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Review

The plant thioredoxin system

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Abstract. Thioredoxins are small proteins catalyzing thiol-disulfide interchange and are involved in the regulation of the redox environment of the cell. In plants, the thioredoxin system is particularly complex since at least 20 thioredoxin isoforms are found in the plant model *Arabidopsis thaliana*. Based upon primary sequence analysis and subcellular localization, thioredoxins can be classified into different groups and subgroups. Different pathways allowing thioredoxin reduction also coexist in the plant involving ferredoxin-thioredoxin reductase, thioredoxin reductases and the glutathione/glutaredoxin system. This review discusses the literature of plant thioredoxins with emphasis on recent findings in the field.

Key words. Disulfide bridge; redox regulation; thioredoxins; thiol reduction.

Introduction

Several fundamental processes mediating and regulating enzyme activities are performed through sensor proteins and transmitters of redox signals. Oxidation-reduction of cysteinyl residues is a key factor in these processes, allowing the activity modulation of several enzymes. Cysteinyl residues can undergo oxidation to form disulfides (S-SR), sulfenic acid (S-OH), sulfinic acid ($-SO_2H$) or sulfonic acid ($-SO_3H$). The development of proteomic as well as other approaches led to the identification, in all kingdoms, of numerous post-translationally redox-regulated proteins. Apart from low molecular weight compounds such as glutathione and ascorbic acid, cellular redox control is mediated mainly by two sets of related proteins: the thioredoxins and glutaredoxins. The plant glutaredoxin system has been recently reviewed [1, 2], and we will focus in this review on current knowledge about the thioredoxin system in plants. Since several aspects of this topic have been reviewed recently [3–9], this review will only deal with most recent advances in the field.

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Sequence comparisons and thioredoxin diversity in plants

Thioredoxins are small proteins (around 12 kDa) that are present in all organisms. In addition to these 'classical' thioredoxins, numerous proteins also exhibit thioredoxin-like domains or multiple thioredoxin domains. These proteins differ from the classical thioredoxins and are not reviewed in this article. In plants, the thioredoxin system is particularly developed in comparison with other organisms. Indeed, in *Arabidopsis thaliana* at least 20 thioredoxin genes have been reported in the whole sequenced genome [10]. An alignment of the amino acid sequences of different thioredoxins found in *A. thaliana* is shown in figure 1. Primary structure analysis allows a classification of the reported isoforms: Trx*f*, Trx*h*, Trx*m*, Trx*o*, Trx*x* and Trx*y* thioredoxins. The increasing number of expressed sequence tags (ESTs) found in databases reveals that the different thioredoxin groups are present in all higher plants studied (poplar, pinus, tomato, soybean and so on) suggesting that the thioredoxin diversity found in *A. thaliana* is representative of all higher plants. Based on the primary structure analysis, a phylogenetic tree was constructed using Clustalw (http://clustalw.genome.

Figure 1. Amino acid sequence comparison of the thioredoxins of *A. thaliana*. The alignment was performed with ClustalW. The protein entry codes are the following: ath1: At3g51030 ; ath2: At5g39950; ath3: At5g42980; ath4: At1g19730 ; ath5: At1g45145; ath7: At1g59730; ath8: At1g69880; ath9: At3g08710; ato1: At2g35010; ato2: At1g31020; Atm1: At1g03680; Atm2: At4g03520; Atm3: At2g15570; Atm4: At3g15360; Atf1: At3g02730; Atf2: At5g16400; Atx: At1g50320; Aty1: At1g76760; Aty2: At1g43560; AtCXXS1: At1g11530. The atypical AtCXXS2 (At2g40790) has been omitted from this alignment. Catalytic sites are in yellow, strictly conserved amino acids in black on blue. The residue 101 important in the thioredoxin *h* classification (Ath3 numeration) is depicted in pink on gray.

