

Redox Regulation and Flower Development: A Novel Function for Glutaredoxins

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Abstract: Glutaredoxins (GRXs) are small, ubiquitous oxidoreductases that have been intensively studied in *E. coli*, yeast and humans. They are involved in a large variety of cellular processes and exert a crucial function in the response to oxidative stress. GRXs can reduce disulfides by way of conserved cysteines, located in conserved active site motifs. As in *E. coli*, yeast, and humans, GRXs with active sites of the CPYC and CGFS type are also found in lower and higher plants, however, little has been known about their function. Surprisingly, 21 GRXs from *Arabidopsis thaliana* contain a novel, plant-specific CC type motif. Lately, information on the function of CC type GRXs and redox regulation, in general, is accumulating. This review focuses on recent findings indicating that GRXs, glutathione and redox regulation, in general, seem to be involved in different processes of development, so far, namely in the formation of the flower. Recent advances in EST and genome sequencing projects allowed searching for the presence of the three different types of the GRX subclasses in other evolutionary informative plant species. A comparison of the GRX subclass composition from *Physcomitrella*, *Pinus*, *Oryza*, *Populus*, and *Arabidopsis* is presented. This analysis revealed that only two CC type GRXs exist in the bryophyte *Physcomitrella* and that the CC type GRXs group expanded during the evolution of land plants. The existence of a large CC type subclass in angiosperms supports the assumption that their capability to modify target protein activity posttranslationally has been integrated into crucial plant specific processes involved in higher plant development.

Key words: Glutaredoxin, flower development, redox regulation, disulfide reduction.

Glutaredoxins: Versatile Oxidoreductases Acting in Redox Regulation

Glutaredoxins (GRXs) are oxidoreductases that belong to the thioredoxin (TRX) superfamily, all mediating reversible reduction of intracellular disulfide bonds *in vivo* (reviewed by Fernandes and Holmgren, 2004; Buchanan et al., 2005). The first

GRX found was a dithiol hydrogen donor system for ribonucleotide reductase in an *E. coli* mutant lacking thioredoxin1 (Holmgren, 1976). Now, GRXs are known to exist in a number of isoforms in prokaryotes, viruses and eukaryotes, including yeast, vertebrates and humans, where they play a major role in the response to oxidative stress. Although several plant cDNAs have been isolated from spinach, poplar, *Rhizinus* and rice (Morell et al., 1995; Rouhier et al., 2003; Szederkényi et al., 1997; Lee et al., 2002), information on the function of plant GRXs has been scarce and analyses have mostly been of a descriptive nature. Recent reviews on plant glutaredoxins and plant redox control, in general, have comprehensively summarized the constantly accumulating knowledge within this field (Dietz, 2003; Lemaire, 2004; Rouhier et al., 2004; Buchanan et al., 2005; Ströher and Dietz, 2006). This review will survey novel findings indicating an intriguing function for plant GRXs and redox signalling in developmental processes. Furthermore, we have conducted a comparative analysis of the GRX organization in representative species from lower plants, gymnosperms and angiosperms, aiming for a better understanding of the evolution of the multiplicity of different GRX isoforms in photosynthetic organisms.

GRXs exert their activity through two different mechanisms that are depicted in Fig. 1. Both mechanisms depend on the presence of motifs containing characteristic cysteines with a sulfhydryl group that are particularly sensitive to reactive oxygen species (ROS) formed during oxidative stress (Gille and Sigler, 1995). In the dithiol reaction, two cysteines, normally arranged in an active site including a conserved C- and N-terminal cysteine (CXXC), are required to reduce protein disulfides. The N-terminal cysteine initiates a nucleophilic attack on one of the sulfur atoms of the disulfide target. A transient disulfide bridge is formed between the two proteins which is resolved by generating an intramolecular disulfide bridge between the two cysteines of the active site, thereby releasing a reduced target protein (Fig. 1A). In the monothiol reaction, a protein tagged via a disulfide bridge with glutathione is reduced by means of only one, typically the N-terminal, cysteine that can be part of either a CXXC or CXXS motif. Here, the GRX reacts exclusively with the glutathione (GSH), for which it possesses a high affinity (Fig. 1B). Glutathionylation can protect sulfhydryl groups from irreversible damage during oxidative stress and, after normalization of the cellular redox state, protein reactivation occurs by deglutathionylation (Ghezzi, 2005). Monothiol reactions are only exerted by GRXs, whereas the di-

