Review

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Cytosolic thiol switches regulating basic cellular functions: GAPDH as an information hub?

Abstract: Cytosolic glyceraldehyde 3-phosphate dehydrogenase (GAPDH, E.C. 1.2.1.12) is present in all organisms and catalyzes the oxidation of triose phosphate during glycolysis. GAPDH is one of the most prominent cellular targets of oxidative modifications when reactive oxygen and nitrogen species are formed during metabolism and under stress conditions. GAPDH harbors a strictly conserved catalytic cysteine, which is susceptible to a variety of thiol modifications, including S-sulfenylation, S-glutathionylation, S-nitrosylation, and S-sulfhydration. Upon reversible oxidative thiol modification of GAPDH, glycolysis is inhibited leading to a diversion of metabolic flux through the pentose-phosphate cycle to increase NADPH production. Furthermore, oxidized GAPDH may adopt new functions in different cellular compartments including the nucleus, as well as in new microcompartments associated with the cytoskeleton, mitochondria and plasma membrane. This review focuses on the recently discovered mechanism underlying the eminent reactivity between GAPDH and hydrogen peroxide and the subsequent redox-dependent moonlighting functions discriminating between the induction either of adaptive responses and adjustment of metabolism or of cell death in yeast, plants, and mammals. In light of the summarized results, cytosolic GAPDH might function as a sensor for redox signals and an information hub to transduce these signals for appropriate responses.

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Introduction

Oxidative stress vs. redox homeostasis vs. redox signaling

The impact of concepts such as “oxidative stress” (Sies, 1985, 1986) or the “free radical theory of aging” (Harman, 1956) on biological research is undisputed. In all kingdoms of life, stress situations caused by disease or adverse environmental changes have been linked to increased cellular levels of reactive oxygen species (ROS) (Dalle-Donne et al., 2009; Cai and Yan, 2013; Ghezzi, 2013 and references therein). ROS are formed along with many processes within the cell and in plants during photosynthesis, in particular when cells have to cope with adverse growth conditions. The formation of reactive nitrogen species (RNS) and of hydrogen sulfide (H_2S) was also found to be of high impact on cellular activities because of their potential to modify cysteine residues, and can result in stress. However, the view on ROS and other reactive molecules has changed during recent years; some ROS and RNS species as well as H_2S are now known to be crucial components of signaling cascades and not only destructive in their nature.

The finding that ROS and RNS can act as important second messengers contributed significantly to the concept of redox signaling (reviewed in: Rhee et al., 2005; Gould et al., 2013). Recently, H_2S – via S-sulfhydration of cysteine residues – was also identified to function as a signaling compound (Bruce King, 2013; Gotor et al., 2013; Kolluru et al., 2013; Hancock and Whiteman, 2014; Kabil

et al., 2014). The concept of redox signaling requires a regulated adjustment of ROS/RNS/H_2S levels. Meanwhile, it is widely accepted that the formation of these small reactive molecules does not occur just accidently in an unregulated manner, but that they are produced by specific enzymes that are activated upon certain stimuli. The emerging importance of redox regulation for a variety of cellular functions led to a new definition of oxidative/nitrosative stress, including the subsequent disruption of redox signaling (see Jones and Sies, 2007).

Redox signaling is performed via modifications of cysteine and methionine residues, the two sulfur-containing amino acids, which serve to modify the property of the protein. In contrast to cysteine thiol groups, methionyl thioether groups are oxidized to two diastereomers, methionine-S-sulfoxide and methionine-R-sulfoxide which are stereospecifically reduced by methionine sulfoxide reductases (Msr) A (S-sulfoxide) and B (R-sulfoxide) (Boschi-Muller et al., 2008). Further reaction with hydrogen peroxide (H_2O_2) leads to irreversible oxidation to methionylsulfone.

In this review, we will focus in more detail on cysteinyl thiol modifications, mainly on the initial reaction of H_2O_2 with a cysteine in a specific protein environment, using the example of the cytosolic GAPDH, and their impact on regulation of cellular functions. As GAPDH belongs to the group of early responsive redox-sensitive proteins (Wang et al., 2012) and can adopt multiple functions in various cell compartments including the nucleus (Sirover, 2012), it appears to be well suited for a central role in redox-sensing and signaling.

Reversible thiol modifications and redoxin targets

Protein thiols with their reversible modifications can serve as signal transmitters and as redox switches for dynamic regulation of cellular functions (Barford, 2004; Spadaro et al., 2010; Cai and Yan, 2013). Thiols can undergo a large variety of modifications including disulfide bridge formation, S-glutathionylation, S-nitrosylation, S-sulfhydration, and formation of sulfenic, sulfinic and sulfonic acids.

Many target proteins for cysteine modifications have been identified in attempts to solve the redox-proteome. Noteworthy, oxidative modifications do not appear randomly, but at very specific cysteines. The specificity of redox signaling is guaranteed by the micro-environment of cysteinyl residues and specific interconverting enzymes. For example, S-nitrosylation is supported by an electrostatic and/or hydrophobic environment (Perez-Mato et al., 1999; Hess et al., 2005). In some cases, S-nitrosylation is considered to be solely a non-enzymatic process orchestrated by the chemistry of the target site environment and driven by an increased level of RNS. However, many other factors in the cell are contributing to generate a specific signal for appropriate responses. S-nitrosylation requires the particular nitric oxide synthetase isoform to come in close proximity to the target site via compartmentalization with supporting factors and the target protein. It can be further orchestrated by enzymes that directly nitrosylate, de-nitrosylate or trans-nitrosylate cysteine residues.

Basic amino acids lower the usual pK_a value of the thiol from 8 to between 5 and 7, increasing the tendency for the thiol to be deprontonated to a thiolate anion. A thiolate anion is a far superior nucleophile compared to a protonated thiol group. Thus, it follows that a low pK_a thiol – which will be mostly deprotonated at physiological pH – increases the availability of the nucleophile and is an important prerequisite for thiol reactivity. However, calculations suggest that nucleophile availability can only explain a reaction-rate increase of about one order of magnitude with respect to free cysteine (Ferrer-Sueta et al., 2011). This is supported by measurements on several proteins with low pK_a thiols including glutaredoxin 1, PTP1B and papain, each of which has reaction rates with H_2O_2 that are hardly better than that of free cysteine (Ferrer-Sueta et al., 2011). Furthermore, the relationship between thiol pK_a and thiol nucleophilicity is more complex than is typically assumed, as described by the Brønsted equation, thiol nucleophilicity may actually decrease with decreasing pK_a (Ferrer-Sueta et al., 2011).

It is clear that pK_a or nucleophile availability alone is not sufficient to explain the exceptional reactivity of some proteins, including cytosolic GAPDH, towards H_2O_2. Other factors, like stabilization of the reaction transition state and promotion of leaving-group departure, play a far more important role in determining the reaction rate of a protein thiol with H_2O_2 (Ferrer-Sueta et al., 2011; Nagy, 2013). Specific structural features, which serve to catalyze the entire reaction, beyond the initial nucleophilic attack are known to exist in both peroxiredoxins and glutathione peroxidases (Flohé et al., 2011).

Moreover, specificity of redox signaling is guaranteed by enzymatic catalysis. ROS scavenging is controlled by a set of enzymes. The main cellular redox couple, GSH/GSSG, during redox regulation is highly controlled, especially by GSH peroxidases or members of the thioredoxin family of proteins (Berndt et al., 2014). Oxido-reductases of the thioredoxin family (redoxins) – thioredoxins, glutaredoxins, and peroxiredoxins – occur in all organisms

and in all cell compartments. Particularly in plants, redoxins are present in very large numbers (for reviews see: Buchanan and Balmer, 2005; Michelet et al., 2006; Rouhier et al., 2008; Meyer et al., 2009). Thioredoxins and glutaredoxins are key enzymes in the maintenance of the protein-thiol pool, thereby regulating reversible oxidative modifications and redox signaling (Hanschmann et al., 2013; Lillig and Berndt, 2013).

Redox-regulated cellular functions

The field of redox regulation and signaling started with the discovery and characterization of thioredoxins 50 years ago (see: Buchanan et al., 2012). After the discovery of thioredoxin in *Escherichia coli* in the 1960s, thioredoxin-mediated regulation of photosynthesis was the first biological function identified to be controlled by cysteinyl thiol modifications. Light-dark modulation in chloroplasts was found to occur because of reversible redox changes of stromal enzymes. This organelle appeared to be the unique site for this type of post-translational modification, as the reductive power from photosynthesis and the simultaneous presence of oxygen are the basis of such regulation (for review see: Buchanan, 1980).

The activity of the chloroplast GAPDH isoforms GapA/B was the first among the various Calvin cycle enzymes to be found responsive to redox changes during light-dark modulation. This enzyme is involved in CO_2 assimilation and primarily directed to reduce 3-phosphoglycerate (3-PGA) via glycerate 1,3-bisphosphate (1,3bisPGA) to glyceraldehyde 3-phosphate (G3P). In the presence of light, the ferredoxin-thioredoxin system continuously reduces the enzyme. Oxidation, and concomitant inactivation, is the result of the formation of a disulfide bridge between two cysteine residues at the unique C-terminal extension of GapB. Subsequent aggregation leads to the formation of the inactive hexadecamer $[GapA_2/B_2]_4$ (Baalmann et al., 1994, 1995). However, even in the light, these enzymes are constantly re-oxidized by the oxygen evolved during photosynthesis, thus requiring continuous re-reduction in order to maintain the assimilatory flux. Such a futile cycle is also the basis for fine-regulation of the activity in the light, as metabolites act as effectors in the reductive step and can shift the steady-state ratio of reduced (active) and oxidized (inactive) enzyme to the actually required *in vivo* activity at this step (Scheibe, 1990). However, it later became clear that this regulatory principle is not only present in the chloroplasts of plants growing under challenging environmental conditions, which require the continuous and rapid adjustment of enzyme activities by post-translational modification. Also in all other organisms, in particular in the context of aging, pathogen attack and disease, redox changes take place. Today, numerous examples became apparent establishing the redox chemistry of thiol groups as a universal mechanism in controlling cellular homeostasis and in sensing and transmitting many types of signals which is reflected in several special issues (e.g., Herrmann and Jakob, 2008; Lillig and Berndt, 2008; Foyer and Noctor, 2012).