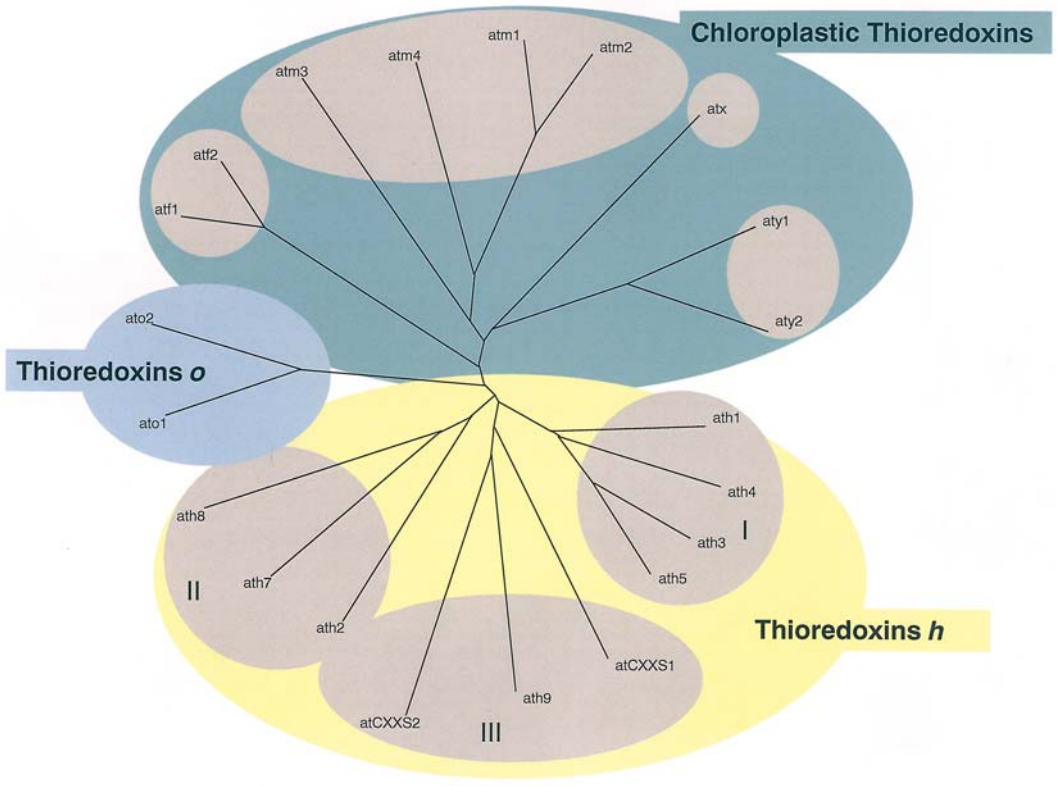

Figure 2. Phylogenetic tree of the *A. thaliana* Trx isoforms. This tree was drawn using ClustalW. The protein entry codes are identical to those of figure 1.

ad.jp/), encompassing the various thioredoxin isoforms found in *A. thaliana* (fig. 2). Comparisons with prokaryotic and eukaryotic sequences show that thioredoxins *m*, *x*, and *y* are of prokaryotic origin [10, 11]. In contrast, phylogenetic analyses suggest an eukaryotic origin for thioredoxins *f*, *h* and *o* [10, 12, 13].

In addition to primary structure analysis, subcellular localization of the different thioredoxins is an important factor in understanding the plant thioredoxin system. The chloroplast contains the thioredoxins *f*, *m*, *x* and *y* and the atypical CDSP32 (chloroplast dought-induced protein). The corresponding amino acid sequences possess a N-terminal extension. These extensions are likely to correspond to a putative transit peptide for targeting to the chloroplast. All sequences are predicted to be addressed to the chloroplast with a very high score by Predotar, iPSORT and TargetP (links available at http://www.expasy.ch). The chloroplastic localization of *f*, *m* and *x* thioredoxins has been confirmed by fusion green fluorescent protein (GFP) experiments using the *A. thaliana* isoforms [14].

The thioredoxins *o*, at least AtTrx*o*1, have been recently demonstrated to be present in mitochondria. These isoforms, which exhibit a N-terminal extension, are predicted to be addressed to the mitochondria. This localization has been confirmed by mitochondrial import experiments [13] and by GFP fusion experiments for AtTrx*o*1 [14]. The mitochondrial system could be completed by at least one isoform of thioredoxin *h*. Based on primary structure analysis, thioredoxin *h* could be divided in to three subgroups [15, 16]. Our group has recently demonstrated that one poplar isoform (PtTrx*h*2) belonging to the subgroup 2 [17] is associated with mitochondria [18].

Besides this isoform, the thioredoxin *h* group found in *A. thaliana* constitutes a large disparate group that includes some thioredoxin isoforms exhibiting N-terminal extensions. Nevertheless, cell sorting prediction programs suggest that these isoforms are cytosolic. This point is in disagreement with previous results showing the purification of thioredoxin *h* from mitochondria [19, 20] and endoplasmic reticulum [19]. Furthermore, Trx*h*1 and Trx*h*2 from soybean belonging to the same subgroup as PtTrx*h*2 exhibit a hydrophobic N-terminal extension, suggesting that they could be bound to membranes [21]. Nuclear localization of thioredoxin *h* has been also demonstrated [22]. Finally, recent data demonstrate that subcellular localization may not depend only on the signal peptide presence [23, 24], suggesting that subcellular localization of thioredoxin *h* needs to be investigated.