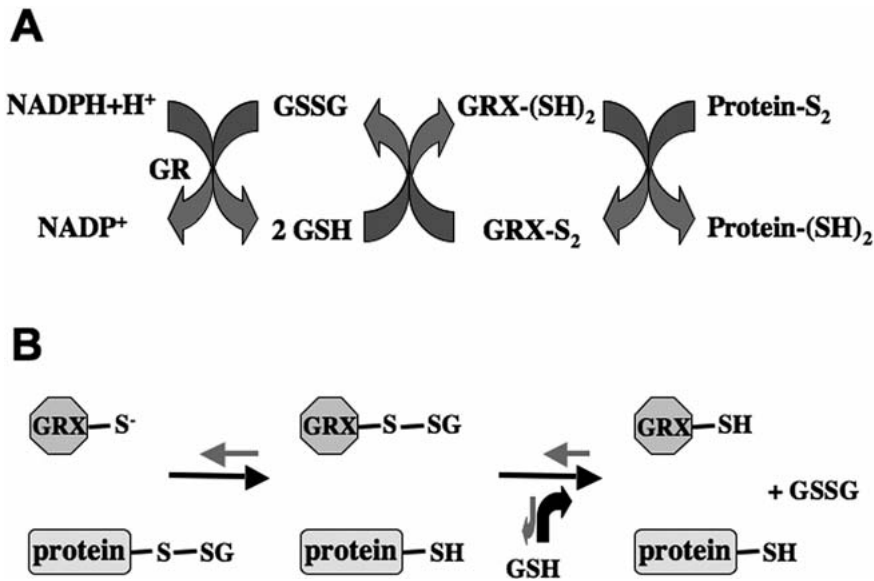


Fig. 1 GRX mechanisms for reduction of disulfides. **(A)** Dithiol mechanism: electrons are transferred from NADPH to glutathione reductase (GR), then to glutathione (GSH), and from there to glutaredoxin (GRX). Finally, GRX reduces disulfides in target proteins. **(B)** Monothiol mechanism: reduction of protein GSH mixed disulfides is called deglutathionylation (black arrows). Glutathionylation, the reverse reaction (grey arrows), generates glutathionylated proteins. (Adapted from Fernandes and Holmgren, 2004.)

thiol mechanism can also be conducted by TRXs (Nulton-Persson et al., 2003). It is assumed that dithiol GRXs such as the CXXC types can exert dithiol and monothiol reductions, whereas monothiol CXXS type GRXs are restricted to reduction of mixed disulfides. Both disulfide reduction mechanisms have in common that they cause posttranslational modifications of GRX target proteins, probably in response to an altered cellular redox state. Consequently, protein structure can be altered, and these modifications represent a means by which the biological activity of target proteins can be regulated.

The Family of Plant Glutaredoxins: Novel GRX Members Broaden the Horizon

Based on the amino acid composition of the active motifs, the 31 members of the *Arabidopsis* GRXs family have been divided into three major subclasses (Lemaire, 2004; Rouhier et al., 2004). Six classical GRXs were identified in *Arabidopsis* and contain a CXXC motif, mostly represented by a CPYC active site (Lemaire, 2004). The monocysteine enzymes of the CXXS type contain predominantly CGFS type active site motifs that were originally identified and characterized in yeast (Rodríguez-Manzanque et al., 1999). In *Arabidopsis*, this group comprises four GRXs. Surprisingly, the largest GRX group is also of the CXXC type, but these GRXs contain an additional, conserved cysteine in the second position of the motif that led to the designation of this novel CC type subclass (Lemaire, 2004). Database searches and protein sequence analysis from Lemaire (2004) and Rouhier et al. (2004) indicated that CC type GRXs might have appeared later during evolution and could be specific to higher plants. Intrigued by this observation, we took advantage of rapidly expanding plant EST and genome sequencing data and conducted a broader GRX search in selected plant species representing different stages of plant evolution. In databases from the cyanobacterium *Synechocystis* and green alga *Chlamydomonas*, we only identified GRXs of the CPYC and CGFS type but not of the CC type (data not shown), confirming earlier searches (Lemaire, 2004). As a representative for lower land plants, we searched the bryophyte model system *Physco-*

mitrella patens EST and genomic sequence databases (the latter being a personal communication, S. Rensing, S. Zachgo). Next, we analyzed GRXs from seed plants and identified GRXs from the large collection of over 300 000 ESTs from the gymnosperm *Pinus taeda*. The sequenced genome of *Oryza sativa* served as a representative for monocots and that of *Populus*, together with described GRXs from *Arabidopsis* (Rouhier et al., 2004; Lemaire, 2004), for dicots. All newly identified GRXs were aligned together using ClustalW. Fig. 2 gives the amino acid similarities, using the tree view program for graphic presentation. Clearly, extended analysis of GRX isoforms shows that they group into three subclasses, CPYC, CGFS and CC type, supporting data from earlier studies (Rouhier et al., 2004; Lemaire, 2004). The different GRXs from each species were listed according to their active site motif sequence and are shown in Fig. 3A. Strikingly, our data allow new insights into the evolution of the CC type GRX subclass. Two ESTs encoding CCMC type GRXs were identified in *Physcomitrella*. This bryophyte, thus far, represents the most basal plant species from which CC type GRXs could be identified. Searches were not successful in the green alga *C. reinhardtii* or in photosynthetically active cyanobacteria, however, with the limitation that these datasets do not yet represent all cDNAs. The occurrence of the CC type GRXs can, thus, probably be dated back about 450 million years, when the first nonvascular land plants evolved (Kenrick and Crane, 1997). In Fig. 3B, a comparison of the number of members of the three GRX subclasses is depicted. This highlights another interesting observation: again, being aware of the limitation that not all GRXs might yet be annotated for all investigated species, it is still evident that the number of the CC type GRXs increases from bryophytes to gymnosperms and finally to angiosperms, represented by mono- and dicots. Numbers of CC type GRXs progressively increase from two for *Physcomitrella* to 22 and 21 CC type GRXs in *Populus* and *Arabidopsis*, respectively. In contrast, sizes of the other two GRX groups are similar, with 4–6 members of CPYC type GRXs and 3–8 members of CGFS type GRXs. Analysis of the different *Arabidopsis* GRXs, using expression profiling databases, revealed that most are expressed, supporting a