GAPDH in glycolysis

GAPDH is an oxido-reductase that catalyzes an important step in the central metabolism, namely the interconversion of 1,3-bisPGA into the carbohydrate G3P and vice versa. A cysteine residue is involved covalently in catalysis here. This cysteine is also particularly reactive with H_2O_2, enabling further oxidative modifications, a fact that will be discussed in more detail below. This aspect is subject of the review, and a short introduction into the structure of GAPDH, the number of cysteines and their location is given for examples taken from the various kingdoms of life (Table 1). Common to all GAPDH proteins is their overall structure and high conservation of the amino acid sequence, resulting in a high similarity of the three-dimensional structure. From the various crystal structures that are available, it is evident that the active site is conserved. Figure 1A shows the structure of human GAPDH (Jenkins and Tanner, 2006) and the distribution of cysteine residues. Bacteria and mammals are equipped with essentially only one gene, while yeast possesses three genes. In contrast, the genome of the model plant *Arabidopsis thaliana* contains seven genes encoding GAPDHs (Table 1). GapA/B is involved in the reductive step in the Calvin cycle and is light-dark modulated. There are two sites of glycolytic activities, namely in plastids (GapCp, two genes) and in the cytosol (GapC, two genes). The cytosolic GapC isoforms are involved in glycolysis, but, as shown in this review, also take part in many other cellular events. Therefore, GapC could be of more general importance in the integration of metabolism and cell fate during developmental and environmental adaptation as is also becoming evident now for the mammalian GAPDH.

It had been assumed that the enzymes operate continuously under the reducing conditions of the cytosol of a well-functioning cell. However, it now became clear that there are indeed situations that can lead to oxidation of the catalytic cysteine of the cytosolic isoforms, inactivation of

Table 1 GAPDH isoforms from different species.

Organism	Gene name	Number of cysteines	Catalytic cysteine	Localization	Accession number[a]
Escherichia coli	gapA	3	150	Cytoplasm	E0W9Y9_ECOLX
Saccharomyces cerevisiae	TDH1	2	150	Cytoplasm	G3P1_YEAST
S. cerevisiae	TDH2	2	150	Cytoplasm	G3P2_YEAST
S. cerevisiae	TDH3	2	150	Cytoplasm	G3P3_YEAST
Nostoc sp.	gap1	4	155	Cytoplasm	G3P1_NOSS1
Nostoc sp.	gap2	4	154	Cytoplasm	G3P2_NOSS1
Nostoc sp.	gap3	4	153	Cytoplasm	G3P3_NOSS1
Homo sapiens	GAPD	3	152	Cytoplasm	G3P_HUMAN
H. sapiens	GAPDS	6	224	Cytoplasm (sperm cell)	G3PT_HUMAN
Arabidopsis thaliana	GAPC1	2	156	Cytoplasm	G3PC1_ARATH
A. thaliana	GAPC2	2	156	Cytoplasm	G3PC2_ARATH
A. thaliana	GAPCP1	3	160	Plastid	G3PP1_ARATH
A. thaliana	GAPCP2	3	168	Plastid	G3PP2_ARATH
A. thaliana	GAPA1	5	153	Chloroplast	G3PA1_ARATH
A. thaliana	GAPA2	5	168	Chloroplast	G3PA2_ARATH
A. thaliana	GAPB	7	155	Chloroplast	G3PB_ARATH

[a]Accession numbers according to UniProtKB (www.uniprot.org).

catalysis, and subsequent "moonlighting" functions that can occur in various places within the cell.

Interaction of GAPDH with hydrogen peroxide

What determines the H_2O_2 sensitivity of GAPDH?

GAPDH is known to be particularly sensitive to H_2O_2-induced oxidation and has been identified as one of the most prominent protein targets of H_2O_2 (Grant et al., 1999; Baty et al., 2005; Hancock et al., 2005). H_2O_2 reacts with the catalytic cysteine of GAPDH, leading to the formation of a cysteine sulfenic acid, which can further react, for example with GSH leading to S-glutathionylation (Shenton and Grant, 2003; Peralta et al., 2015).

GAPDH represents a particularly interesting case in terms of its reactivity towards H_2O_2 as compared to other proteins. The second order rate constant for the reaction with H_2O_2, to yield the sulfenic acid, is in the order of 10^2–10^3 M^{-1} s^{-1} (Little and O'Brien, 1969; Stone, 2004). This is considerably faster than the reaction rate of free cysteine, glutathione, and most known redox-regulated proteins (1–10 M^{-1} s^{-1}) with H_2O_2, but nonetheless still slower than dedicated H_2O_2-reacting enzymes such as peroxiredoxins (10^5–10^7 M^{-1} s^{-1}) (Winterbourn and Hampton, 2008). The catalytic cysteine in GAPDH forms an ion pair with a neighboring histidine residue in the active site (His179 in the human enzyme) (Polgar, 1975) this leads to de-protonation of the catalytic cysteine (Cys152 in the human enzyme), which at least partially explains its reactivity towards G3P. It is generally assumed that this mechanism is also sufficient to explain the high reactivity of the catalytic cysteine with H_2O_2. This assumption is based on the line of argumentation that a thiol may be "generally reactive", or "generally unreactive", such that a thiol that is found to be reactive towards one substrate is also likely to be reactive towards other substrates. A recent study shows the exceptional H_2O_2 reactivity of GAPDH (compared to all but a few highly evolved and specialized enzymes) is based on specific structural features as observed for other highly reactive enzymes (see "Reversible thiol modifications and redoxin targets", above).

H_2O_2-binding pocket and transition-state stabilization promoting Cys152-H_2O_2 reactivity

Peralta and colleagues from the lab of Tobias Dick recently investigated the H_2O_2-reactivity of GAPDH using an elegant combination of molecular dynamics (MD) and quantum mechanics (QM) simulations together with wet-lab experiments and bioinformatics. Intriguingly, they provide strong evidence for the existence of a dedicated proton-relay mechanism within GAPDH, which serves to promote the reactivity of the catalytic cysteine with H_2O_2.

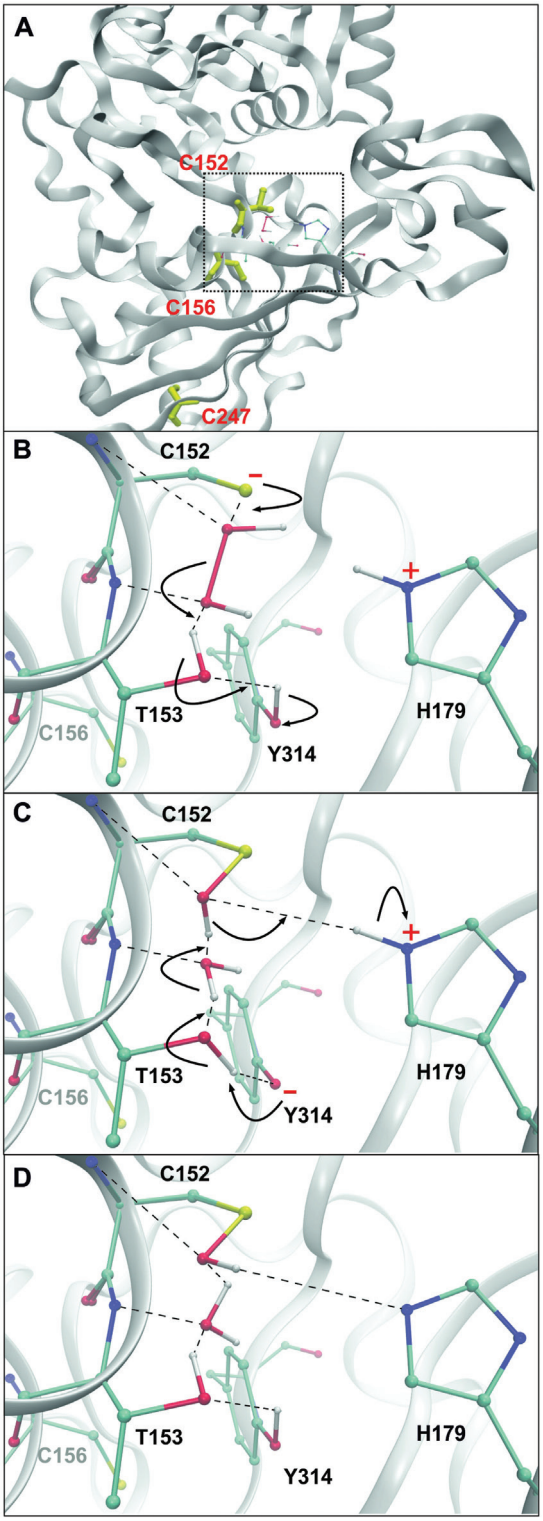

Figure 1 Structure of human GAPDH and reaction of active-site residues with H_2O_2.
(A) GAPDH monomer (PDB entry 1U8F) with cysteine residues highlighted in yellow. (B) Forward directed proton relay leads to protonation of Tyr314 and water formation. (C) De-protonation of Tyr314 initiates the proton relay backward reaction. (D) Final state of the protonation cycle with water and sulfenic acid as reaction products.