Physical and mechanistic aspects of thioredoxin

A thioredoxin is defined both by its structural and catalytic characteristics. It is able to reduce disulfide bonds (the model used is insulin). In addition, thioredoxins are very stable proteins containing around 110 amino acids in their mature form (excluding the transit peptides of the nuclear encoded chloroplastic and mitochondrial isoforms). The major feature of thioredoxins is the presence of two cysteinyl residues involved in the very conserved catalytic site WC[G/P]PC. A few thioredoxin *h* isoforms belonging to subgroup 3 [10, 16] exhibit an unusual catalytic site where the C-terminal cysteine residue is changed to a serine (CXXS). In classical catalytic sites, both cysteinyl residues are involved in the catalytic activities of the enzyme, allowing disulfide bridge reduction of the target protein (fig. 3). The redox potential of thioredoxin is also critical in governing its activity. The redox potential of plant thioredoxins ranges between –285 and –350 mV [14, 25], with the notable exception of PtTrx*h*4. This poplar isoform belongs to thioredoxin *h* subgroup 3 [16] and exhibits a redox potential around –200 mV [unpublished results], explaining its unusual reduction pathway, which involves glutaredoxins (see below). The amino acid environment of the cysteinyl residues is critical for thioredoxin disulfide reductase activity. Protein disulfide isomerases (PDIs) catalyze the formation of disulfide bridges and also possess a CXXC active site (CGHC). Eukaryotic PDIs exhibit an E_m ranging from –147 to –175 mV [26]. In Dsba, which exhibits a CGHC catalytic site and oxidase activity, the pKa of the N-terminal active site cysteine is decreased in comparison to thioredoxin. Stabilization of the thiolate is due to an additional hydrogen bond with the active site histidine [27]. Different mutagenesis studies have shown that the sequences of the X–X dipeptides are a determinant of the redox potential of the thioredoxin superfamily of oxidoreductases and are also important in interactions with target proteins [28–30]. In particular, several thioredoxins *h* exhibiting the unusual catalytic site WCPPC have been reported [10]. In *A. thaliana*, among five isoforms belonging to subgroup I, three are WCPPC [10]. The redox potential of these isoforms is similar to the WCGPC isoforms (around –285 mV) [25]. Nevertheless, yeast complementation experiments have shown that modification of AtTrx*h*3 active site (WCPPC to WCGPC) restores a partial sulfate assimilation phenotype [31]. Furthermore, mutation of the PtTrx*h*3 active site (WCGPC to WCPPC) strongly modifies the protein conformation [32]. All these data suggest that the prolyl residue could play a role in the active site conformation, leading to specific interactions with target proteins.

Thioredoxins as well as the other members of the thioredoxin superfamily exhibit similar three-dimensional (3D) architecture. The description of these 3D structures has been reviewed recently [4, 6, 8] and will be not discussed here.

The chloroplastic thioredoxin system: Composition, mode of reduction and target enzymes

The multiplicity of the different thioredoxin isoforms found in chloroplasts 4*m*, 2*f*, 1*x* and 2*y* in *A. thaliana* raises the question of thioredoxin specificity and function. It is well documented that chloroplastic thioredoxins are associated with light regulation of carbon metabolism through regulation of the reducing pentose phosphate pathway and also of the C_4 pathway. Under oxidizing conditions prevailing in the dark, a number of enzymes are inactive due to the oxidized state of critical cysteinyl residues. Following a dark-to-light transition, these residues are reduced to the thiol state by thioredoxins present in the chloroplast stroma, leading in general to activation of the biocatalysts with the notable exception of glucose-6-phosphate dehydrogenase, which is inhibited during a dark-to-light transition [33]. The reducing power provided by light is mediated by the ferredoxin/thioredoxin system composed of ferredoxin, ferredoxin-thioredoxin reductase (FTR) and thioredoxins (fig. 3). This system has recently been reviewed and will not be detailed here [8, 34, 35]. Only two thioredoxin types have been implicated together with the ferredoxin/thioredoxin system, thioredoxins *f* and *m*. Historically, the Trx*m*s were

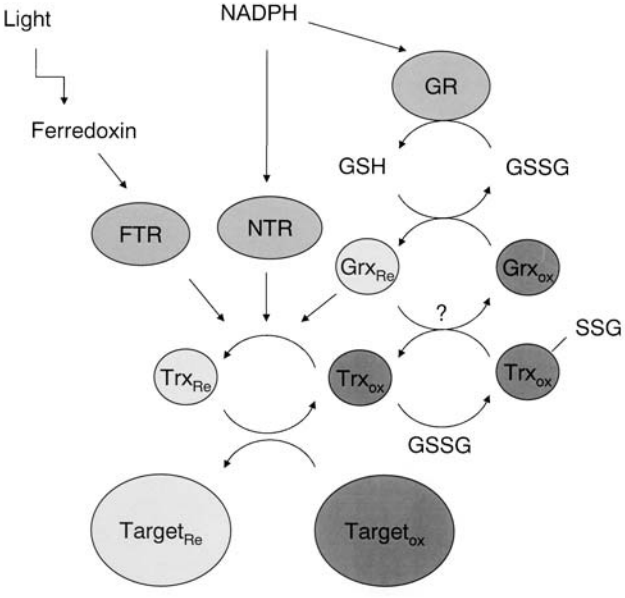

Figure 3. Different pathways of Trx reduction. The chloroplastic pathway involves ferredoxin-thioredoxin reductase (FTR). In mitochondria and cytoplasm, thioredoxins could be reduced by NADP-thioredoxin reductase (NTR). Particular thioredoxins could also be reduced via the glutathione (GSH/GSSG)/glutaredoxin (Grx) system. Glutathionylation of Trx has also been demonstrated.

so named because they were able to activate the well-characterized NADP malate dehydrogenase (NADP-MDH) [7], while Trx*f* has been demonstrated as a specific activator of fructose-1,6-biphosphatase (FBPase). Among the different protein targets biochemically characterized so far, many Calvin cycle enzymes are redox regulated via specific interactions with Trx*f* [7]. This suggests a specificity of thioredoxin *f* for photosynthetic carbon assimilation.

