast enzymes. Plant Physiol. **96:** 1–3

70 Ruelland E. and Miginiac-Maslow M. (1999) Regulation of chloroplast enzyme activities by thioredoxins: activation or relief from inhibition? Trends Plant Sci. **4:** 136–141

71 Villeret V., Huang S. H., Zhang Y. P., Xue Y. F. and Lipscomb W. N. (1995) Crystal structure of spinach chloroplast fructose-1,6-bisphosphatase at 2.8 Angstrom resolution. Biochemistry **34:** 4299–4306

72 Jacquot J. P., López-Jaramillo J., Chueca A., Cherfils J., Lemaire S., Chedozeau B. et al. (1995) High-level expression of recombinant pea chloroplast fructose-1,6-bisphosphatase and mutagenesis of its regulatory site. Eur. J. Biochem. **229:** 675–681

73 Rodriguez-Suarez R. J., Mora-Garcia S. and Wolosiuk R. A. (1997) Characterization of cysteine residues involved in the

reductive activation and the structural stability of rapeseed (*Brassica napus*) chloroplast fructose-1,6-bisphosphatase. Biochem. Biophys. Res. Commun. **232:** 388–393

74 Jacquot J. P., López-Jaramillo J., Miginiac-Maslow M., Lemaire S., Cherfils J., Chueca A. et al. (1997) Cysteine-153 is required for redox regulation of pea chloroplast fructose-1,6-bisphosphatase. FEBS Lett. **401:** 143–147

75 Balmer Y. and Schürmann P. (2001) Heterodimer formation between thioredoxin f and fructose 1,6-bisphosphatase from spinach chloroplasts. FEBS Lett. **492:** 58–61

76 Sahrawy M., Chueca A., Hermoso R., Lázaro J. J. and Gorge J. L. (1997) Directed mutagenesis shows that the preceding region of the chloroplast fructose-1,6-bisphosphatase regulatory sequence is the thioredoxin docking site. J. Mol. Biol. **269:** 623–630

77 Miginiac-Maslow M., Johansson K., Ruelland E., Issakidis-Bourguet E., Schepens I., Goyer A. et al. (2000) Light-activation of NADP-malate dehydrogenase: a highly controlled process for an optimized function. Physiol. Plant. **110:** 322–329

78 Ashton A. R., Trevanion S. J., Carr P. D., Verger D. and Ollis D. L. (2000) Structural basis for the light regulation of chloroplast NADP malate dehydrogenase. Physiol. Plant. **110:** 314–321

79 Carr P. D., Verger D., Ashton A. R. and Ollis D. L. (1999) Chloroplast NADP-malate dehydrogenase: structural basis of light-dependent regulation of activity by thiol oxidation and reduction. Structure **7:** 461–475

80 Ruelland E., Lemaire-Chamley M., Lemarechal P., Issakidis-Bourguet E., Djukic,N. and Miginiac-Maslow M. (1997) An internal cysteine is involved in the thioredoxin-dependent activation of sorghum leaf NADP-malate dehydrogenase. J. Biol. Chem. **272:** 19851–19857

81 Goyer A., Decottignies P., Lemaire S., Ruelland E., Issakidis-Bourguet E., Jacquot J. P. et al. (1999) The internal Cys-207 of sorghum leaf NADP-malate dehydrogenase can form mixed disulphides with thioredoxin. FEBS Lett. **444:** 165–169

82 Issakidis E., Miginiac-Maslow M., Decottignies P., Jacquot J. P., Cretin C. and Gadal P. (1992) Site-directed mutagenesis reveals the involvement of an additional thioredoxin-dependent regulatory site in the activation of recombinant sorghum leaf NADP-malate dehydrogenase. J. Biol. Chem. **267:** 21577–21583

83 Issakidis E., Saarinen M., Decottignies P., Jacquot J. P., Cretin C., Gadal P. et al. (1994) Identification and characterization of the 2nd regulatory disulfide bridge of recombinant sorghum leaf NADP-malate dehydrogenase. J. Biol. Chem. **269:** 3511–3517