A combination of MD, QM and molecular docking experiments performed by Peralta and colleagues suggest that GAPDH maintains a specific H_2O_2-binding pocket (Figure 1B). This shallow binding pocket is lined by the Cys152 thiolate as well as the γ-OH group of Thr153 and the two backbone amide groups of Cys152 and Thr153. The orientation of Thr153 is itself stabilized by H-bonding to the Tyr314-OH group. Upon positioning itself into the binding pocket, one H_2O_2 oxygen atom is positioned close to the Cys152 thiolate, which performs an S_N2-type nucleophilic attack on the peroxide molecule. The second oxygen atom of H_2O_2 forms an H-bond with the Thr153 γ-OH group. The interaction of H_2O_2 with Cys152 and Thr153 leads to a stretching of the H_2O_2 molecule and promotes reaction completion (Peralta et al., 2015).

A proton relay promotes leaving-group departure

In addition to a specific H_2O_2-binding pocket and transition-state stabilization, GAPDH also appears to harbor a dedicated proton-relay mechanism, which serves to protonate the leaving hydroxyl anion, thereby converting it to water, a much more efficient leaving group. Specifically, the hydroxyl anion accepts a proton from the γ-OH group of Thr153, which is itself re-protonated by the ζ-OH group of Tyr314. Following the de-protonation of Tyr314 the proton relay runs backwards, ultimately taking a proton from His179, which is passed via the sulfenic acid on Cys152, the water molecule and the Thr153 hydroxyl group, back to Tyr314 (Peralta et al., 2015).

The mechanism predicted by MD and QM simulations is well supported by wet-lab experiments. Mutation of Thr153, Tyr314 and another residue Thr177, which also forms an H-bond with Tyr314, all lead to a strong decrease in the H_2O_2 reactivity of recombinant GAPDH in vitro. In further support of a mechanism specifically promoting reactivity with H_2O_2, the reactivity of Cys152 towards an alternative peroxide (tert.-butyl hydroperoxide) is much lower than with H_2O_2 and is unaffected by any of the mutations described above.

GAPDH contains a second highly conserved cysteine residue (Cys156 in the human protein). Mutation of Cys156 also strongly decreases the H_2O_2-reactivity of Cys152. However, surprisingly, rather than performing a redox-related function, it seems that the role of Cys156 is purely structural. MD simulations of wild-type GAPDH show that the H-bond present between the side-chain OH groups of Thr153 and Tyr314 is stable throughout the time-course of the simulation. However, upon mutation of Cys156 to

either an alanine or a serine, the side-chain of Tyr314 becomes more mobile and consequently destabilizes the Thr153-Tyr314 H-bond. Cys156 therefore seems to serve purely to stabilize Tyr314 in the correct orientation to allow for efficient functioning of the proton relay. Consistent with a non-redox role of Cys156, both crystal structures and MD simulations indicate that Cys156 is buried deeply within GADPH and is therefore unavailable to take part in redox chemistry.

The key proton-relay residues, Thr153 and Tyr314 as well as Thr177 and Cys156 are all highly conserved in both bacteria and eukaryotes, although not in the archaea. However, in about 5% of non-archaeal GAPDH sequences Cys156 is replaced with either an alanine, glycine or serine residue. Interestingly, in the vast majority (98.3%) of these sequences a serine or threonine is found at position 243 where there is normally a conserved valine. Analysis of the two available crystal structures of such GAPDH variants, one with Ser243 (*Thermus aquaticus*) and the other with Thr243 (*Staphylococcus aureus*), reveals that in both cases the hydroxyl group of the serine or threonine at position 243 forms an H-bond with the hydroxyl group of Ser156. It appears that this H-bond is sufficient to enable the serine at position 156 to perform an equivalent role to the cysteine, which is normally present in most GAPDH proteins. Indeed, in MD simulations of human GAPDH containing a Cys156Ser mutation, together with a Val-243Thr, the Thr153-Tyr314 H-bond was stabilized compared to the Cys156Ser mutant alone. More excitingly, and a final convincing proof, the H_2O_2 sensitivity of the double mutant GAPDH is restored to that of the wild-type protein. Therefore, it appears that at least in some species other residues effectively replace Cys156 and stabilize the key proton-relay residues.

The proton-relay mechanism also explains the propensity of GAPDH to S-glutathionylation

The propensity of GAPDH towards S-glutathionylation can also be explained by the existence of the proton-relay mechanism. It was determined that the dominant mechanism for S-glutathionylation on Cys152 involves the initial formation of sulfenic acid, followed by a condensation reaction between the sulfenic acid and GSH. Thus, formation of a sulfenic acid on Cys152 is a prerequisite for S-glutathionylation, and therefore the existence of a dedicated mechanism for promoting H_2O_2-reactivity also explains the propensity of Cys152 to S-glutathionylation.

Separate mechanisms promote G3P and H_2O_2 reactivity – implications for our understanding of thiol reactivity

Intriguingly, it was observed that mutation of the key proton-relay residues, while severely affecting the reactivity of Cys152 towards H_2O_2, has little or no impact upon the reaction with the glycolytic substrate G3P. Conversely, it was observed that mutation of Arg234, a key residue in the GAPDH active site and well established to contribute to G3P binding (Reis et al., 2013), leads to a decrease in the rate of glycolytic activity, but has no effect on the reactivity of Cys152 towards H_2O_2.

These findings demonstrate that GAPDH maintains separate and distinct mechanisms, which serve to promote the reactivity of Cys152 towards G3P and H_2O_2, respectively. It is possible to disrupt the reactivity of GAPDH towards H_2O_2 without influencing the reactivity towards G3P and vice versa. More broadly, these findings challenge conventional ideas surrounding "generally reactive" or "generally unreactive" thiols, which are widely assumed to be largely dependent upon thiol pK_a and thus nucleophilicity. On the contrary, it is now becoming clearer that thiol reactivity is a highly tuned and evolved feature, which is frequently very specific towards the intended substrate. Indeed, for both G3P and H_2O_2, GAPDH has highly evolved, specific binding sites and harbors residues that serve to stabilize the reaction transition states and catalyze all steps of the reaction.

In summary, while nucleophile (deprotonated thiol) availability is undoubtedly an essential prerequisite for a reaction to occur, other factors are far more important for determining with which substrates a cysteine residue will react and the kinetics of the reaction. The example of GAPDH tells us that two distinct mechanisms can exist side by side, with each mechanism promoting reactivity of the same cysteine towards a different substrate. These observations thus challenge the long-held concept of 'generally reactive' or 'generally unreactive' thiols.

Redox-regulated functions of GAPDH in physiology and stress

GAPDH functions in primary metabolism

The possibility to disrupt the reactivity of Cys152 towards H_2O_2 without affecting its reactivity with G3P and therefore its glycolytic function, afforded for the first time, the

chance to test the actual physiological relevance of the redox sensitivity of a cysteine residue, where that cysteine residue is also the functional catalytic residue. It has been reported previously that oxidation of GAPDH allows for metabolic re-routing during times of oxidative stress, leading to a diversion of glycolytic flux through the oxidative pentose-phosphate cycle to increase NADPH production (Grant et al., 1999; Ralser et al., 2007). In the cytosol of plant cells, a non-phosphorylating GAPDH exists, namely NP-GAPDH, which is irreversibly oxidizing G3P yielding 3-PGA directly, and not 1,3-bisPGA that would allow for ATP formation in a next step. Interestingly, NP-GAPDH is found to be 63 times less sensitive to oxidative modification and subsequent inactivation than is GapC (Piattoni et al., 2013). NP-GAPDH is thought to produce NADPH, when GapC is inactivated under oxidative stress. The oxidized GapC then could function in signaling (see "Moonlighting functions of GAPDH" below for its many moonlighting functions).

In particular, S-sulfenylation, S-glutathionylation, and S-nitrosylation at the catalytic cysteine are known to inhibit GAPDH glycolytic activity (Little and O'Brien, 1969). Additionally, peroxynitrite inactivates GAPDH by oxidation (Buchczyk et al., 2000). However, it had not been possible to test the specific adaptive function of GAPDH oxidation, as it was not possible to disrupt H_2O_2 reactivity without also abolishing glycolytic activity. With their knowledge of the proton-relay residues present within GAPDH, Peralta and colleagues were able to engineer a yeast strain deleted for all three endogenous GAPDH isoforms with viability maintained by a human GAPDH expressed from a plasmid (Peralta et al., 2015). The yeast growth rate was observed to be indistinguishable in cells expressing either wild-type GAPDH or the Cys156Ser and Tyr314Thr mutants, supporting the observation that these mutations have no effect on the glycolytic activity of GAPDH. However, following both acute and chronic exposure to H_2O_2, cell growth and viability was significantly affected in cells expressing "proton-relay" mutants of GAPDH, as compared to those expressing wild-type GAPDH. Consistent with this observation, the NADPH/$NADP^+$ ratio is increased following an H_2O_2 treatment in cells expressing wild-type GAPDH, presumably as a result of glycolytic flux being diverted through the oxidative pentose-phosphate cycle. However, in yeast cells expressing Cys156Ser and Tyr314Thr mutant GAPDH with an impaired proton relay, the NADPH/$NADP^+$ ratio is decreased following an H_2O_2 challenge, consistent with an inability to divert glycolytic flux to meet the increased NADPH demand.

Moonlighting functions of GAPDH

The responses of the target proteins upon cysteine modifications can be many-fold, because of changed properties and cellular localizations, and often in connection with moonlighting (Dalle-Donne et al., 2009; Hwang et al., 2009; Marozkina and Gaston, 2012). In the past two decades, numerous studies have broadened the classical view of GAPDH as a glycolytic housekeeping gene to a wide range of non-glycolytic functional roles in different cellular locations apart from the cytosol. GAPDH has been identified in the nucleus, ER-Golgi vesicles, plasma membrane and even in the extracellular space. GAPDH, while being inactivated by most modifications, can acquire new properties that might result in the formation of various different microcompartments (Zachgo et al., 2013).