Among the four AtTrx*m*s, AtTrx*m*1, *m*2 and *m*4 have been shown to efficiently activate NADP-MDH [14]. Furthermore, using proteomic tools, these three isoforms have been found associated with the stromal side of the thylakoid membrane [36], even if this association seems to be very weak [37]. This localization has also been shown for 2-Cys peroxiredoxin [36–38]. Peroxiredoxins constitute a multigenic family involved notably in reactive oxygen species reduction (ROS) [39]. Among the nine expressed peroxiredoxins found in the *A. thaliana* genome, four isoforms are found in chloroplasts, two dimeric 2-Cys Prx, one type II Prx and one Prx Q [40]. Nevertheless, different chloroplastic thioredoxins (*f*, *m*, *x* and *y*) as well as one thioredoxin-like chloroplast drought-induced protein of 32 kDa [41] are able to reduce in vitro 2-Cys AtPrx as well as poplar PrxQ [14, 42]. AtTrx*x* is the most efficient thioredoxin tested in the reduction of 2-Cys Prx, suggesting that this isoform could be involved in vivo in peroxide detoxication [14].

AtTrx*m*3 exhibits specials properties. In in vitro tests, this isoform is not able either to activate NADH-MDH and FBPase or to reduce 2-Cys Prx [14], and its expression pattern differs from the other thioredoxins *m* [43], suggesting that this isoform could be involved in specific functions in non-green plastids.

One Trx*y* isoform conserved in photosynthetic organisms has been characterized in the green algae *Chlamydomonas reinhardtii* [11]. *Cr*Trx*y* is efficient in reduction of various Prxs [11, 42], but is probably not involved in, in vivo activation of FBPase or NADP-MDH [11].

The thioredoxin reduction pathway in chloroplasts is probably not only dependent on the FTR system. Indeed, *A. thaliana* genome analysis has suggested the presence of a putative NADPH-thioredoxin reductase (NTR, see below and fig. 3) containing also a thioredoxin domain in chloroplasts [22, 44]. This protein has been recently characterized in rice as a bifunctional enzyme exhibiting both thioredoxin and NTR activity but not a NTR/thioredoxin system. Nevertheless, the NTR domain is unable to reduce the thioredoxins tested, particularly thioredoxins *f* and *m*. Knockout experiments have suggested that this protein is probably involved in protecting of chloroplasts against oxidative damage [45].

The diversity of chloroplastic thioredoxin isoforms is in accordance with the number of demonstrated or putative target proteins. Nevertheless, in most cases the specificity of these protein-thioredoxin interactions remains undefined. Recently, the development of proteomics coupled with the identification of redox-regulated proteins led to a dramatic increase of the number of potential thioredoxin targets in chloroplasts [46–48]. The potential thioredoxin-interacting proteins are listed in table 1. The number of potential functions cannot be detailed here and remain to be confirmed in several cases. Apart from its well-documented dark-to-light transition, the thioredoxin system participates in responses against environmental stresses. In most cases, abiotic or biotic stresses are linked to the production of ROS. The thioredoxin system is involved in ROS detoxication at least through several peroxiredoxin isoforms [38, 42], glutathione peroxidases [49] and peptide methionine sulfoxide reductases [50, 51].

The mitochondrial thioredoxin system: composition, mode of reduction and target enzymes

The plant mitochondrial thioredoxin system involving thioredoxin *o* and NTR was recently described in *A. thaliana* [13]. In this system, reducing equivalents for thioredoxin reduction are provided by NADPH through NTR (fig. 3). This system is ubiquitous, but despite their similarities the NTRs of plants and animals are fundamentally different. In animals, NTRs are very homologous to glutathione reductases [52]. These homodimers of 55-kDa subunits usually contain a selenocysteine residue present in a carboxy-terminal active site [53]. In plants, as well as in fungi, archea and bacteria, the NTR proteins are found as homodimers of 35-kDa subunits without selenocysteine residues [54, 55]. *Chlamydomonas reinhardtii* exhibits both NTR forms [56]. In *A. thaliana*, two genes encoding typical NTRs have been found in the whole sequenced genome and called *ntrA* and *ntrB* [10]. *ntrA* produced two different messengers: one shorter, encoding a cytosolic protein, and one longer, featuring a signal peptide that allows mitochondrial importation of the protein [13]. This mitochondrial NTRA has been shown to efficiently reduce AtTrxo1, the first characterized plant mitochondrial thioredoxin [13]. Two thioredoxins *o* have been described in *A. thaliana*, AtTrx*o*1 and AtTrx*o*2; nevertheless, only AtTrx*o*1 has been shown to be localized in mitochondria, while the subcellular localization of AtTrx*o*2 remains unclear [13]. Thioredoxin *o* genes have been found in other plant genomes [25]. Besides the *o* type of thioredoxins, plant mitochondria could also contain thioredoxin *h*. The poplar thioredoxin *h*2 (PtTrx*h*2) [17] was recently shown to be associated with mitochondria using both GFP fusion and immunolocalization experiments [18]. PtTrx*h*2 is reduced efficiently in vitro by AtNTRA, suggesting

Table 1. Potential target of thioredoxins in the chloroplasts.