84 Issakidis E., Decottignies P. and Miginiac-Maslow M. (1993) A thioredoxin-independent fully active NADP-malate dehydrogenase obtained by site-directed mutagenesis. FEBS Lett. **321:** 55–58

85 Ross S. A., Zhang M. X. and Selman B. R. (1996) A role for the disulfide bond spacer region of the *Chlamydomonas reinhardtii* coupling factor 1 gamma-subunit in redox regulation of ATP synthase. J. Bioenerg. Biomem. **28:** 49–57

86 Ross S. A., Zhang M. X. and Selman B. R. (1995) Role of the *Chlamydomonas reinhardtii* coupling factor 1 γ-subunit cysteine bridge in the regulation of ATP synthase. J. Biol. Chem. **270:** 9813–9818

87 Werner-Grüne S., Gunkel D., Schumann J. and Strotmann H. (1994) Insertion of a chloroplast-like regulatory segment responsible for thiol modulation into gamma-subunit of F_0F_1-ATPase of the cyanobacterium *Synechocystis 6803* by mutagenesis of ATPc. Mol. Genet. Gen. **244:** 144–150

88 Krenn B. E., Aardewijn P., Vanwalraven H. S., Werner-Grüne S., Strotmann H. and Kraayenhof R. (1995) ATP synthase from a cyanobacterial *Synechocystis 6803* mutant containing the regulatory segment of the chloroplast gamma subunit shows thiol modulation. Biochem. Soc. Trans. **23:** 757–760

89 Groth G. and Pohl E. (2001) The structure of the chloroplast F_1-ATPase at 3.2 Angstrom resolution. J. Biol. Chem. **276:** 1345–1352

90 Konno H., Yodogawa M., Stumpp M. T., Kroth P., Strotmann H., Motohashi K. et al. (2000) Inverse regulation of F_1-ATPase activity by a mutation at the regulatory region on the gamma subunit of chloroplast ATP synthase. Biochem. J. **352:** 783–788

91 Wendt U. K., Wenderoth I., Tegeler A. and Schaewen A. von (2000) Molecular characterization of a novel glucose-6-phosphate dehydrogenase from potato (*Solanum tuberosum* L.). Plant J. **23:** 723–733

92 Austin P. A., Ross I. S. and Mills J. D. (1996) Regulation of pigment content and enzyme activity in the cyanobacterium *Nostoc* sp Mac grown in continuous light, a light-dark photoperiod, or darkness. Biochim. Biophys. Acta **1277:** 141–149

93 Gleason F. K. (1996) Glucose-6-phosphate dehydrogenase from the cyanobacterium, *Anabaena* sp pcc 7120 – purification and kinetics of redox modulation. Arch. Biochem. Biophys. **334:** 277–283

94 Wendt U. K., Hauschild R., Lange C., Pietersma M., Wenderoth I. and Schaewen A. von (1999) Evidence for functional convergence of redox regulation in G6PDH isoforms of cyanobacteria and higher plants. Plant Mol. Biol. **40:** 487–494

95 Scheibe R., Baalmann E., Backhausen J. E., Rak C. and Vetter S. (1996) C-terminal truncation of spinach chloroplast NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase prevents inactivation and reaggregation. Biochim. Biophys. Acta **1296:** 228–234

96 Reichert A., Baalmann E., Vetter S., Backhausen J. E. and Scheibe R. (2000) Activation properties of the redox-modulated chloroplast enzymes glyceraldehyde 3-phosphate dehydrogenase and fructose-1,6-bisphosphatase. Physiol. Plant. **110:** 330–341

97 Baalmann E., Scheibe R., Cerff R. and Martin W. (1996) Functional studies of chloroplast glyceraldehyde-3-phosphate dehydrogenase subunits a and b expressed in *Escherichia coli*: formation of highly active A4 and B4 homotetramers and evidence that aggregation of the B4 complex is mediated by the B subunit terminus. Plant Mol. Biol. **32:** 505–513

98 Li A. D. and Anderson L. E. (1997) Expression and characterization of pea chloroplastic glyceraldehyde-3-phosphate dehydrogenase composed only of the B-subunit. Plant Physiol. **115:** 1201–1209