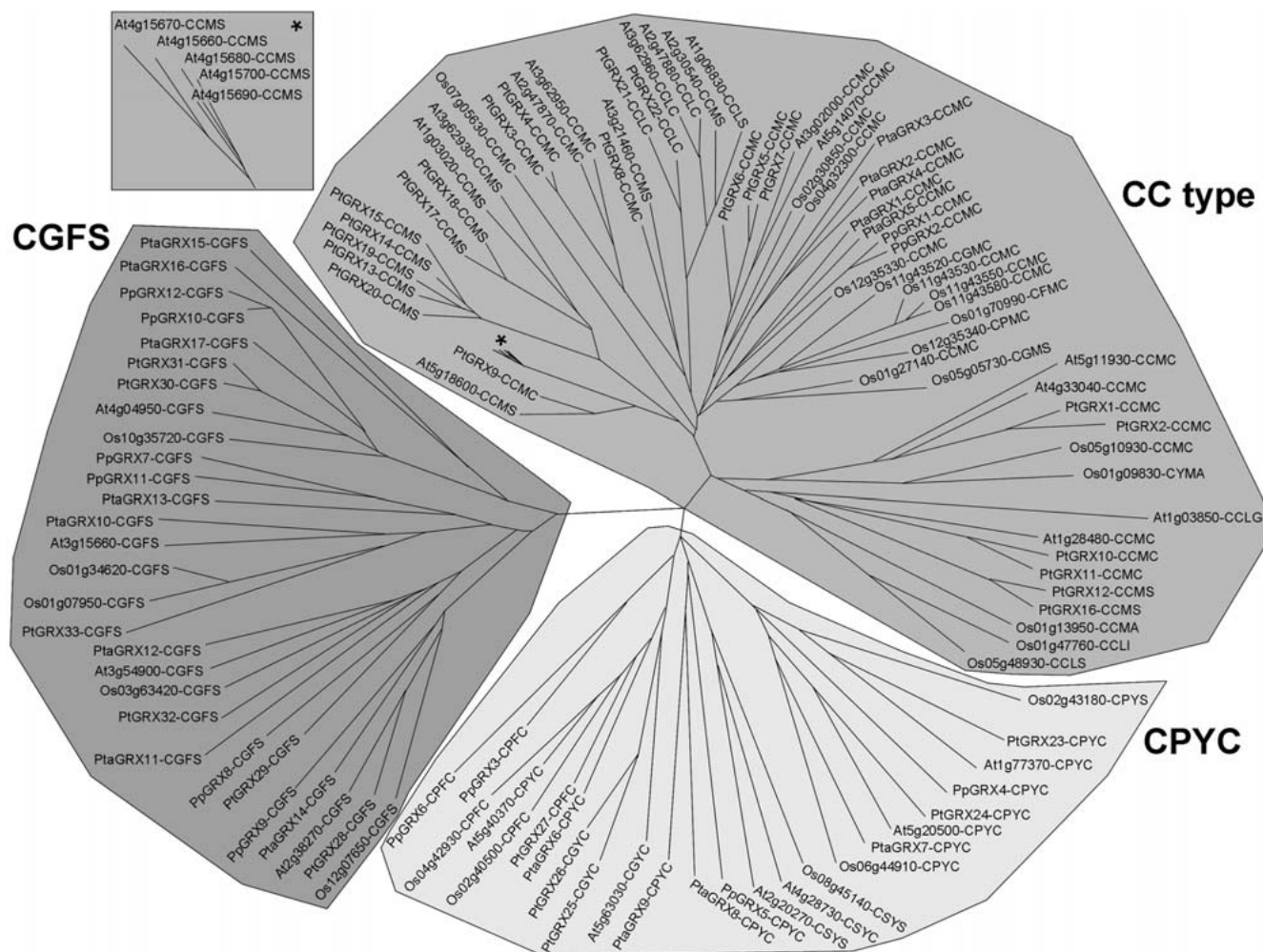


Fig. 2 Schematic representation of amino acid similarities between GRXs from *Physcomitrella patens*, *Pinus taeda*, *Oryza sativa*, *Populus trichocarpa*, and *Arabidopsis thaliana*. In order to retrieve the GRX amino acid sequences from *Physcomitrella patens* (Pp), *Pinus taeda* (Pta), and *Oryza sativa* (Os), the publicly available nucleotide sequences (containing ESTs and, for Pp, Os and Pt also genomic sequences) were searched using the tblastn program. Pp: <http://moss.nibb.ac.jp>, <http://www.cosmos.org>; Pta: <http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=3352>; Os: <http://www.tigr.org/tdb/e2k1/osa1/index.shtml>; Pt: <http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Popotr1&advanced=1>. The predicted amino acid sequences of ROXY1 (At3g02000), At4g04950 and At5g63030, representatives for the three different GRX subclasses from *Arabidopsis*, were used for the searches. Only nucleotide sequences from predicted proteins with an E value < 10⁻⁵ were further grouped into contigs to determine the longest detectable unigene sequence. The corresponding translated amino acid

sequence was submitted to the rpsblast program at the NCBI website in order to discriminate between GRX-encoding sequences and to exclude false positives, mainly originating from closely related TRX-encoding sequences. The list of *Arabidopsis* GRXs was obtained from Rouhier et al. (2004). The predicted amino acid sequences from all the investigated organisms were aligned using the ClustalW program (<http://www.ebi.ac.uk/clustalw>) with the default settings. The alignment was then visualized with the treeview program (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). Amino acid sequences deduced from assembled contig sequences from *Physcomitrella*, *Pinus*, and *Populus* were numbered consecutively, following the order of those belonging to the CCMC, CPYC, and CGFS subclasses, and will be provided upon request. For identification of GRXs from *Arabidopsis*, the AGI code is given, and for GRXs from *Oryza*, locus identifiers from the TIGR rice genome annotation nomenclature are used.