	References		References
Carbon assimilation		**Photorespiration**	
Transketolase	[47]	Glycerate kinase	[93]
Triose phosphate isomerase	[47]		
Ribulose-P-3-epimerase	[47]	**Translation**	
Carbonic anhydrase	[47]	RB60	[94]
Sedoheptulose-1,7-biphosphatase	[78]	Elongation factor Tu	[47]
Phosphoribulokinase	[79]	Elongation factor g	[47]
Glyceraldehyde-3P- dehydrogenase	[80]	Ribonucleoprotein	[47]
Rubisco activase	[81]	30S ribosomal protein S1	[47]
Rubisco small subunit	[47]	Ribosomal protein S6 (PrpS6)	[47]
Fructose-1,6-biphosphatase	[82]		
		DNA replication/transcription	
Metabolism		ATP-dependent DNA helicase	[47]
Glutamate synthase			
3-deoxy-D-arabino-heptulosonate 7-phosphate synthase	[83, 84]	**Plastid division**	
		FtsZ protein	[47]
NADP-malate dehydrogenase	[85]		
Acetyl-CoA carboxylase	[86]	**Protein degradation**	
CF1 ATPase	[87]	ATP dependent clp protease	[47]
Glucose-6P-dehydrogenase	[88]	Magnesium chelatase	[47]
Cysteine synthase	[47]		
ADP-glucose pyrophosphorylase	[89]	**Vitamin biosynthesis**	
Cyclophilin	[90]	Thiamin biosynthesis protein	[47]
Phosphoglycerate dehydrogenase	[47]	Thiazole biosynthetic enzyme	[47]
6-phosphogluconate dehydrogenase	[47]		
Enolase	[91]	**Stress coupled reactions**	
β-amylase	[47]	Peroxiredoxin Q	[46]
		2-Cys Peroxiredoxin	[95]
Photosystem		Glutathione peroxidase	[96]
LHCII protein kinase	[92]	Methionine sulfoxide reductase	[16]

that this isoform could also be reduced in vivo. These data confirm previous studies demonstrating the presence of Trx*h* in mitochondria [19]. The presence of at least two distinct thioredoxins in mitochondria raises the question of the specificity of each type. Recent proteomics experiments have detected numerous potential thioredoxin targets in mitochondria involved in several fundamental processes [57]. Indeed, up to 50 potential Trx-linked proteins are potentially involved in 12 processes: photorespiration, citric acid cycle, lipid metabolism, electron transport, ATP synthesis, membrane transport, translation, protein folding, nitrogen metabolism, sulfur metabolism, hormone synthesis and stress-related reactions. Biochemical experiments now need to be performed to determine the possible specificity of each mitochondrial thioredoxin type towards these targets. In addition, PtTrx*h*2 as well as AtTrx*o*1 have been shown to activate alternative oxidase (AOX) [18]. AOX couples the oxidation of ubiquinol to the complete reduction of oxygen to water. This pathway does not contribute to ATP synthesis but can dampen the mitochondrial generation of ROS. AOX activity is submitted to post-translational regulation involving the reduction of an intersubunit disulfide bridge and allowing AOX activation by α-keto acids (for review, see [58]). This disulfide bridge reduction could be performed by thioredoxins. AOX could play a role in the regulation of the programmed cell death [59]. In addition, mitochondrial thioredoxins could play another role in apoptosis regulation, modulating porin conformation [57, 60]. The mitochondrial redox state and also probably the mitochondrial thioredoxin system play a key role in plant redox homeostasis and particularly in stress resistance induction [59].

The thioredoxin *h* families and cytososolic thioredoxins

Based on primary structure analysis, thioredoxins *h* constitute a large disparate group which can be divided in the three different subgroups [6, 10, 16], called subgroups I, II and III [9].

The classification of thioredoxins h needs to be improved by considering both their subcellular localization and their participation in distinct reduction pathways. We also propose a more detailed classification, presented below.

The subgroup I comprises thioredoxins, which are believed to be cytosolic. An analysis of 41 subgroup I