Nuclear functions of GAPDH

Most stress conditions result in a changed gene expression, requiring a signal translocation to the nucleus where interaction with the transcription machinery or with the chromatin structure initiates the required transcriptional change. Upon translocation into the nucleus, GAPDH orchestrates apoptosis, DNA replication, DNA repair, and participates in telomere maintenance (see also: Tristan et al., 2011; Seidler, 2013; Sirover, 2012). In many studies, it has been demonstrated that GAPDH has the property to bind to various DNA and RNA species (for review see: Nicholls et al., 2012).

In mammals, GAPDH translocates into the nucleus upon S-nitrosylation by nitric oxide (NO) (Figure 2). Mammalian GAPDH variants have either three or four cysteine residues that could serve as target sites for covalent attachment of NO. However, increased NO levels under apoptotic conditions lead to S-nitrosylation solely of the active-site cysteine (Cys152 in human, Cys149 in rabbit and Cys150 in mouse), which abolishes the glycolytic activity. S-nitrosylated GAPDH (SNO-GAPDH) then binds to Siah1, an E3-ubiquitin protein ligase with high cytosolic turnover-rate under basal conditions. Upon complex formation, Siah1 becomes stabilized and shuttles SNO-GAPDH into the nucleus in a NO-dependent manner (Hara et al., 2005). On the mechanistic level, the SNO-GAPDH active site (aa 220–238) associates with Siah1, with Lys225 being the critical binding partner as it has been demonstrated with mutant variants. Hence, SNO-GAPDH does not directly interact with its newly formed nitrosylated site, but with an adjacent peptide motif that became accessible as the result of covalent attachment of NO

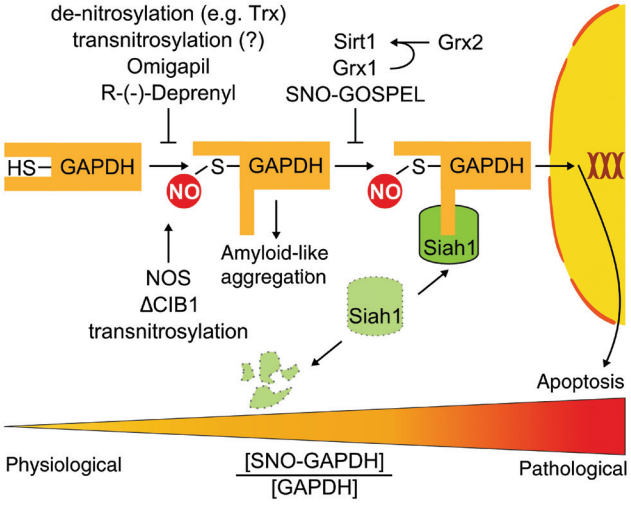

Figure 2 Induction of apoptosis upon nitrosylation of mammalian GAPDH.
Siah-1 shuttles nitrosylated GAPDH into the nucleus in a NO-dependent manner and initiates apoptosis. GAPDH-mediated apoptosis is attenuated prior to binding to Siah-1 because of the action of denitrosylating enzymes or deprenyl derivatives. Furthermore, nitrosylated GOSPEL competes with Siah1 for SNO-GAPDH and prevents nuclear transfer. Sirt-1, which in turn is subjected to redox-modification by glutaredoxin 2, also prevents nuclear accumulation.

(Hara et al., 2005). Once inside the nucleus, SNO-GAPDH/Siah1 initiates apoptotic pathways, e.g. in neurons and macrophages.

A number of anti-apoptotic factors have been described to interfere with GAPDH-Siah1 formation and prevent nuclear translocation of GAPDH under apoptotic conditions (Figure 2), thus constituting a molecular threshold for the manifestation of GAPDH-mediated apoptosis. GAPDH's competitor of Siah Protein Enhances Life (GOSPEL) is a 52 kDa cytosolic protein expressed in high levels in tissues with high glycolytic activity such as brain, heart, lung, and skeletal muscle. After S-nitrosylation of Cys47, it binds to SNO-GAPDH and competes with Siah1 for complex formation as it has been shown in primary neuronal cultures (Sen et al., 2009). Noteworthy, S-nitrosylation of GOSPEL is much faster than that of GAPDH, which allows a fast cytoprotective response after nitrosative stress induction.

Another mechanism that prevents nuclear import of GAPDH under apoptotic conditions induced by irradiation has been described by Hyun-Yoo Joo and colleagues in 293T and Hela cells (Joo et al., 2012). They propose that Sirtuin1 (Sirt1) expression, but not its enzymatic activity, retains GAPDH in the cytosol. Sirt1 is an epigenetic NAD^+-dependent protein deacetylase that regulates gene expression in response to the metabolic state. Surprisingly, when Sirt1 was depleted, Joo and coworkers observed that GAPDH translocates into the nucleus per se, even in the absence of any apoptotic trigger. Thus, solely Sirt1 knockout initiates GAPDH-mediated apoptosis. However, in another context, Sirt1 activity in turn is regulated by redox-modification at the post-translational level. A conserved cysteine in the catalytic domain of Sirt1 serves as a substrate for glutaredoxin 2. Glutaredoxin 2 deglutathionylates the cysteinyl residue and thereby orchestrates Sirt1-dependent vascular development in zebrafish embryos (Bräutigam et al., 2013). A morpholino-based glutaredoxin 2 knock-down lead to delayed and disordered vascular development in zebrafish indicating the physiological relevance of Sirt1 post-translational (redox-)regulation. Regulators of GAPDH export such as Sirt1 might also be subjected to redox-modification, which increases the complexity of GAPDH-mediated apoptosis. Similar to the animal system with the Sirt1 binding to GAPDH, in yeast the Sirt1 homologue Sir2 interacts with TDH3 resulting in histone deacetylation and chromatin restructuring (Ringel et al., 2013).

In plants, nuclear localization of GAPDH was observed upon cadmium stress. Cadmium treatment induces oxidative stress and NO accumulation in roots of Arabidopsis seedlings leading to nuclear localization of GapC (Vescovi et al., 2013) (Figure 3A). GapC and thioredoxin h were previously also found to interact in the nucleus of Arabidopsis protoplasts expressing the respective FP-GapC fusion constructs (Scheibe, R., unpublished data). GapC had been identified to bind to the NADP-MDH gene (Hameister et al., 2007), and its transcript and protein level increase upon sustained over-reduction and initial formation of ROS (Becker et al., 2006). A function of nuclear localized GapC might indicate its role as a co-activator for controlling NADP-MDH expression allowing for an increased capacity of the malate-valve required to export and balance excess reducing equivalents in the chloroplast. Under physiological conditions, for example upon changes in photosynthesis, malate valves adjust the interchange of reducing equivalents over compartmental boundaries (Scheibe, 2004). Such role of GAPDH as a co-activator has previously been demonstrated for histone expression (Zheng et al., 2003).

Cytosolic microcompartments containing GAPDH

GAPDH-containing metabolons associated with the cytoskeleton are the classical case of transient complex formation in the cytosol of animal cells (Ovádi and Srere,

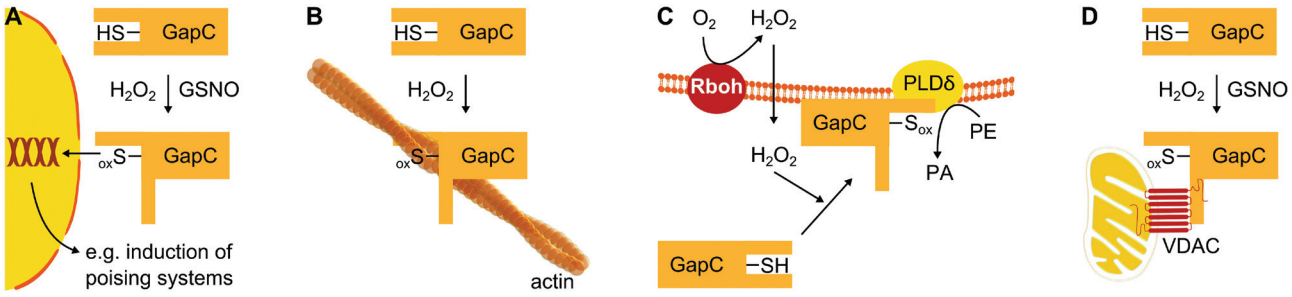

Figure 3 Localizations and moonlighting functions of plant GAPDH found to occur upon redox changes.
(A) Oxidative cysteine modification initiates nuclear localization of GapC and its suggested function as a co-activator of gene expression. (B) Binding of oxidized GapC to actin filaments leading to actin bundling. (C) Plasma membrane binding of GapC and activation of phosphatidic acid (PA) signaling pathway inducing stomata closure. (D) Binding of GapC to VDAC altering VDAC properties and mitochondria localization.

1996). S-sulfhydration at Cys152 of human GAPDH was identified and related to its activity and cellular function such as actin polymerization (Mustafa et al., 2011). In plants, actin-binding of GapC was shown to be redox-dependent *in vitro*, leading to bundling of actin filaments (Wojtera-Kwiczor et al., 2012) (Figure 3B).

The redox-dependent binding of GAPDH to the inositol 1,4,5-triphosphate (IP_3) receptor at the plasma membrane of human cell lines was shown to result in Ca^{2+} release by the local NADH production (Patterson et al., 2005). Such a link between metabolism and signaling pathways can also be deduced from the role of Arabidopsis GapC1 in guard cells. Its interaction with the phospholipase PLD mediates crosstalk with ABA signaling and stomatal closure as shown by Guo et al. (2012) (Figure 3C).