function for the CC type GRXs (data not shown). This observation raises the question of whether a relationship might exist between the development of more complex plant structures and the concomitant expansion of the CC type GRXs in land plants.

The CC type motif shows some features that distinguish this group from the other two subclasses. The function of the second conserved cysteine next to the N-terminal cysteine remains elusive: is it also involved in disulfide reduction?; if so, via the

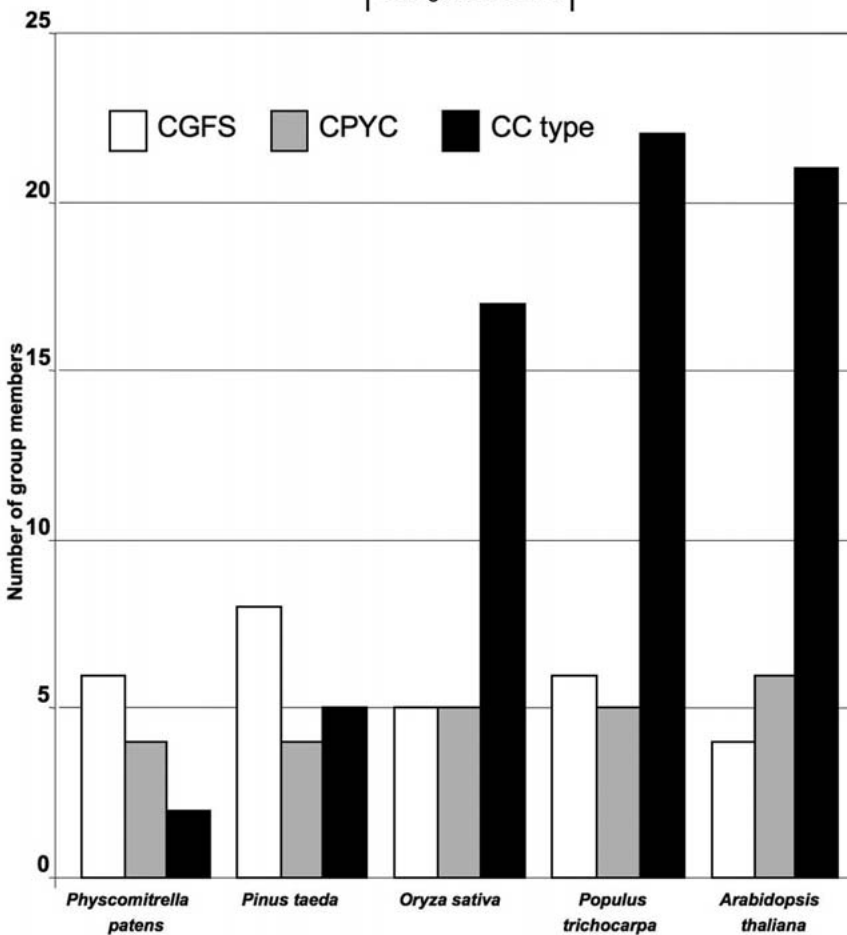
monothiol or dithiol mechanism? However, this conserved cysteine was replaced in four GRXs from *Oryza* (Os01g70990 CFMC, Os11g43520 CGMC, Os05g05730 CGMS, Os01g09830 CYMA, Os12g35340 CPMC), although their overall amino acid similarity still groups these GRXs into the CC type subclass (Fig. 2).

The comparative study across different species allows further insights into deviations from the two active site motifs from the CPYC and CGFS subclasses (Fig. 3C). Proteins of the CPYC