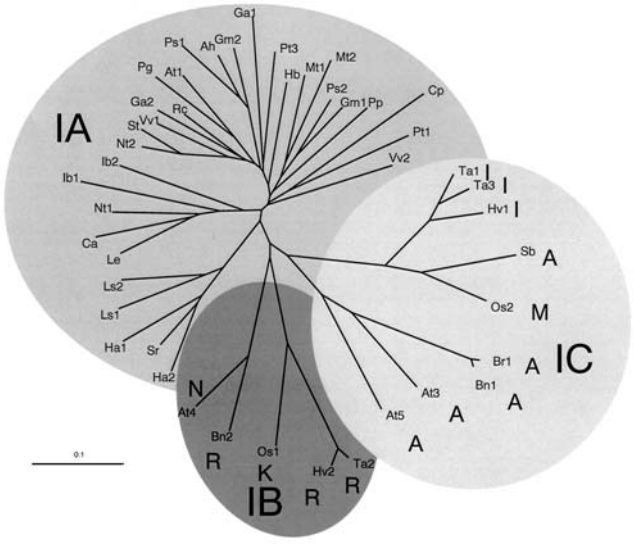

Figure 4. Phylogenetic tree of several thioredoxins *h* belonging to the subgroup I. This tree was drawn using ClustalW. Three clusters named IA, IB and IC can be distinguished according to their primary sequence. In clusters IB and IC, residue 101 is shown. The codes used are the following: Ah: *Arachnis hypogaea* (CD038084); At1: *A. thaliana* (At3g51030); At3: *A. thaliana* (At5g42980); At4: *A. thaliana* (At1g19730); At5: *A. thaliana* (At1g45145); Bn1: *Brassica napus* (U59379); Bn2: *Brassica napus* (U59380); Br1: *Brassica rapa* (AB10434); Ca: *Capsicum annuum* (AY496104); Cp: *Citrus paradisi* (AY271308); Ga1: *Gossypium arboreum* (BQ408049); Ga2: *Gossypium arboreum* (TC25963); Gm1: *Glycine max* (AI461219); Gm2: *Glycine max* (BI699372) Ha1: *Helianthus annus* (TC12357); Ha2: *Helianthus annus* (TC12867); Hb: *Hevea brasiliensis* (CB377001); Hv1: *Hordeum vulgare* (AY245454); Hv2: *Hordeum vulgare* (AY245455); Ib1: *Ipomoea batatas* (AY344230); Ib2: *Ipomoea batatas* (BJ561302); Le: *Lycopersicon esculentum* (TC116392); Mt1: *Medicago truncatula* (TC85696); Mt2: *Medicago truncatula* (TC87208); Ls1: *Lactuca sativa* (TC9289); Ls2: *Lactuca sativa* (BQ873424); Nt1: *Nicotiana tabacum* (X58527); Nt2: *Nicotiana tabacum* (Z11803); Os1: *Oryza sativa* (Q42443); Os2: *Oryza sativa* (Q9FRT3); Pg: *Peppermint glandular* (AW255457); Pp: *Prunus persica* (AF323593); Ps1: *Pisum sativum* (AY170650); Ps2: *Pisum sativum* (AJ310990); Pt1: *Populus tricocharpa cv. Trichobel* (AF483625); Pt3: *Populus tricocharpa cv. Trichobel* (BU822062); Rc: *Ricinus communis* (Z70677); Sb: *Sorghum bicolor* (TC87208); Sr: *Stevia rebaudiana* (BG525644); St: *Solanum tuberosum* (BM111010); Ta1: *Triticum aestivum* (CD886902); Ta2: *Triticum aestivum* (CD892602); Ta3: *Triticum aestivum* (BJ210524); Vv1: *Vitis vinifera* (CF216136); Vv2: *Vitis vinifera* (CB348011).

thioredoxins *h* shows that these proteins can be classified into three different clusters, which we have named IA, IB and IC (fig. 4). *A. thaliana* thioredoxins *h*3, *h4* and *h5* as well as brassica sequences are related to cereal thioredoxins in cluster IB and IC, whereas AtTrx*h*1 is present in cluster IA. A classification has been recently proposed based on the residue at position 101 [15, 61]. This residue could be present on the protein surface from ca. 12 Å of the active site [61]. AtTrx*h*3 and AtTrx*h*5 cluster in IC with thioredoxins exhibiting a hydrophobic residue in this position (A, I or M). In contrast, AtTrx*h*4 is related to thioredoxins harbouring a charged or hydrophilic residue (R, K or N) in cluster IB. This 101 residue is also involved in a structural motif [R_{101}KDD] critical for transfer from companion cells to sieve-tube elements [62, 63]. Indeed, thioredoxins *h* have been found abundantly in phloem sieve tubes from several plants [62, 64, 66]. Nevertheless, AtTrx*h*5 exhibits a [A_{101}KDE] motif and has also been reported to be expressed in vascular tissues [65]. Besides this C-terminal motif, the N-terminal sequence MAAEE also seems to be required for transfer through plasmodesmata [63].

Recent studies have shown that *A. thaliana* thioredoxins *h* differ by their cell type and specificity of expression [65, 67]. Among the thioredoxins *h* subgroup I, Attrx*h*1 and Attrx*h*4 expressions are correlated with the cell cycle, suggesting a role in redox control of cell proliferation [68]. In contrast, AtTrx*h*5 seems to be specifically involved in response to pathogens and oxidative stresses [67]. In cereals, thioredoxins *h* are found throughout the plant but are more abundant in mature seeds [64, 69]. During seed maturation, TaTrx*h*A has been detected in the nucleus of aleurone and scutellum cells, this localization corresponding to oxidative conditions in these tissues [22].

The second subgroup of thioredoxins *h* encloses proteins harbouring N-terminal extensions. Primary analysis of 28 sequences show that these thioredoxins can be divided in different clusters, one corresponding to cereal sequences called IIA, one containing the mitochondrial PtTrx*h*2, AtTrx*h*7 and AtTrx*h*8 (see above) named IIB and one containing thioredoxins related to AtTrx*h*2 named IIC (fig. 5). Concerning proteins of cluster IIB, the different reported sequences have been submitted to the prediction program (http://hypothesiscreater.net/iPSORT/), showing that these isoforms could also possess a signal peptide. Nevertheless, AtTrx*h*7, which belongs to this cluster, is not currently predicted to be associated with mitochondria, which does not exclude a possible mitochondrial localization. Despite this obvious exception, cluster IIB may include mitochondrial thioredoxins *h*.