GAPDH also associates with the outer mitochondrial membrane (Sweetlove et al., 2002; Giegé, 2003; Graham et al., 2007). VDAC-binding appears to be redox-dependent *in vitro* leading to a transient microcompartment attached to the voltage-dependent anion channel (VDAC) at the outer mitochondrial membrane (Wojtera-Kwiczor et al., 2012) (Figure 3D). Whether the formation of this complex is part of a redox-dependent signal *in vivo* inducing or preventing cell death is not yet known, as plants do not possess the specific proteins involved in animal apoptosis (van Doorn, 2011). For heart cells, the trans-nitrosylating activity of GAPDH was related even to the mitochondrial localization of the modified enzyme and S-nitrosylation of mitochondrial proteins such as VDAC1 (Kohr et al., 2014).

Much attention is paid to downstream signaling events initiated by S-nitrosylation leading to the unexpected functional roles of GAPDH. Recently, members of the thioredoxin family were found to reversibly nitrosylate GAPDH. In glutaredoxin 1 overexpressing H9c2 cells nuclear translocation of GAPDH is inhibited, suggesting that glutaredoxin 1 might denitrosylate SNO-GAPDH (Inadomi et al., 2012). The cytosolic enzyme GAPDH with its active-site cysteine residue that is prone to oxidation can be found in lists of interaction partners for thioredoxins when affinity chromatography has been performed on immobilized thioredoxin (Yamazaki et al., 2004; Marchand et al., 2006). As most signals appear transiently, it is conceivable that the modifications and their reversal are catalyzed by members of the thioredoxin family. Conversely, S-nitrosylation of plant GAPDH has been shown to be merely dependent upon the GSH/GSNO ratio (Zaffagnini et al., 2013).

Fine-control and specificity of GAPDH as a thiol switch

GAPDH in pathogenesis and medical treatments

Accumulation of nuclear GAPDH has been recognized long before the discovery of the SNO-GAPDH/Siah1 pathway, e.g., in post-mortem brain samples from patients with Parkinson's disease (PD) (Tatton, 2000). These findings already suggested a causative role of nuclear GAPDH in PD and other neurodegenerative diseases (Chuang et al., 2005). This hypothesis has been followed later on by Hara et al. in a study with a MPTP-based PD mouse model. Hara et al. were able to demonstrate increased levels of SNO-GAPDH and SNO-GAPDH/Siah1 in their PD mouse model (Hara et al., 2006), suggesting that SNO-GADPH/Siah1 pathway may indeed be involved in PD pathogenesis. Although no drug that specifically prevents nuclear translocation of GAPDH has been listed by the FDA, it is now known that MAO inhibitors such as selegilin (R-(-)-deprenyl) also exert a neuroprotective side effect ascribed

to interaction with SNO-GAPDH and inhibition of complex formation with Siah1 (Hara et al., 2006).

Based on this, Omigapil (TCH346), an R-(-)-deprenyl derivative without MAO inhibitory but neuroprotective properties, has been developed (Kragten et al., 1998). However, a neuroprotective effect in clinical trials involving PD and amyotrophic lateral sclerosis patients failed to show any beneficial effect during therapy (Olanow et al., 2006; Miller et al., 2007). Currently, Omigapil is under investigation in a study involving patients with congential muscular dystrophy.

Huntington's disease is caused by a mutational elongation of the trinucleotide CAG repetitive segment of huntingtin protein (htt). Disease severity depends on the length of the elongated polyglutamine segment which can differ by 215 glutamines compared to the healthy counterpart (6–35). Pathomechanism of trinucleotide repeat disorders is linked to nuclear accumulation of their mutated proteins and subsequent cytotoxic action. Based on the observation that GAPDH interacts with mutated htt (mhtt) but not wild-type (Burke et al., 1996), Bae and coworkers found that SNO-GAPDH/Siah1 shuttles mhtt into the nucleus and elicits cytotoxicity (Bae et al., 2006). With a mutant variant of Siah1 which is no longer pro-apoptotic, but is still stabilized by SNO-GAPDH and translocated into the nucleus (Siah1ΔRING), cytotoxicity elicited by nuclear SNO-GAPDH-Siah1 was decoupled from cytotoxicity exerted by nuclear mhtt.

Interaction of GAPDH with other mutant polyglutamine proteins involved in neurodegenerative disorders like ataxin-1 (spinobulbar muscular atrophy) (Koshy et al., 1996) and atrophin-1 (dentatorubral-pallidoluysian atrophy) (Burke et al., 1996) was demonstrated about two decades ago, but still there is no study that addresses the possibility of a SNO-GAPDH-Siah1-mediated transfer of both candidates.

Taken together, the current data underscore the therapeutic potential of targeting SNO-GADPH/Siah1 formation in neurodegenerative diseases in general. So far, only a limited number of *in vivo* studies address this issue. Interestingly, SNO-GAPDH-Siah1 association could not be observed in the cardiomyocyte-derived cell line H9c2 (Inadomi et al., 2012). This suggests the existence of cell-type specific routes by which GAPDH is shuttled into the nucleus in a NO-dependent manner.

NO transfer is more complex than assumed in some studies and occurs in a specific spatio-temporal manner involving the specificity of enzymes. Also, the nitrosylated thiol can be further oxidized to sulfenic, sulfinic or sulfonic acids, a fact that again increases complexity. A better understanding of GAPDH-mediated apoptosis on the atomic and molecular level will contribute to the development of novel and promising drugs for clinical intervention with SNO-GAPDH/Siah1-complex formation.

Role of GAPDH in acclimation and cell protection

In plants, cytosolic GAPDH was suggested as H_2O_2 sensor (Hancock et al., 2006) and as a central hub in the plant signaling network. In addition, there are various indications that S-nitrosylation of GAPDH can lead either to adaptation and acclimation of metabolism under stress conditions, or to cell death in order to prevent any further metabolism and ROS/RNS generation (Romero-Puertas et al., 2013). Arabidopsis GapC1 and 2 are modified and thereby reversibly inactivated, and S-nitrosylation as well as S-glutathionylation could be seen *in vitro* to occur at both cysteine residues (Holtgrefe et al., 2008; Zaffagnini et al., 2013). Velocity and extent of the modifications likely depend on the presence of metabolites similar to light-dark modulation of chloroplast enzymes. Here, specific metabolite effects on the interconverting reactions in each of these cases allow for fine-tuning of the individual enzyme activities *in situ* (Scheibe, 1990). In an analogous way, GapC modification and inactivation by GSNO is decreased in the presence of the substrate G3P (Holtgrefe et al., 2008). This suggests that the metabolic activities fine-modulate the modification reaction, i.e. it only occurs to the extent that the glycolytic flux is inhibited because of incoming oxidative stress. Some difference in the redox-responsiveness of the two Arabidopsis GAPDH isoforms GapC1 and GapC2 have been observed *in vitro* when exposed to increasing concentrations of GSNO on a high GSH background level as would be expected in a cellular environment under incoming stress (Holtgrefe et al., 2008). However, there is still no information on the differential role of these two highly related isoforms.

In electron-transport chains for energy conversion, or upon activation of the respiratory-burst oxidase in the plasma membrane, H_2O_2 is formed that can lead to oxidative cysteine modifications as described in "Reversible thiol modifications and redoxin targets", above. The source of H_2O_2 production and its spatial and temporal distribution is recognized and elicits different signal transduction pathways for an appropriate. Upon light stress, this can be either photoacclimation or cell death (Mullineaux et al., 2006). H_2O_2 is sensed already at low levels and leads to the induction of either antioxidant systems or of genes for re-routing of metabolic pathways

in order to cope with changed demand for electron acceptors for maintenance of redox homeostasis (Piattoni et al., 2013; Selinski and Scheibe, 2014).

In yeast, reductive stress leads to increased expression of one of the three GAPDH isoforms, namely TDH1, suggesting a link between metabolism, stress and gene expression (Valadi et al., 2004). Yeast mutants lacking the GAPDH isoform TDH3 were shown to be more sensitive to oxidative stress (Grant et al., 1999). TDH3 is reversibly protected by mixed disulfide formation with GSH, and TDH2 is maintained in the active form under low oxidative stress, while it is irreversibly damaged in an oxidative burst. Protection of TDH3 and its re-activation after the H_2O_2 challenge was thus essential for survival.

Importance of GAPDH and its central role in stress responses

GAPDH is constitutively expressed in virtually all cell types and tissues under normal and most pathophysiological conditions. Therefore, GAPDH was commonly accepted as a reference gene for transcript and protein analyses. Conversely, under a variety of stress conditions applied to plants, expression of cytosolic GAPDH has been found to be increased. There are various examples for Arabidopsis with increased expression of cytosolic GAPDH upon stress such as heat shock, anaerobiosis, increased sugar supply (Yang et al., 1993), cold (Bae et al., 2003), cadmium (Roth et al., 2006; Sarry et al., 2006) or addition of salicylic acid (Rajjou et al., 2006). Cytosolic GAPDH expression is also increased by biotic stress treatment in potato (Laxalt et al., 1996). Conversely, overexpression of GapC3 confers salt tolerance in rice (Zhang et al., 2011).

Some interesting clues as to a potential role of plant GAPDH in sensing and signaling as opposed to its glycolytic function come from the analysis of mutant lines lacking GAPDH: GapC1-KO plants were characterized and found to suffer from defects in fertility and increased oxidative stress (Rius et al., 2008). GapC2-KO leads to hypersensitivity towards low-phosphate stress (Wang et al., 2007). In contrast to wild-type plants, GapC1/2 knockdown plants were found not to respond to drought by closing their stomata (Guo et al., 2012). Here, it was suggested that oxidized GapC interacts with phospholipase D leading to its activation (Guo et al., 2012). Phosphatidic acid (PA)-dependent signaling and its integration with H_2O_2 and ABA signaling have been suggested to operate to control stomatal opening and closure (Figure 3C). Osmotic stress-activated kinase (NtOSAK) was found to interact with GAPDH, and to directly or indirectly respond to NO. As salt stress induces activation of the kinase, correlating with the S-nitrosylation of GAPDH, it is assumed that it is involved in signaling for induction of salt resistance in tobacco (Wawer et al., 2010).