A

| <i>Physcomitrella patens</i> | | <i>Pinus taeda</i> | | <i>Oryza sativa</i> | | <i>Populus trichocarpa</i> | | | | <i>Arabidopsis thaliana</i> | | | |
|------------------------------|-------------|--------------------|-------------|---------------------|-------------|----------------------------|-------------|---------|-------------|-----------------------------|-------------|-----------|-------------|
| name | active site | name | active site | name | active site | name | active site | name | active site | name | active site | name | active site |
| PpGRX1 | CCMC | PtaGRX1 | CCMC | Os02g30850 | CCMC | PtGRX1 | CCMC | PtGRX18 | CCMS | At1g28480 | CCMC | At5g18600 | CCMS |
| PpGRX2 | CCMC | PtaGRX2 | CCMC | Os04g32300 | CCMC | PtGRX2 | CCMC | PtGRX19 | CCMS | At2g47870 | CCMC | At2g47880 | CCLC |
| PpGRX3 | CPFC | PtaGRX3 | CCMC | Os05g10930 | CCMC | PtGRX3 | CCMC | PtGRX20 | CCMS | At3g02000 | CCMC | At3g62960 | CCLC |
| PpGRX4 | CPYC | PtaGRX4 | CCMC | Os07g05630 | CCMC | PtGRX4 | CCMC | PtGRX21 | CCLC | At3g62950 | CCMC | At1g03850 | CCLG |
| PpGRX5 | CPYC | PtaGRX5 | CCMC | Os11g43530 | CCMC | PtGRX5 | CCMC | PtGRX22 | CCLC | At4g33040 | CCMC | At1g06830 | CCLC |
| PpGRX6 | CPFC | PtaGRX6 | CPYC | Os11g43550 | CCMC | PtGRX6 | CCMC | PtGRX23 | CPYC | At5g11930 | CCMC | At1g77370 | CPYC |
| PpGRX7 | CGFS | PtaGRX7 | CPYC | Os11g43580 | CCMC | PtGRX7 | CCMC | PtGRX24 | CPYC | At5g14070 | CCMC | At5g20500 | CPYC |
| PpGRX8 | CGFS | PtaGRX8 | CPYC | Os12g35330 | CCMC | PtGRX8 | CCMC | PtGRX25 | CGYC | At1g03020 | CCMS | At5g40370 | CPYC |
| PpGRX9 | CGFS | PtaGRX9 | CPYC | Os01g27140 | CCMC | PtGRX9 | CCMC | PtGRX26 | CGYC | At2g30540 | CCMS | At5g63030 | CGYC |
| PpGRX10 | CGFS | PtaGRX10 | CGFS | Os01g13950 | CCMA | PtGRX10 | CCMC | PtGRX27 | CPFC | At3g21460 | CCMS | At4g28730 | CSYC |
| PpGRX11 | CGFS | PtaGRX11 | CGFS | Os01g47760 | CCLI | PtGRX11 | CCMC | PtGRX28 | CGFS | At3g62930 | CCMS | At2g20270 | CSYS |
| PpGRX12 | CGFS | PtaGRX12 | CGFS | Os05g48930 | CCLS | PtGRX12 | CCMS | PtGRX29 | CGFS | At4g15660 | CCMS | At2g38270 | CGFS |
| | | PtaGRX13 | CGFS | Os01g70990 | CFMC | PtGRX13 | CCMS | PtGRX30 | CGFS | At4g15670 | CCMS | At3g15660 | CGFS |
| | | PtaGRX14 | CGFS | Os11g43520 | CGMC | PtGRX14 | CCMS | PtGRX31 | CGFS | At4g15680 | CCMS | At3g54900 | CGFS |
| | | PtaGRX15 | CGFS | Os05g05730 | CGMS | PtGRX15 | CCMS | PtGRX32 | CGFS | At4g15690 | CCMS | At4g04950 | CGFS |
| | | PtaGRX16 | CGFS | Os01g09830 | CYMA | PtGRX16 | CCMS | PtGRX33 | CGFS | At4g15700 | CCMS | | |
| | | PtaGRX17 | CGFS | Os12g35340 | CPMC | PtGRX17 | CCMS | | | | | | |
| | | | | Os06g44910 | CPYC | | | | | | | | |
| | | | | Os02g43180 | CPYS | | | | | | | | |
| | | | | Os08g45140 | CSYS | | | | | | | | |
| | | | | Os02g40500 | CPFC | | | | | | | | |
| | | | | Os04g42930 | CPFC | | | | | | | | |
| | | | | Os01g07950 | CGFS | | | | | | | | |
| | | | | Os01g34620 | CGFS | | | | | | | | |
| | | | | Os12g07650 | CGFS | | | | | | | | |

B



C



Fig. 3

class were characterized initially from *E. coli* and *S. cerevisiae*. In plants, the most frequent sequence of their active site is CPYC. However, proline (P) is replaced three times by a glycine (G) and three times by a serine (S) in 24 investigated CPYC motifs. The tyrosine (Y) can be exchanged for a phenylalanine (F), and the C-terminal cysteine (C) for a serine (S). Members of the CGFS class are also present in many prokaryotic and eukaryotic organisms. These proteins show two peculiarities: firstly, the active site, CGFS, is absolutely conserved and we could not find a divergence from this motif composition, even in distantly-related species, such as *Arabidopsis*, *Physcomitrella*, and *Agrobacterium tumefaciens* (data not shown). Secondly, some members of this group, i.e., At4g04950 and PpGRX10, have a chimeric structure: two or three GRX domains at the C-terminus of the protein follow a TRX domain. This feature of CGFS type GRXs has also been demonstrated for proteins from many other organisms (Isakov et al., 2000).

Function of Plant Glutaredoxins: Insights into a Black Box?