Cluster IIC also constitutes a homogeneous group which exhibits a N-terminal extension. In soybean, a thioredoxin belonging to this subgroup has been shown to be anchored to the plasma membrane [21]. Very few studies have been performed on these thioredoxins, preventing any conclusion about the significance of this N-terminal extension. Complementation experiments have shown that AtTrx*h*2 allows yeast Trx mutants to grow on sulfate [70]. Furthermore, Attrx*h*2 gene expression is correlated to the cell cycle, exhibiting a peak of expression in G2 phase [68].

The third subgroup of thioredoxins *h* comprises the atypical thioredoxins CXXS and thioredoxins exhibiting the usual catalytic site WCGPC (fig. 6). The CXXS thioredoxins cannot be considered as true thioredoxins and are

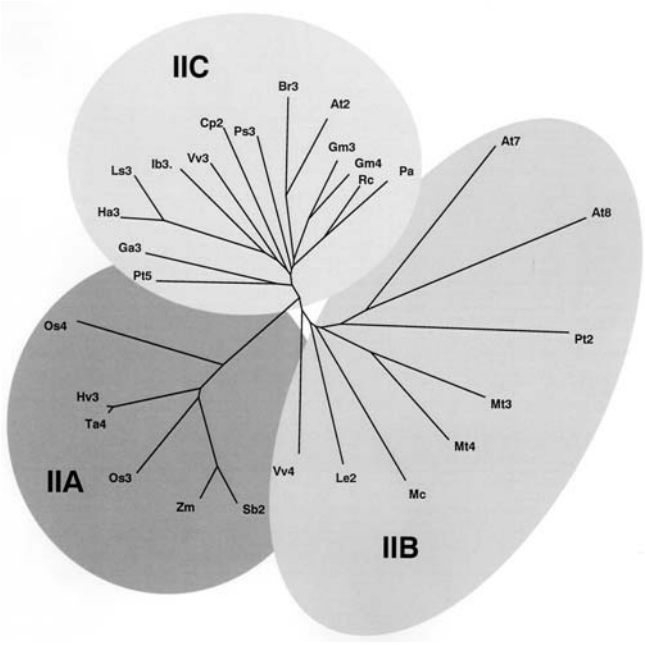

Figure 5. Phylogenetic tree of different thioredoxins *h* belonging to subgroup II. This tree was drawn using ClustalW. Three clusters named IIA, IIB and IIC can be distinguished according to their primary sequence. The codes used are the following: At2: *Arabidopsis thaliana* (At5g39950); At7: *Arabidopsis thaliana* (At1g59730); At8: *Arabidopsis thaliana* (At1g69880); Cp2: *Citrus paradisi* (CF837405); Br3: *Brassica rapa* (AF352030); Ga3: *Gossypium arboreum* (BG440056) Gm3: *Glycine max* (AW620807); Gm4: *Glycine max* (BM188930); Ha3: *Helianthus annus* (TC13569); Ib3: *Ipomoea batatas* (AY344228); Hv3: *Hordeum vulgare* (BI960260); Le2: *Lycopersicon esculentum* (TC135169); Ls3: *Lactuca sativa* (TC9551); Mc: *Mesembryanthemum crystallinum* (BE033809); Mt3: *Medicago truncatula* (AW560796); Mt4: *Medicago truncatula* (AW686237); Os3: *Oryza sativa* (AK062383); Os4: *Oryza sativa* (CB681257); Pa: *Prunus armeniaca* (CB818939); Ps3: *Pisum sativum* (AY170651); Pt2: *Populus tricocharpa cv. Trichobel* (AF483266); Pt5: *Populus tricocharpa cv. Trichobel* (BU869308); Rc: *Rosa chinensis* (BI978567); Sb2: *Sorghum bicolor* (TC76743); Ta4: *Triticum aestivum* (CD894811); Vv3: *Vitis vinifera* (CF513794); Vv4: *Vitis vinifera* (TC25464); Zm: *Zea mays* (AY104013).