Redox-switching mechanisms are highly suitable for crosstalk between metabolism and gene expression during development and upon stress. In addition to stress, the changing metabolic requirements during development in the various tissues need to be met under undisturbed conditions. It is essential that any imbalance is sensed in time and the cellular redox-state is returned back to normal in order to avoid oxidative stress and subsequent damage. This is also true for photoautotrophic as well as for heterotrophic growth conditions in all organisms. Minor deviations from steady-state are sensed and the signals are transferred to the nucleus to induce expression (or repression) of the required genes to regain homeostasis. Therefore, complete removal of ROS would be even counterproductive.

As the various regulated pathways are distributed between cellular compartments, transport of signals and metabolites is essential. In addition, basic functions need to respond to changes caused by developmental programs and to external impact from abiotic and biotic factors. All this requires regulatory systems for integration of these signals for appropriate responses of growth, differentiation and cell fate (Neill et al., 2002; Potters et al., 2010; Kocsy et al., 2013). GAPDH appears to be a versatile and flexible hub because of its spatial distribution over many locations and its appearance in multiple non-glycolytic functions. Its affinity to a variety of protein complexes as well as nucleic acids indicates the potential to influence a wide range of biological processes and to serve as target for clinical intervention and diagnosis as well as for improving crops for adaptation of growth and resistance under unfavorable conditions.

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References

Baalmann, E., Backhausen, J.E., Kitzmann, C., and Scheibe, R. (1994). Regulation of NADP-dependent glyceraldehyde 3-phosphate dehydrogenase activity in spinach chloroplasts. Bot. Acta *107*, 313–320.

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.

Bae, M.S., Cho, E.J., Choi, E.Y., and Park, O.K. (2003). Analysis of the *Arabidopsis* nuclear proteome and its response to cold stress. Plant J. *36*, 652–663.

Bae, B.I., Hara, M.R., Cascio, M.B., Wellington, C.L., Hayden, M.R., Ross, C.A., Ha, H.C., Li, X.J., Snyder, S.H., and Sawa, A. (2006). Mutant huntingtin: nuclear translocation and cytotoxicity mediated by GAPDH. Proc. Nat. Acad. Sci. USA *103*, 3405–3409.

Barford, D. (2004). The role of cysteine residues as redox-sensitive regulatory switches. Curr. Opin. Struct. Biol. *14*, 679–686.

Baty, J.W., Hampton, M.B., and Winterbourn, C.C. (2005). Proteomic detection of hydrogen peroxide-sensitive thiol proteins in Jurkat cells. Biochem. J. *389*, 785–795.

Becker, B., Holtgrefe, S., Jung, S., Wunrau, C., Kandlbinder, A., Baier, M., Dietz, K.J., Backhausen, J.E., and Scheibe, R. (2006). Influence of the photoperiod on redox regulation and stress responses in *Arabidopsis thaliana* L. (Heynh.) plants under long- and short-day conditions. Planta *224*, 380–393.

Berndt, C., Lillig, C.H., and Flohé L. (2014). Redox regulation by glutathione needs enzymes. Front. Pharmacol. *5*, 168.

Boschi-Muller, S., Gand, A., and Branlant, G. (2008). The methionine sulfoxide reductases: catalysis and substrate specificities. Arch. Biochem. Biophys. *474*, 266–273.

Bräutigam, L., Jensen, L.D., Poschmann, G., Nystrom, S., Bannenberg, S., Dreij, K., Lepka, K., Prozorovski, T., Montano, S.J., Aktas, O., et al. (2013). Glutaredoxin regulates vascular development by reversible glutathionylation of sirtuin 1. Proc. Nat. Acad. Sci. USA *110*, 20057–20062.

Bruce King, S. (2013). Potential biological chemistry of hydrogen sulfide (H_2S) with the nitrogen oxides. Free Radic. Biol. Med. *55*, 1–7.

Buchanan, B.B. (1980). Role of light in the regulation of chloroplast enzymes. Annu. Rev. Plant Physiol. *31*, 341–374.

Buchanan, B.B. and Balmer, Y. (2005). Redox regulation: A broadening horizon. Annu. Rev. Plant Biol. *56*, 187–220.

Buchanan, B.B., Holmgren, A., Jacquot, J.P., and Scheibe, R. (2012). Fifty years in the thioredoxin field and a bountiful harvest. Biochim. Biophys. Acta *1820*, 1822–1829.

Buchczyk, D.P, Briviba, K., Hartl, U., and Sies, H. (2000). Responses to peroxynitrite in yeast: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a sensitive intracellular target for nitration and enhancement of chaperone expression and ubiquitination. Biol. Chem. *381*, 121–126.

Burke, J.R., Enghild, J.J., Martin, M.E., Jou, Y.S., Myers, R.M., Roses, A.D., Vance, J.M., and Strittmatter, W.J. (1996). Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. Nat. Med. *2*, 347–350.

Cai, Z. and Yan, L.-J. (2013). Protein oxidative modifications: Beneficial roles in disease and health. J. Biochem. Pharmacol. Res. *1*, 15–26.

Chuang, D.M., Hough, C., and Senatorov, V.V. (2005). Glyceraldehyde-3-phosphate dehydrogenase, apoptosis, and neurodegenerative diseases. Annu. Rev. Pharmacol. Toxicol. *45*, 269–290.

Dalle-Donne, I., Rossi, R., Colombo, G., Giustarini, D., and Milzani, A. (2009). Protein S-glutathionylation: a regulatory device from bacteria to humans. Trends Biochem. Sci. *34*, 85–96.

Ferrer-Sueta, G., Manta, B., Botti, H., Radi, R., Trujillo, M., and Denicola, A. (2011). Factors affecting protein thiol reactivity and specificity in peroxide reduction. Chem. Res. Toxicol. *24*, 434–450.

Flohé, L., Toppo, S., Cozza, G., and Ursini, F. (2011). A comparison of thiol peroxidase mechanisms. Antioxid. Redox Signal. *15*, 763–780.

Foyer, C.H. and Noctor, G. (2012). Managing the cellular redox hub in photosynthetic organisms. Plant Cell Environ. *35*, 199–201.

Ghezzi, P. (2013). Protein glutathionylation in health and disease. Biochim. Biophys. Acta *1830*, 3165–3172.

Giegé, P. (2003). Enzymes of glycolysis are functionally associated with the mitochondrion in *Arabidopsis* cells. Plant Cell *15*, 2140–2151.

Gotor, C., Garcia, I., Crespo, J.L., and Romero, L.C. (2013). Sulfide as a signaling molecule in autophagy. Autophagy *9*, 609–611.

Gould, N., Doulias, P.T., Tenopoulou, M., Raju, K., and Ischiropoulos, H. (2013). Regulation of protein function and signaling by reversible cysteine S-nitrosylation. J. Biol. Chem. *288*, 26473–26479.

Graham, J.W.A., Williams, T.C.R., Morgan, M., Fernie, A.R., Ratcliffe, R.G., and Sweetlove, L.J. (2007). Glycolytic enzymes associate dynamically with mitochondria in response to respiratory demand and support substrate channeling. Plant Cell *19*, 3723–3738.

Grant, C.M., Quinn, K.A., and Dawes, I.W. (1999). Differential protein S-thiolation of glyceraldehyde-3-phosphate dehydrogenase isoenzymes influences sensitivity to oxidative stress. Mol. Cell. Biol. *19*, 2650–2656.

Guo, L., Devaiah, S.P., Narasimhan, R., Pan, X., Zhang, Y., Zhang, W., and Wang, X. (2012). Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the *Arabidopsis* response to stress. Plant Cell *24*, 2200–2212.

Hameister, S., Becker, B., Holtgrefe, S., Strodtkötter, I., Linke, V., Backhausen, J.E., and Scheibe, R. (2007). Transcriptional regulation of NADP-dependent malate dehydrogenase: comparative genetics and identification of DNA-binding proteins. J. Mol. Evol. *65*, 437–455.

Hancock, J.T. and Whiteman, M. (2014). Hydrogen sulfide and cell signaling: team player or referee? Plant Physiol. Biochem. *78*, 37–42.

Hancock, J.T., Henson, D., Nyirenda, M., Desikan, R., Harrison, J., Lewis, M., Hughes, J., and Neill, S.J. (2005). Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in *Arabidopsis*. Plant Physiol. Biochem. *43*, 828–835.

Hancock, J., Desikan, R., Harrison, J., Bright, J., Hooley, R., and Neill, S. (2006). Doing the unexpected: proteins involved in hydrogen peroxide perception. J. Exp. Bot. *57*, 1711–1718.

Hanschmann, E.M., Godoy, J.R., Berndt, C., Hudemann, C., and Lillig, C.H. (2013). Thioredoxins, glutaredoxins, and peroxiredoxins – molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. Antioxid. Redox Signal. *19*, 1539–1605.

Hara, M.R., Agrawal, N., Kim, S.F., Cascio, M.B., Fujimuro, M., Ozeki, Y., Takahashi, M., Cheah, J.H., Tankou, S.K., Hester, et al. (2005). S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. Nat. Cell. Biol. *7*, 665–674.

Hara, M.R., Thomas, B., Cascio, M.B., Bae, B.I., Hester, L.D., Dawson, V.L., Dawson, T.M., Sawa, A., and Snyder, S.H. (2006).

Neuroprotection by pharmacologic blockade of the GAPDH death cascade. Proc. Nat. Acad. Sci. USA *103*, 3887–3889.

Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. J. Gerontol. *11*, 298–300.