Information on the biochemical activity, structure and function of plant glutaredoxins has thus far been limited to characterization of a few cDNAs, namely of the CPYC type, that were isolated from rice, spinach, *Ricinus* and poplar (Minakuchi et al., 1994; Szederkényi et al., 1997; Morell et al., 1995; Rouhier et al., 2001). Further analysis showed that some proteins possess activity in reduction assays using 2-hydroxyethyl disulfide (HED) and dehydroascorbate (DHA). The poplar GRX is a good electron donor for a cytosolic peroxiredoxin (Rouhier et al., 2001; Rouhier et al., 2002) and an *Arabidopsis* CC type GRX can regenerate a mitochondrial peroxiredoxin (Finkemeier et al., 2005). So far, the putative intracellular localization of GRXs has been determined using prediction software programs and, thereby, GRXs are expressed in all cellular compartments, except for the nucleus. However, localization is isoform-specific and different isoforms are restricted to different compartments (Rouhier et al., 2004). Evidence is accumulating that plant GRX proteins can be detected in the phloem sap in sieve tubes, indicating a function in long-distance signalling (Balachandran et al., 1997; Szederkényi et al., 1997; Rouhier et al., 2004).

However, recently, the first GRX mutant from *Arabidopsis* was isolated, allowing investigation of the function of a plant GRX *in vivo*. The gene was named *ROXY1* and belongs to the CC type subclass. Three independent mutant alleles, all displaying the same phenotype, have been isolated from the GABI-KAT T-DNA collection (Xing et al., 2005). The *roxy1* mutants show a floral

phenotype restricted to one floral organ. The number of *roxy1* petals is reduced from 4.0 to an average of 2.5. Petals that are formed also show defects during later morphogenesis and are often abnormally bent and form a reduced blade. *ROXY1* mRNA expression was detected transiently in young floral buds and then in young floral organ primordia. Site-directed mutagenesis revealed that only the N-terminal cysteine in the CCMC motif is crucial for *ROXY1* function (Xing et al., 2005). This indicates that *ROXY1* could exert its activity via the monothiol mechanism and posttranslationally modify target proteins by deglutathionylation. Further insight into *ROXY1* function was obtained by double mutant analysis combined with expression studies. *ROXY1* is required to restrict the expression of the MADS-box gene *AGAMOUS* (*AG*), a key regulatory transcription factor controlling stamen and carpel organogenesis (Yanofsky et al., 1990) to the reproductive organs during wild-type flower development (Xing et al., 2005). Given the fact that *AG* expression is controlled by a group of repressors (Conner and Liu, 2000; Franks et al., 2002), *ROXY1* could exert its function by modifying the activity of *AG* repressor proteins. Together, these results indicate an intriguing connection between redox regulation and control of floral organogenesis.

Plant Glutaredoxin Targets

Crucial to understanding the function of GRXs is the identification and analysis of the target proteins. Cellular redox homeostasis depends on the balance between oxidation and reduction of target thiols and dithiols, respectively. GRXs can either affect the glutathionylation status of target proteins via the monothiol mechanism or reduce intra- or intermolecular disulfide bridges via the dithiol mechanism.

In plants, the family of TRXs and their target proteins has been intensively studied and the number of TRX targets is rapidly expanding (Gelhaye et al., 2005; Buchanan and Balmer, 2005). Most TRX targets were identified using affinity chromatography approaches (Motohashi et al., 2001; Balmer et al., 2003; Lemaire et al., 2004). TRXs are stronger reductants than GRXs, with a midpoint redox potential of around -300 mV, compared to -200 mV for GRXs (Åslund et al., 1997). Recently, Rouhier et al. (2005) identified targets for a plant GRX. Using liquid chromatography with a mutagenized second active site cysteine in the CPYC motif coupled to tandem mass spectrometry, over 90 targets of a polar GRX were isolated. Similar to observations for the TRX targets, the spectrum of these target proteins is very broad. The poplar GRX targets are involved in several processes, such as stress response, photorespiration, translation, nitrogen, sulfur and carbon metabolism and protein folding. Most of these proteins, being involved in house-keeping functions, are likely to be expressed at high levels and will therefore probably be detected with the techniques used by Rouhier et al. (2005). However, proteins involved in signalling or transcription factors that are normally expressed at a lower level might have escaped detection thus far. Using the tomato Cf-9 resistance protein in a yeast two-hybrid screen, a TRX-related protein (*CITRX*) from *Lycopersicon esculentum* was identified as an interaction partner. *CITRX* can alter protein kinase activity and is a negative regulator of cell death and defence mechanisms induced through the C-9 protein (Rivas et al., 2004). In the future, the challenges will be to isolate more targets and to test their specificity with complemen-

◀ **Fig. 3** Content of GRXs from five plant species. (A) GRXs identified from *Physcomitrella patens*, *Pinus taeda*, *Oryza sativa*, *Populus trichocarpa*, and GRXs reported from *Arabidopsis thaliana* were grouped together according to the amino acid composition of their active site. (B) Distribution of GRXs into different subclasses. For determining subclass membership, priority was given to overall amino acid similarity, even if amino acids in the name-giving motifs are not totally conserved. (C) Ratio composition of the active site sequence for CC type and CPYC proteins. Residue letter size is proportional to occurrence among the studied proteins. CPYC (n = 24): first position 100% C; second position 75% P, 9% G, 12.5% S, 12.5% G; third position 79% Y, 21% F; fourth position 87.5% C, 12.5% S. CC type (n = 67): first position 100% C; second position 92.5% C, 1.5% Y, 1.5% F, 1.5% P, 3.0% G; third position 88% M, 12% L; fourth position 61% C, 33% S, 3% A, 1.5% G, 1.5% I.