99 Qi J., Isupov M. N., Littlechild J. A. and Anderson L. E. (2001) Chloroplast glyceraldehyde-3-phosphate dehydrogenase contains a single disulfide located in the C-terminal extension to the b subunit. J. Biol. Chem. **276:** 35247–35252

100 Baalmann E., Backhausen J. E., Rak C., Vetter S. and Scheibe R. (1995) Reductive modification and nonreductive activation of purified spinach chloroplast NADP-dependent glyceraldehyde-3-phosphate dehydrogenase. Arch. Biochem. Biophys. **324:** 201–208

101 Sasaki Y., Kozaki A. and Hatano M. (1997) Link between light and fatty acid synthesis – thioredoxin-linked reductive activation of plastidic acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA **94:** 11096–11101

102 Kozaki A. and Sasaki Y. (1999) Light-dependent changes in redox status of the plastidic acetyl-CoA carboxylase and its regulatory component. Biochem. J. **339:** 541–546

103 Kozaki A., Kamada K., Nagano Y., Iguchi H. and Sasaki Y. (2000) Recombinant carboxyltransferase responsive to redox of pea plastidic acetyl-CoA carboxylase. J. Biol. Chem. **275:** 10702–10708

104 Lichter A. and Häberlein I. (1998) A light-dependent redox signal participates in the regulation of ammonia fixation in chloroplasts of higher plants – ferredoxin-glutamate synthase