Herrmann, J.M. and Jakob, U. (2008). Special issue: redox regulation of protein folding. Preface. Biochim. Biophys. Acta *1783*, 519.

Hess, D.T., Matsumoto, A., Kim, S.O., Marshall, H.E., and Stamler, J.S. (2005). Protein S-nitrosylation: purview and parameters. Nat. Rev. Mol. Cell. Biol. *6*, 150–166.

Holtgrefe, S., Gohlke, J., Starmann, J., Druce, S., Klocke, S., Altmann, B., Wojtera, J., Lindermayr, C., and Scheibe, R. (2008). Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications. Physiol. Plant. *133*, 211–228.

Hwang, N.R., Yim, S.H., Kim, Y.M., Jeong, J., Song, E.J., Lee, Y., Choi, S., and Lee, K.J. (2009). Oxidative modifications of glyceraldehyde-3-phosphate dehydrogenase play a key role in its multiple cellular functions. Biochem. J. *423*, 253–264.

Inadomi, C., Murata, H., Ihara, Y., Goto, S., Urata, Y., Yodoi, J., Kondo, T., and Sumikawa, K. (2012). Overexpression of glutaredoxin protects cardiomyocytes against nitric oxide-induced apoptosis with suppressing the S-nitrosylation of proteins and nuclear translocation of GAPDH. Biochem. Biophys. Res. Commun. *425*, 656–661.

Jenkins, J.L. and Tanner, J.J. (2006). High-resolution structure of human D-glyceraldehyde-3-phosphate dehydrogenase. Acta Crystallogr. D. Biol. Crystallogr. *62*, 290–301.

Jones, D.P. and Sies, H. (2007). Oxidative stress. In: Encyclopaedia of Stress, G. Fink, ed. (San Diego, CA: Elsevier), pp. 45–48.

Joo, H.Y., Woo, S.R., Shen, Y.N., Yun, M.Y., Shin, H.J., Park, E.R., Kim, S.H., Park, J.E., Ju, Y.J., Hong, S.H., et al. (2012). SIRT1 interacts with and protects glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from nuclear translocation: implications for cell survival after irradiation. Biochem. Biophys. Res. Commun. *424*, 681–686.

Kabil, O., Motl, N., and Banerjee, R. (2014). H_2S and its role in redox signaling. Biochim. Biophys. Acta *1844*, 1355–1366.

Kocsy, G., Tari, I., Vankova, R., Zechmann, B., Gulyas, Z., Poor, P., and Galiba, G. (2013). Redox control of plant growth and development. Plant Sci. *211*, 77–91.

Kohr, M.J., Murphy, E., and Steenbergen, C. (2014). Glyceraldehyde-3-phosphate dehydrogenase acts as a mitochondrial trans-S-nitrosylase in the heart. PLoS One *9*, e111448.

Kolluru, G.K., Shen, X., and Kevil, C.G. (2013). A tale of two gases: NO and H_2S, foes or friends for life? Redox Biol. *1*, 313–318.

Koshy, B., Matilla, T., Burright, E.N., Merry, D.E., Fischbeck, K.H., Orr, H.T., and Zoghbi, H.Y. (1996). Spinocerebellar ataxia type-1 and spinobulbar muscular atrophy gene products interact with glyceraldehyde-3-phosphate dehydrogenase. Human Mol. Gen. *5*, 1311–1318.

Kragten, E., Lalande, I., Zimmermann, K., Roggo, S., Schindler, P., Muller, D., van Oostrum, J., Waldmeier, P., and Furst, P. (1998). Glyceraldehyde-3-phosphate dehydrogenase, the putative target of the antiapoptotic compounds CGP 3466 and R-(-)-deprenyl. J. Biol. Chem. *273*, 5821–5828.

Laxalt, A.M., Cassia, R.O., Sanllorenti, P.M., Madrid, E.A., Andreu, A.B., Daleo, G.R., Conde, R.D., and Lamattina, L. (1996). Accumulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase RNA under biological stress conditions and elicitor treatments in potato. Plant Mol. Biol. *30*, 961–972.

Lillig, C.H. and Berndt, C. (2008). Preface to the special issue on redox control of cell function. Biochim. Biophys. Acta *1780*, 1169.

Lillig, C.H. and Berndt, C. (2013). Glutaredoxins in thiol/disulfide exchange. Antioxid. Redox Signal. *18*, 1654–1665.

Little, C. and O'Brien, P.J. (1969). Mechanism of peroxide-inactivation of the sulphydryl enzyme glyceraldehyde-3-phosphate dehydrogenase. Eur. J. Biochem. *10*, 533–538.

Marchand, C., Le Marechal, P., Meyer, Y., and Decottignies, P. (2006). Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. Proteomics *6*, 6528–6537.

Marozkina, N.V. and Gaston, B. (2012). S-Nitrosylation signaling regulates cellular protein interactions. Biochim. Biophys. Acta *1820*, 722–729.

Meyer, Y., Buchanan, B.B., Vignols, F., and Reichheld, J.P. (2009). Thioredoxins and glutaredoxins: unifying elements in redox biology. Ann. Rev. Genet. *43*, 335–367.

Michelet, L., Zaffagnini, M., Massot, V., Keryer, E., Vanacker, H., Miginiac-Maslow, M., Issakidis-Bourguet, E., and Lemaire, S.D. (2006). Thioredoxins, glutaredoxins, and glutathionylation: new crosstalks to explore. Photosynth. Res. *89*, 225–245.

Miller, R., Bradley, W., Cudkowicz, M., Hubble, J., Meininger, V., Mitsumoto, H., Moore, D., Pohlmann, H., Sauer, D., Silani, V., et al. (2007). Phase II/III randomized trial of TCH346 in patients with ALS. Neurology *69*, 776–784.

Mullineaux, P.M., Karpinski, S., and Baker, N.R. (2006). Spatial dependence for hydrogen peroxide-directed signaling in light-stressed plants. Plant Physiol. *141*, 346–350.

Mustafa, A.K., Gadalla, M.M., Sen, N., Kim, S., Mu, W., Gazi, S.K., Barrow, R.K., Yang, G., Wang, R., and Snyder, S.H. (2011). H_2S signals through protein S-sulfhydration. Sci. Signal. *2*, ra72.

Nagy, P. (2013). Kinetics and mechanisms of thiol-disulfide exchange covering direct substitution and thiol oxidation-mediated pathways. Antioxid. Redox Signal. *18*, 1623–1641.

Neill, S.J., Desikan, R., Clarke, A., Hurst, R.D., and Hancock, J.T. (2002). Hydogen peroxide and nitric oxide as signalling molecules in plants. J. Exp. Bot. *53*, 1237–1247.

Nicholls, C., Li, H., and Liu, J.P. (2012). GAPDH: a common enzyme with uncommon functions. Clin. Exp. Pharmacol. Physiol. *39*, 674–679.

Olanow, C.W., Schapira, A.H., LeWitt, P.A., Kieburtz, K., Sauer, D., Olivieri, G., Pohlmann, H., and Hubble, J. (2006). TCH346 as a neuroprotective drug in Parkinson's disease: a double-blind, randomised, controlled trial. Lancet Neurol. *5*, 1013–1020.

Ovádi, J. and Srere, P.A. (1996). Metabolic consequences of enzyme interactions. Cell Biochem. Funct. *14*, 249–258.

Patterson, R.L., van Rossum, D.B., Kaplin, A.I., Barrow, R.K., and Snyder, S.H. (2005). Inositol 1,4,5-trisphosphate receptor/GAPDH complex augments Ca^{2+} release via locally derived NADH. Proc. Nat. Acad. Sci. USA *102*, 1357–1359.

Peralta, D., Bronowska, A.K., Morgan, B., Dóka, É., Van Laer, K., Nagy, P., Gräter, F., and Dick, T.P. (2015). A proton relay enhances H_2O_2 sensitivity of GAPDH to facilitate metabolic adaptation. Nat. Chem. Biol. *11*, 156–163.

Perez-Mato, I., Castro, C., Ruiz, F.A., Corrales, F.J., and Mato, J.M. (1999). Methionine adenosyltransferase S-nitrosylation is regulated by the basic and acidic amino acids surrounding the target thiol. J. Biol. Chem. *274*, 17075–17079.

Piattoni, C.V., Guerrero, S.A., and Iglesias, A.A. (2013). A differential redox regulation of the pathways metabolizing glyceraldehyde-3-phosphate tunes the production of reducing power in the cytosol of plant cells. Int. J. Mol. Sci. *14*, 8073–8092.

Polgar, L. (1975). Ion-pair formation as a source of enhanced reactivity of the essential thiol group of D-glyceraldehyde-3-phosphate dehydrogenase. Eur. J. Biochem. *51*, 63–71.

Potters, G., Horemans, N., and Jansen, M.A. (2010). The cellular redox state in plant stress biology – a charging concept. Plant Physiol. Biochem. *48*, 292–300.

Rajjou, L., Belghazi, M., Huguet, R., Robin, C., Moreau, A., Job, C., and Job, D. (2006). Proteomic investigation of the effect of salicylic acid on *Arabidopsis* seed germination and establishment of early defense mechanisms. Plant Physiol. *141*, 910–923.

Ralser, M., Wamelink, M.M., Kowald, A., Gerisch, B., Heeren, G., Struys, E.A., Klipp, E., Jakobs, C., Breitenbach, M., Lehrach, H., et al. (2007). Dynamic rerouting of the carbohydrate flux is key to counteracting oxidative stress. J. Biol. *6*, Article 10.

Reis, M., Alves, C.N., Lameira, J., Tunon, I., Marti, S., and Moliner, V. (2013). The catalytic mechanism of glyceraldehyde 3-phosphate dehydrogenase from *Trypanosoma cruzi* elucidated via the QM/MM approach. Phys. Chem. Chem. Phys. *15*, 3772–3785.