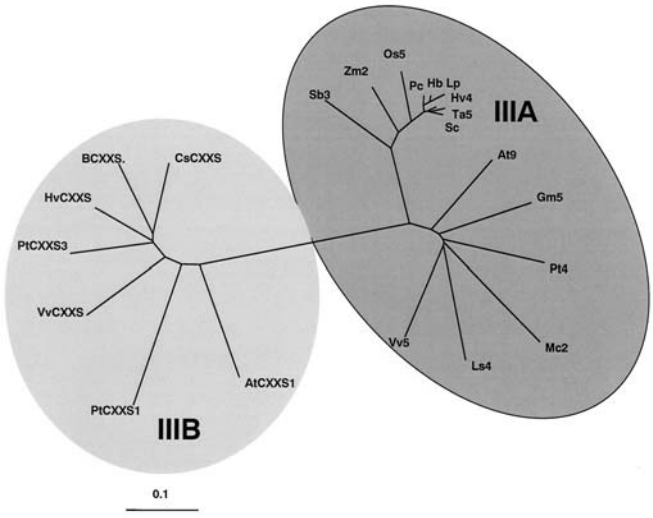

Figure 6. Phylogenetic tree of different thioredoxins *h* belonging to the subgroup III. Two clusters named IIIA and IIIB can be distinguished according to their primary sequence. Cluster IIIA enclosed the thioredoxins harbouring the usual active site WCGPC, whereas the members of cluster IIIB exhibit a CXXS catalytic site. The codes used are the following: At9: *Arabidopsis thaliana* (At3g08710); Gm5: *Glycine max* (CA799351); Hb: *Hordeum bulbosum* (AF159385); Hv4: *Hordeum vulgare* (AF435815); Lp: *Lolium perenne* (159387); Ls4: *Lactuca sativa* (TC9851); Mc2: *Mesembryanthemum crystallinum* (CA838461); Os5: *Oryza sativa* (AF435817); Pc: *Phalaris coerulescens* (AF159388); Pt4: *Populus tricocharpa cv. Trichobel* (BU835000); Sb3: *Sorghum bicolor* (TC72759); Sc: *Secale cereale* (AF159386); Ta5: *Triticum aestivum* (AF438359); Vv5: *Vitis vinifera* (CB004453); Zm2: *Zea mays* (AF435816); AtCXXS1: *Arabidopsis thaliana* (At1g11530); BpCXXS: Betula pendula (CD278293); CsCXXS: *Citrus sinensis* (BQ623126); HbCXXS: *Hevea brasiliensis* (AF133127); PtCXXS1: *Populus tricocharpa cv. Trichobel* (CA823821); PtCXXS3: *Populus tricocharpa cv. Trichobel* (BU874060); VvCXXS: *Vitis vinifera* (CF204852). The atypical AtCXXS2 clusters separately. Databases analysis did not reveal any plant AtCXXS2 orthologues. Consequently, AtCXXS2 has been omitted in the construction of this phylogenetic tree.

more likely related to the monothiol glutaredoxin superfamily [1]. Indeed, the absence of the second cysteinyl residue in the catalytic site prevents 'true' thioredoxin activity. In vitro experiments performed with PtCXXS3 have confirmed this hypothesis [16]. Nevertheless, these atypical thioredoxin genes have introns at the same positions than the other thioredoxins *h* [10]. In fact, the main feature of the third subgroup is possible interactions with the glutaredoxin/glutathione system. Indeed, in contrast with the other thioredoxins *h* (belonging to subgroups I and II), these proteins are not reduced by NTRs [15, 16]. PtCXXS3 is active in a glutathione: hydroxyethyldisulfide (HED) transhydrogenase assay, suggesting glutaredoxin-like activity [1]. Furthermore, PtTrx*h*4, which possesses a classical WCGPC active site, exhibits true thioredoxin activity but is reduced by glutaredoxins. An amino acid comparison of different PtTrx*h*4-related thioredoxins shows that a third cysteinyl is conserved in the N-terminal part of the protein (fig. 7). Mutagenesis experiments have shown that this third residue is probably involved in protein reduction by glutaredoxin [E. Gelhaye et al., unpublished]. PtTrx*h*4 exhibits an E_m value around −200 mV at pH 7 [E. Gelhaye et al., unpublished], a value quite elevated over the one reported for other thioredoxins [18, 25], but adequate to allow reduction by glutaredoxins [1]. This subgroup also constitutes a direct interconnection between the two major systems involved in cellular redox regulation.

The number of potential targets of thioredoxins *h* has considerably increased with the development of proteomics [71–75]. Nevertheless, the as yet undefined specificity of interactions between thioredoxins and target proteins prevents firm conclusions at this point.

Figure 7. Amino acid sequence comparison of thioredoxins belonging to the cluster IIIA. The alignment was performed with ClustalW. The codes used are identical to those used in figure 6. Catalytic sites and the third conserved cysteine are in white on black.

Conclusion and perspectives

The thioredoxin system is particularly complex in higher plants, involving numerous isoforms present in all plant compartments. The chloroplast system is the best documented; nevertheless, the function of each isoform remains unclear. The development of proteomics tools led to the identification of numerous potential thioredoxin-regulated proteins; biochemical studies are needed to confirm these interactions. The plant mitochondrial system was discovered only very recently, and other thioredoxins may also be present in this organelle. Mitochondrial thioredoxins seem to be involved in fundamental processes in particular in apoptosis regulation; again these interactions have to be confirmed.

In the field of redox regulation, it is obvious that the different systems are directly connected. Indeed, human thioredoxin activity is modulated by glutathionylation [76], and yeast Grx5 may be reduced by Trx [77]. In plants, PtTrx*h*4 is directly reduced by Grx, and PtCXXS3 exhibits a glutaredoxin-like activity. Furthermore, it was recently established that PtTrx*h*2 redox potential is modulated by glutathionylation (fig. 3) [18]. These interconnections between major redox regulating systems are probably physiologically important. The study of these relationships will certainly lead to a better understanding of plant cell redox regulation.

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