is a thioredoxin-dependent enzyme. J. Plant Physiol. **153:** 83–90
105 Choi Y. A., Kim S. G. and Kwon Y. M. (1999) The plastidic glutamate synthetase activity is directly modulated by means of redox change at two cysteine residues. Plant Sci. **149:** 175–182
106 Motohashi K., Kondoh A., Stumpp M. T. and Hisabori T. (2001) Comprehensive survey of proteins targeted by chloroplast thioredoxin. Proc. Natl. Acad. Sci. USA **98:** 11224–11229
107 Ballicora M. A., Frueauf J. B., Fu Y. B., Schürmann P. and Preiss J. (2000) Activation of the potato tuber ADP-glucose pyrophosphorylase by thioredoxin. J. Biol. Chem. **275:** 1315–1320
108 Hannah L. C., Shaw J. R., Giroux M. J., Reyss A. and Prioul J.-L. (2001) Maize genes encoding the small subunit of ADP-glucose pyrophosphorylase. Plant Physiol. **127:** 173–183
109 Carlberg I., Rintamäki E., Aro E. M. and Andersson B. (1999) Thylakoid protein phosphorylation and the thiol redox state. Biochemistry **38:** 3197–3204
110 Rintamäki E., Martinsuo P., Pursiheimo S. and Aro E. M. (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. Proc. Natl. Acad. Sci. USA **97:** 11644–11649
111 Kong W., Shiota S., Shi Y. X., Nakayama H. and Nakayama K. (2000) A novel peroxiredoxin of the plant *Sedum lineare* is a homologue of *Escherichia coli* bacterioferritin co-migratory protein (bcp). Biochem. J. **351:** 107–114
112 Baier M. and Dietz K. J. (1996) Primary structure and expression of plant homologues of animal and fungal thioredoxin-dependent peroxide reductases and bacterial alkyl hydroperoxide reductases. Plant Mol. Biol. **31:** 553–564
113 Baier M. and Dietz K. J. (1997) The plant 2-Cys peroxiredox in *bas1* is a nuclear-encoded chloroplast protein – its expression regulation, phylogenetic origin, and implications for its specific physiological function in plants. Plant J. **12:** 179–190
114 Baier M. and Dietz K. J. (1999) Protective function of chloroplast 2-cysteine peroxiredoxin in photosynthesis: evidence from transgenic *Arabidopsis*. Plant Physiol. **119:** 1407–1414
115 Hirasawa M., Schürmann P., Jacquot J. P., Manieri W., Jacquot P., Keryer E. et al. (1999) Oxidation-reduction properties of chloroplast thioredoxins, ferredoxin: thioredoxin reductase, and thioredoxin f-regulated enzymes. Biochemistry **38:** 5200–5205
116 Geck R. K., Larimer F. W. and Hartman F. C. (1996) Identification of residues of spinach thioredoxin f that influence interactions with target enzymes. J. Biol. Chem. **271:** 24736–24740
117 Schürmann P., Maeda K. and Tsugita A. (1981) Isomers in thioredoxins of spinach chloroplasts. Eur. J. Biochem. **116:** 37–45
118 López Jaramillo J., Chueca A., Jacquot J. P., Hermoso R., Lázaro J. J., Sahrawy M. et al. (1997) High-yield expression of pea thioredoxin m and assessment of its efficiency in chloroplast fructose-1,6-bisphosphatase activation. Plant Physiol. **114:** 1169–1175
119 Geck M. K. and Hartman F. C. (2000) Kinetic and mutational analyses of the regulation of phosphoribulokinase by thioredoxins. J. Biol. Chem. **275:** 18034–18039
120 Wangensteen O. S., Chueca A., Hirasawa M., Sahrawy M., Knaff D. B. and López Gorgé J. (2001) Binding features of chloroplast fructose-1,6-bisphosphatase-thioredoxin interaction. Biochim. Biophys. Acta **1547:** 156–166
121 Duek P. D. and Wolosiuk R. A. (2001) Rapeseed chloroplast thioredoxin-m – modulation of the affinity for target proteins. Biochim. Biophys. Acta **1546:** 299–311
122 Pedrajas J. R., Kosmidou E., Miranda-Vizuete A., Gustafsson J. A., Wright A. P. H. and Spyrou G. (1999) Identification and functional characterization of a novel mitochondrial thioredoxin system in *Saccharomyces cerevisiae*. J. Biol. Chem. **274:** 6366–6373
123 Lee S. R., Kim J. R., Kwon K. S., Yoon H. W., Levine R. L., Ginsburg A. et al. (1999) Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver. J. Biol. Chem. **274:** 4722–4734
124 Miranda-Vizuete A., Damdimopoulos A. E., Pedrajas J. R., Gustafsson J. A. and Spyrou G. (1999) Human mitochondrial thioredoxin reductase – cDNA cloning, expression and genomic organization. Eur. J. Biochem. **261:** 405–412
125 Konrad A., Banze M. and Follmann F. (1996) Mitochondria of plant leaves contain two thioredoxins – completion of the thioredoxin profile of higher plants. J. Plant Physiol. **149:** 317–321
126 Bunik V., Shoubnikova A., Loeffelhardt S., Bisswanger H., Borbe H. O. and Follmann H. (1995) Using lipoate enantiomers and thioredoxin to study the mechanism of the 2-oxoacid-dependent dihydrolipoate production by the 2-oxoacid dehydrogenase complexes. FEBS Lett. **371:** 167–170
127 Bunik V., Follmann H. and Bisswanger H. (1997) Activation of mitochondrial 2-oxoacid dehydrogenases by thioredoxin. Biol. Chem. **378:** 1125–1130
128 Watabe S., Hiroi T., Yamamoto Y., Fujioka Y., Hasegawa H., Yago N. et al. (1997) Sp-22 is a thioredoxin-dependent peroxide reductase in mitochondria. Eur. J. Biochem. **249:** 52–60
129 Araki M., Nanri H., Ejima K., Murasato Y., Fujiwara T., Nakashima Y. et al. (1999) Antioxidant function of the mitochondrial protein Sp-22 in the cardiovascular system. J. Biol. Chem. **274:** 2271–2278
130 Vanlerberghe G. C. and McIntosh L. (1997) Alternative oxidase: from gene to function. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 703–734
131 Lozano R. M., Wong J. H., Yee B. C., Peters A., Kobrehel K. and Buchanan B. B. (1996) New evidence for a role for thioredoxin h in germination and seedling development. Planta **200:** 100–106
132 Yano H., Wong J. H., Lee Y. M., Cho M. J. and Buchanan B. B. (2001) A strategy for the identification of proteins targeted by thioredoxin. Proc. Natl. Acad. Sci. USA **98:** 4794–4799
133 Kobrehel K., Wong J. H., Balogh A., Kiss F., Yee B. C. and Buchanan B. B. (1992) Specific reduction of wheat storage proteins by thioredoxin h. Plant Physiol. **99:** 919–924
134 Kobrehel K., Yee B. C. and Buchanan B. B. (1991) Role of the NADP/thioredoxin system in the reduction of alpha-amylase and trypsin inhibitor proteins. J. Biol. Chem. **266:** 16135–16140
135 Jiao J., Yee B. C., Kobrehel K. and Buchanan B. B. (1992) Effect of thioredoxin-linked reduction on the activity and stability of the Kunitz and Bowman-Birk soybean trypsin inhibitor proteins. J. Agric. Food Chem. **40:** 2333–2336
136 Besse I., Wong J. H., Kobrehel K. and Buchanan B. B. (1996) Thiocalsin – a thioredoxin-linked, substrate-specific protease dependent on calcium. Proc. Natl. Acad. Sci. USA **93:** 3169–3175
137 Cho M. J., Wong J. H., Marx C., Jiang W., Lemaux P. G. and Buchanan B. B. (1999) Overexpression of thioredoxin h leads to enhanced activity of starch debranching enzyme (pullulanase) in barley grain. Proc. Natl. Acad. Sci. USA **96:** 14641–14646
138 Hirota K., Matsui M., Iwata S., Nishiyama A., Mori K. and Yodoi J. (1997) Ap-1 transcriptional activity is regulated by a direct association between thioredoxin and ref-1. Proc. Natl. Acad. Sci. USA **94:** 3633–3638
139 Minakuchi K., Yabushita T., Masumura T., Ichihara K. and Tanaka K. (1994) Cloning and sequence analysis of a cDNA encoding rice glutaredoxin. FEBS Lett. **337:** 157–160
140 Szederkenyi J., Komor E. and Schobert C. (1997) Cloning of the cDNA for glutaredoxin, an abundant sieve-tube exudate protein from *Ricinus communis* L. and characterisation of the