Rhee, S.G., Kang, S.W., Jeong, W., Chang, T.-S., Yang, K.-S., and Woo, H.A. (2005). Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. Curr. Op. Cell Biol. *17*, 183–189.

Ringel, A.E., Ryznar, R., Picariello, H., Huang, K.L., Lazarus, A.G., and Holmes, S.G. (2013). Yeast Tdh3 (glyceraldehyde 3-phosphate dehydrogenase) is a Sir2-interacting factor that regulates transcriptional silencing and rDNA recombination. PLoS Genet. *9*, e1003871.

Rius, S.P., Casati, P., Iglesias, A.A., and Gomez-Casati, D.F. (2008). Characterization of *Arabidopsis* lines deficient in GAPC-1, a cytosolic NAD-dependent glyceraldehyde-3-phosphate dehydrogenase. Plant Physiol. *148*, 1655–1667.

Romero-Puertas, M.C., Rodriguez-Serrano, M., and Sandalio, L.M. (2013). Protein S-nitrosylation in plants under abiotic stress: an overview. Front. Plant Sci. *4*, 373.

Roth, U., von Roepenack-Lahaye, E., and Clemens, S. (2006). Proteome changes in *Arabidopsis thaliana* roots upon exposure to Cd^{2+}. J. Exp. Bot. *57*, 4003–4013.

Rouhier, N., Lemaire, S.D., and Jacquot, J.P. (2008). The role of glutathione in photosynthetic organisms: emerging functions for glutaredoxins and glutathionylation. Annu. Rev. Plant. Biol. *59*, 143–166.

Sarry, J.E., Kuhn, L., Ducruix, C., Lafaye, A., Junot, C., Hugouvieux, V., Jourdain, A., Bastien, O., Fievet, J.B., Vailhen, D., et al. (2006). The early responses of *Arabidopsis thaliana* cells to cadmium exposure explored by protein and metabolite profiling analyses. Proteomics *6*, 2180–2198.

Scheibe, R. (1990). Light/dark modulation: Regulation of chloroplast metabolism in a new light. Bot. Acta *103*, 327–334.

Scheibe, R. (2004). Malate valves to balance cellular energy supply. Physiol. Plant. *120*, 21–26.

Seidler, N.W. (2013). GAPDH: Biological Properties and Diversity (Dordrecht, The Netherlands: Springer).

Selinski, J. and Scheibe, R. (2014). Lack of malate valve capacities lead to improved N-assimilation and growth in transgenic *arabidopsis thaliana* plants. Plant Signal. Behav. *9*, e29057.

Sen, N., Hara, M.R., Ahmad, A.S., Cascio, M.B., Kamiya, A., Ehmsen, J.T., Agrawal, N., Hester, L., Dore, S., Snyder, S.H., et al. (2009). GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation. Neuron *63*, 81–91.

Shenton, D. and Grant, C.M. (2003). Protein S-thiolation targets glycolysis and protein synthesis in response to oxidative stress in the yeast *Saccharomyces cerevisiae*. Biochem. J. *374*, 513–519.

Sies, H. (1985). Oxidative stress. (London: Academic Press).

Sies, H. (1986). Biochemistry of oxidative stress. Angew. Chem. *25*, 1058–1071.

Sirover, M.A. (2012). Subcellular dynamics of multifunctional protein regulation: mechanisms of GAPDH intracellular translocation. J. Cell. Biochem. *113*, 2193–2200.

Spadaro, D., Yun, B.W., Spoel, S.H., Chu, C., Wang, Y.Q., and Loake, G.J. (2010). The redox switch: dynamic regulation of protein function by cysteine modifications. Physiol. Plant. *138*, 360–371.

Stone, J.R. (2004). An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. Arch. Biochem. Biophys. *422*, 119–124.

Sweetlove, L.J., Heazlewood, J.L., Herald, V., Holtzapffel, R., Day, D.A., Leaver, C.J., and Millar, A.H. (2002). The impact of oxidative stress on *Arabidopsis* mitochondria. Plant J. *32*, 891–904.

Tatton, N.A. (2000). Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. Exp. Neurol. *166*, 29–43.

Tristan, C., Shahani, N., Sedlak, T.W., and Sawa, A. (2011). The diverse functions of GAPDH: views from different subcellular compartments. Cell Signal. *23*, 317–323.

Valadi, H., Valadi, Ansell, R., Gustafsson, L., Adler, L., Norbeck, J., and Blomberg, A. (2004). NADH-reductive stress in *Saccharomyces cerevisiae* induces the expression of the minor isoform of glyceraldehyde-3-phosphate dehydrogenase (TDH1). Curr. Genet. *45*, 90–95.

van Doorn, W.G. (2011). Classes of programmed cell death in plants, compared to those in animals. J. Exp. Bot. *62*, 4749–4761.

Vescovi, M., Zaffagnini, M., Festa, M., Trost, P., Lo Schiavo, F., and Costa, A. (2013). Nuclear accumulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase in cadmium-stressed *Arabidopsis* roots. Plant Physiol. *162*, 333–346.

Wang, X., Chen, Y., Zou, J., and Wu, W. (2007). Involvement of a cytoplasmic glyceraldehyde-3-phosphate dehydrogenase GapC-2 in low-phosphate-induced anthocyanin accumulation in *Arabidopsis*. Chin. Sci. Bull. *52*, 1764–1770.

Wang, H., Wang, S., Lu, Y., Alvarez, S., Hicks, L.M., Ge, X., and Xia, Y. (2012). Proteomic analysis of early-responsive redox-sensitive proteins in *Arabidopsis*. J. Prot. Res. *11*, 412–424.

Wawer, I., Bucholc, M., Astier, J., Anielska-Mazur, A., Dahan, J., Kulik, A., Wyslouch-Cieszynska, A., Zareba-Koziol, M., Krzywinska, E., Dadlez, M., et al. (2010). Regulation of *Nicotiana tabacum* osmotic stress-activated protein kinase and its cellular partner GAPDH by nitric oxide in response to salinity. Biochem. J. *429*, 73–83.

Winterbourn, C.C. and Hampton, M.B. (2008). Thiol chemistry and specificity in redox signaling. Free Radic. Biol. Med. *45*, 549–561.

Wojtera-Kwiczor, J., Gross, F., Leffers, H.M., Kang, M., Schneider, M., and Scheibe, R. (2012). Transfer of a redox-signal through the cytosol by redox-dependent microcompartmentation of

glycolytic enzymes at mitochondria and actin cytoskeleton. Front. Plant. Sci. *3*, 284.

Yamazaki, D., Motohashi, K., Kasama, T., Hara, Y., and Hisabori, T. (2004). Target proteins of the cytosolic thioredoxin in *Arabidopsis thaliana*. Plant Cell Physiol. *45*, 18–27.

Yang, Y., Kwon, H.B., Peng, H.P., and Shih, M.C. (1993). Stress responses and metabolic regulation of glyceraldehyde-3-phosphate dehydrogenase genes in *Arabidopsis*. Plant Physiol. *101*, 209–216.

Zachgo, S., Hanke, G.T., and Scheibe, R. (2013). Plant cell microcompartments: a redox-signaling perspective. Biol. Chem. *394*, 203–216.

Zaffagnini, M., Morisse, S., Bedhomme, M., Marchand, C.H., Festa, M., Rouhier, N., Lemaire, S.D., and Trost, P. (2013). Mechanisms of nitrosylation and denitrosylation of cytoplasmic glyceraldehyde-3-phosphate dehydrogenase from *Arabidopsis thaliana*. J. Biol. Chem. *288*, 22777–22789.

Zhang, X.-H., Rao, X.-L., Shi, H.-T., Li, R.-J., and Lu, Y.-T. (2011). Overexpression of a cytosolic glyceraldehyde-3-phosphate dehydrogenase gene OsGAPC3 confers salt tolerance in rice. Plant Cell Tissue Org. Cult. *107*, 1–11.

Zheng, L., Roeder, R.G., and Luo, Y. (2003). S phase activation of the histone H2B promoter by OCA-S, a coactivator complex that contains GAPDH as a key component. Cell *114*, 255–266.

Bionotes

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Thomas Hildebrandt (right) and Carsten Berndt (left) investigate redoxin-mediated regulation of neuronal functions in the Department of Neurology at Heinrich Heine University, Düsseldorf, Germany. Thomas Hildebrandt studied Biotechnology at Technische Universität Berlin and did his Diploma Thesis at the Charité in Berlin. During his Diploma thesis he investigated the impact of nanoparticle labeling on mesenchymal stromal cells. Carsten Berndt received both the Diploma (Department of Plant Physiology) and a PhD degree (Department of Biochemistry of Plants) from the Ruhr-University, Bochum, Germany. Afterwards he joined the Medical Nobel Institute for Biochemistry, Karolinska Institutet, Stockholm, Sweden, headed by Arne Holmgren. During his PhD he investigated the role of FeS clusters in sulfur assimilation. As a postdoctoral fellow, Carsten Berndt contributed to the characterizartion of FeS coordinating glutaredoxins and their role during embryonic development.

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Renate Scheibe studied Pharmaceutical Chemistry in Munich, and then worked with Erwin Beck for her PhD in Plant Physiology at the Botanical Institute of the LMU, and later at the Universities of Illinois at Chicago and of Bayreuth. She received the *venia legendi* in Botany in Bayreuth in 1984 and continued in the research group of Erwin Beck as an Assistant Professor working on light-dark modulation of chloroplast enzymes. During these years, she also took part in ecophysiological studies of the afroalpine vegetation in East Africa and in the Andes in Ecuador. She has been Head of the Department of Plant Physiology at the University of Osnabrück since 1990. Her research interest is the redox regulation of basic metabolism in plants where her group studies the mechanisms of flexible adjustment of energy production and consumption at the various cellular levels.