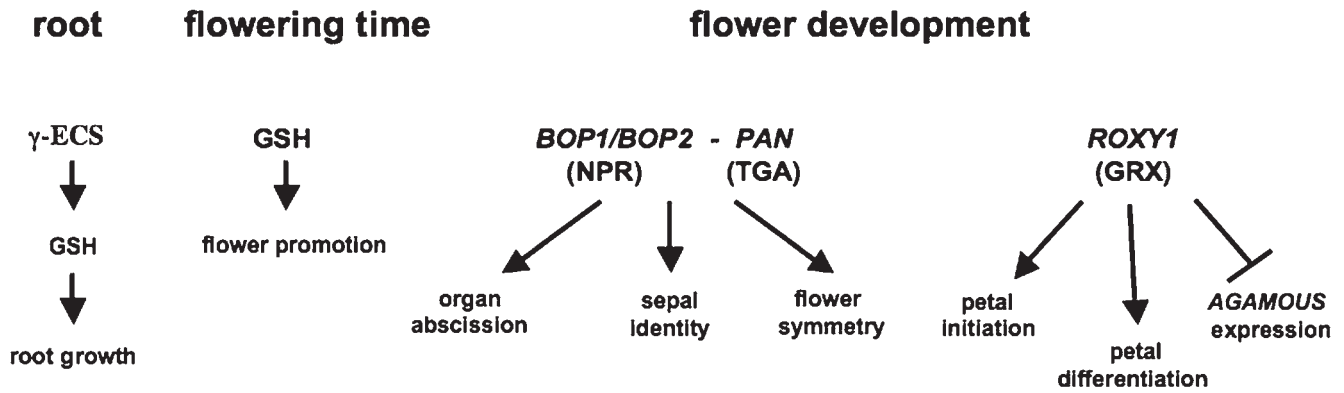


Fig. 4 Overview of redox-sensitive developmental processes. Different molecules, such as γ -ECS, GSH, ROXY1 (a CC type GRX), BOP1, and BOP2 (transcriptional coactivators belonging to the NPR family), as well as the TGA transcription factor PAN, likely participate in the redox-sensitive

regulation of developmental processes. Thus far, genetic and molecular studies have revealed that root and flower development and the control of flowering time are affected.

tary approaches and thus determine if targets are modified by the dithiol or monothiol mechanism.

Further Support for the Importance of Redox Regulation during Developmental Processes

In yeast, the redox potential varies throughout the growth, division and apoptotic phases of its life cycle (Schafer and Buettner, 2001). Consequently, altered cellular redox states seem to correlate with altered developmental fates. In Fig. 4, a survey of currently known plant developmental processes affected by redox regulation is presented. This suggests that, besides participation in plant stress defence, redox-sensitive mechanisms seem to also participate in the control of flower and root development. In contrast to animals, plants use GSH as an electron donor for both the dithiol and monothiol reduction mechanisms (Fig. 1). This emphasizes the importance of GSH as a redox buffer that is present in plant cells at high levels of up to millimolar concentrations (Noctor and Foyer, 1998).

Are similar signalling mechanisms involved in plant defence and floral development?

Salicylic acid (SA) plays an important role in the onset of defence reactions against avirulent pathogens and mediates systemic acquired resistance (SAR) in plants (Delaney et al., 1994). SAR is a systemic and broad disease resistance characterized by the induction of pathogen-related (PR) genes exerting antimicrobial activity (Van Loon and Van Strien, 1999). NPR1 (NON-EXPRESSOR OF PR GENES) is a positive regulator of the defence reaction in *Arabidopsis* (Dong, 2004). Upon SA accumulation, the intracellular redox potential is altered, intermolecular disulfide bonds holding NPR1 proteins together are reduced and thereby NPR1 is converted into active monomers that are translocated from the cytoplasm into the nucleus (Mou et al., 2003). There, NPR1 acts as a coactivator of TGA transcription factors that recognize binding motifs in the PR-1 promoter and can activate PR-1 transcription. The NPR1/TGA interaction depends on the redox state of two cysteines in the TGA1 protein (Després et al., 2003). If an intramolecular disulfide bridge is formed between the two thiol groups in the TGA1 protein, interaction with NPR1 is abolished. NPR1 can only stimulate DNA binding activity of the reduced TGA1 protein. NPR1 con-

tains a BTB/POZ domain and four ankyrin repeats mediating interaction with TGAs. These structural motifs are shared between five genes forming a small *Arabidopsis* gene family. Simultaneous inactivation of two other closely-related family members, BOP1 and BOP2, revealed a broad function for these NPR1-like genes in flower and leaf development (Ha et al., 2004; Hepworth et al., 2005; Norberg et al., 2005). Flowers of the double mutant form ectopic bracts and show organ abscission defects. Wild-type *Arabidopsis* flowers form four symmetrical sepals in the first whorl of the flower. In *bop1 bop2* flowers, an extra organ is formed in the first whorl and the two abaxial sepals are transformed into petaloid organs, thus giving rise to an asymmetric flower. Although most TGA genes are associated with the regulation of PR genes, it has been shown that one member of this bZIP transcription factor family, PERIANTHIA (PAN), is involved in the regulation of floral organ development (Chuang et al., 1999). In the *pan* mutant, an extra organ is formed in the first and second whorl in an abaxial position, disrupting flower symmetry and generating a similar phenotype to that found in *bop1 bop2* flowers (Runnig and Meyerowitz, 1996). Biochemical interaction studies revealed that BOP1 and BOP2 proteins interact in yeast with PAN. As their expression patterns overlap in young flowers, this interaction is likely to occur *in vivo*. Further support for a function of BOP and PAN in the same genetic pathway was provided by analysis of the triple mutant (Hepworth et al., 2005). Given the similarities between the NPR1/TGA1 and BOP/PAN interaction it will be interesting to see if the BOP/PAN interaction might also be redox-controlled and thereby affects the activity of the PAN transcription factor during flower development.