glutathione-dependent thiol-reduction system in sieve tubes. Planta **202:** 349–356

141 Fisher D. B., Wu Y. and Ku M. S. B. (1992) Turnover of soluble proteins in the wheat sieve tube. Plant Physiol. **100:** 1433–1441

142 Sakuth T., Schobert C., Pecsvaradi A., Eichholz A., Komor E. and Orlich G. (1993) Specific proteins in the sieve tube exudate of *Ricinus communis* L. seedlings: separation, characterisation and in vivo labelling. Planta **191:** 207–213

143 Sjölund R. D. (1997) The phloem sieve element: a river runs through it. Plant Cell **9:** 1137–1146

144 Ishiwatari Y., Fujiwara T., McFarland K. C., Nemoto K., Hayashi H., Chino M. et al. (1998) Rice phloem thioredoxin h has the capacity to mediate its own cell-to-cell transport through plasmodesmata. Planta **205:** 12–22

145 Balachandran S., Xiang Y., Schobert C., Thompson G. A. and Lucas W. J. (1997) Phloem sap proteins from *Curcurbita maxima* and *Ricinus communis* have the capacity to traffic cell to cell through plasmadesmata. Proc. Natl. Acad. Sci. USA **1994:** 14150–14155

146 Bower M. S., Matias D. D., Fernandes-Carvalho E., Mazzurco M., Gu T., Rothstein S. J. et al. (1996) Two members of the thioredoxin-h family interact with the kinase domain of a *Brassica* S locus receptor kinase. Plant Cell **8:** 1641– 1650

147 Mazzurco M., Sulaman W., Elina H., Cock J. M. and Goring D. R. (2001) Further analysis of the interactions between the *Brassica* S receptor kinase and three interacting proteins (Arc1, Thl1 and Thl2) in the yeast two-hybrid system. Plant Mol. Biol. **45:** 365–376

148 Cabrillac D., Cock J. M., Dumas C. and Gaude T. (2001) The S-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. Nature **410:** 220–223

149 Laloi C., Rayapuram N., Chartier Y., Grienenberger J.-M., Bonnard G. and Meyer Y. (2001) Identification and characterisation of a mitochondrial thioredoxin system in plants. Proc. Natl. Acad. Sci. USA **98:** 14144–14149


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