Flowering time and redox regulation: a connection?

Glutathione (GSH) is a key component of plant antioxidant defence and is required for the dithiol and monothiol mechanism (Fig. 2). GSH is synthesized in two ATP-dependent steps, catalyzed by γ -glutamylcysteine synthase (γ -ECS) and glutathione synthase.

Recent data indicate that GSH plays a role in determining flowering time. Ogawa et al. (2004) investigated the relationship between GSH levels, photosynthesis and flowering in *Arabi-*

dopsis and found that the level of GSH increases with light intensity. This requires γ -ECS catalyzing the key reaction of GSH synthesis. The level of GSH might be important in the regulation of flowering in *Arabidopsis* as it represents a pool of molecules that can be added posttranslationally to flowering time-associated proteins and thereby influence their activity. Furthermore, mutants defective in glutathione biosynthesis or light harvesting antenna in photosystem II show reduced levels of GSH and have a late flowering phenotype (Ogawa et al., 2004). Thus, the level of GSH seems to be important in the regulation of flowering in *Arabidopsis*, which is further supported by the finding that a γ -ECS mutant (see below) also exhibits a late flowering phenotype (Cobbett et al., 1998).

GSH participates in the regulation of cell division in the root apical meristem

The rate-limiting step in synthesis of the tripeptide GSH is catalyzed by γ -ECS (May and Leaver, 1994). A mutation in the *Arabidopsis* γ -ECS (*ROOT MERISTEMLESS1;RML1/CADMIUM SENSITIVE2*) revealed that a glutathione-dependent pathway is required for correct cell division during postembryonic root development (Vernoux et al., 2000; Cobbett et al., 1998). Knockdown *rml1* mutants are almost devoid of GSH and, consequently, cells in the root tip do not divide after germination, causing an arrest in root development at a length of about 1 mm (Vernoux et al., 2000). These data confirm earlier observations that GSH has a role in controlling cell division. High concentrations of GSH increased the number of cells undergoing mitosis, while depletion of GSH had the opposite effect (Sánchez-Fernández et al., 1997). The existence of a GSH-dependent transduction pathway has also been postulated for mammalian cells (Liang et al., 1989; Suthantrian et al., 1990). The *rml1* phenotype is suggestive for the existence of a general GSH-dependent mechanism to regulate cell division and operates in a broad range of organisms.

Conclusions and Outlook

GRXs are oxidoreductases that can modify proteins posttranslationally and therefore have the potential to alter protein activity. They can reduce intra- and intermolecular disulfide bridges and also, in contrast to TRXs, reduce glutathionylated proteins and thus release deglutathionylated proteins. Evidence is accumulating for redox-regulated disulfide bridge formation, affecting DNA binding activity of plant transcription factors. Interestingly, these posttranslational modifications of TGA proteins are likely not only to be involved in signalling processes in stress responses, but also govern developmental processes. Glutathionylation of chaperones, kinases, metabolic enzymes and also cytoskeletal proteins occurs in bacteria, yeast and animal cells, especially in response to oxidative stress (Lind et al., 2002). Thus far, in *Arabidopsis*, two enzymes of sugar metabolism, a triose-phosphate isomerase and an aldolase, were shown to be glutathionylated and thus represent putative targets for GRXs (Ito et al., 2003). Furthermore, similar to disulfide bridge formation, glutathionylation represents a means by which the DNA binding activity of mammalian transcription factors can be altered, as shown for NF-kappaB and c-Jun (Pineda-Molina et al., 2001; Klatt et al., 1999). The evolution of regulatory transcription factors through gene duplication and subsequent functional divergence has been hypothesized to be a major driving force in the development of

extant plants (Otto and Yong, 2002). In the future, it will be interesting to investigate if and how plant transcription factor activity is posttranslationally regulated, in addition to known modifications such as phosphorylation and prenylation, the latter also requiring a conserved cysteine (Fujita et al., 2003; Yalovsky et al., 2000).

The plant GRX family comprises three different subclasses: the classical CPYC and CGFS GRXs are present throughout prokaryotic and eukaryotic organisms, whereas the CC type GRXs have, thus far, only been identified from land plants. Our comparative analysis suggests that the expansion of this GRX subclass coincided with the evolution of more complex plants, increasing especially in seed plants, as compared to a more steady level in the other two subclasses. Analysis of mutants with defects at different levels of redox regulation supports the importance of this novel mechanism in the control of developmental processes, as outlined in Fig. 4. Genomic tools and recent progress in the field of plant proteomics will be extremely valuable in characterizing more GRXs and identifying and analyzing their targets in order to advance our understanding of how they might have contributed to the evolution of complex plant reproductive structures, namely the flower.

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