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The redox switch: regulation of protein function by cysteine modifications

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Abstract

Reactive oxygen and nitrogen intermediates (ROIs and RNIs, respectively) have now become well established as important signalling molecules in physiological settings, within microorganisms, mammals and plants. These intermediates are routinely synthesized, in a highly controlled and transient fashion, by NADPH-dependent enzymes, which constitute key regulators of redox signalling. Mild oxidants such as hydrogen peroxide (H$_2$O$_2$) and especially nitric oxide (NO) signal through chemical reactions with specific atoms of target proteins that result in covalent protein modifications. Specific, highly reactive cysteine (Cys) residues of low pK$_a$ are a major site of action for these intermediates. The oxidation of target Cys residues can result in either of a number of distinct, stable, redox-based, post-translational modifications including: S-nitrosylation, glutathionylation; and sulphenic acid, sulphinic acid and disulphide formation. Such modifications can regulate protein function resulting in distinct physiological outputs.

These Cys-based, redox-switches are increasingly being found to underpin a variety of signalling systems across kingdoms.
Introduction

Both prokaryotes and eukaryotes produce reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates (RNIs) as a consequence of either aerobic or anaerobic metabolism. Further, the transient synthesis of these intermediates in response to exogenous and endogenous cues underpins a plethora of signalling systems across kingdoms (Hong et al. 2008, Dröge, 2002). Consequently, organisms have evolved robust, redundant and often elaborate mechanisms with which to defend themselves against oxidative and nitrosative insults (Mittler et al. 2004, Feechan et al. 2005). The chemistries of these intermediates have also supported their development as key cellular signalling molecules. In this context, the emerging data suggests that oxidants and nitrosants are selectively generated and employed as cues in the regulation of a myriad of cellular functions (Nathan and Xie, 1994, Grant and Loake 2000). However, in contrast to classical signal transduction pathways that depend upon interactions based upon macromolecular shapes, ROIs and RNIs signal through chemical reactions with specific atoms of target proteins that result in covalent modifications (Nathan, 2003). Hence, signalling by these intermediates occurs at the atomic rather than the macromolecular level.

In metazoans, RNIs and ROIs are both routinely synthesized by isoforms of NADPH-dependent enzymes such as nitric oxide synthase (NOS) (NOS review) and NADPH oxidase (Nathan and Xie 1994, Sagi and Fluhr 2001, Torres et al. 2002), which produce nitric oxide (NO) and superoxide (O$_2^-$), respectively. In plants, the sources of these intermediates are not so well defined. In some physiological settings, careful genetic analysis has revealed that Arabidopsis homologs of NADPH oxidases are required for ROI synthesis (Torres et al. 2002, Foreman et al. 2003). Although alternative or complementary strategies of ROI generation have also been proposed, including cell wall bound peroxidase (Bindschedler et al. 2006), germin-like oxalate oxidase (Zhang et al. 1995) and apoplastic amine, diamine and polyamine oxidase-type enzymes (Alan and Fluhr 1997). In contrast, the source(s) of NO in plants remain to be vigorously determined (Zemojtel et al. 2006, Crawford et al. 2006). Nitrate reductase (NR) that can catalyse the synthesis of NO and N$_2$O from nitrite has been proposed as a candidate (Harper 1981, Desikan et al. 2002). However, significant NO production from NR is dependent upon high levels of nitrite and anoxia (Vacheret et al. 1992) or the absence of photosynthetic activity (Botrel et al. 1996), implying that NR is unlikely to be a major generator of NO. Mitochondrial electron transport has also been proposed as a possible generator of this redox signalling molecule (Planchet et al. 2005) and an NO associated protein (NOA) identified that might be closely linked to an alternative source of NO (Crawford et al. 2006).

Redox-based signalling by RNIs and ROIs is largely governed by their targeted modifications of key, reactive cysteine (Cys) amino acids in proteins. Numerous classes of proteins contain free Cys residues
that are highly conserved across species, suggesting regulatory possibilities beyond structural roles or metal ion coordination. Further, Cys residues can be modified by a variety of stable, redox-based, post-translational modifications which may convey unique effects on protein function (Kim et al. 2002, Ji et al. 1999). Further, distinct and reversible Cys modifications also constitute a mechanism through which differential responsiveness of the target protein can be established to a given cue.

**Requirements for redox-based signalling**

Oxidants and nitrosants utilized in cellular signalling must exhibit specificity for their target substrate. Further, the given redox-based modification must be reversible. Some intermediates such as the hydroxyl radical, peroxynitrite, ozone and nitrogen dioxide do not exhibit specificity in their reactions with biomolecules (Halliwell and Gutteridge, 1999). Thus, modifications elaborated by these extremely active intermediates, which include the formation of protein carbonyls or 3-nitrosotyrosine, are difficult to reduce and are therefore less likely to function in signal transduction. Conversely, H$_2$O$_2$, O$_2^{-}$ and NO execute Cys-based modifications that are generally reversible and consequently these molecules are thought to be integral to a plethora of redox-based signalling pathways (D’Autréaux and Toledano, 2007).

Cys targets of reversible modification routinely possess a low-pK$_a$ sulphahydryl group which supports susceptibility to oxidation in contrast to typical Cys residues (Meng et al. 2002). Thus, while a given protein may contain numerous Cys amino acids, only a minority of these will have the chemical properties to function as possible target sites for oxidants or nitrosants (Boehning and Snyder 2003, Campbell et al. 1996). The chemical environment surrounding protein Cys largely determines their potential reactivities. Thus, many Cys targets modified by NO are embedded within a consensus motif (Stamler et al 1997), akin to a variety of other diverse post-translation modifications. Hydrophobic regions can also drive some of these modifications (Hess et al. 2001), because the reaction between NO and oxygen is promoted in such environments, producing species that support Cys modification (Liu et al. 1998). In certain settings, a high concentration of nitrosant / oxidant is required to support modification of a given Cys. In this context, isoforms of NOS have been shown to physically interact with the protein possessing the target Cys, generating local concentrations of NO sufficient to drive such changes (Kim et al. 2005, Fang et al. 2000).

To be biologically relevant events, rather than manifestations of co-lateral damage, Cys modifications should also occur under physiological time-lines and significantly be reversible, ideally via an enzymatic mechanism. A large tranche of data has demonstrated that time-scales for redox-based post-translational modifications are entirely compatible with those of cellular signalling systems (Gow et al. 2002, Delaunay et al. 2002, Lee et al. 1998). Moreover, most can be rapidly reversed in response to changing
physiological circumstances and some of these Cys modifications can be enzymatically overturned (Benhar et al. 2008; Tada et al. 2008, Jacquot et al. 1997). Thus, redox-based post-translational modifications of Cys amino acids therefore meet a variety of important criteria that underscore their potential biological significance as key regulators of protein function.

S-nitrosylation

S-nitrosylation, the covalent attachment of an NO group to a reactive Cys thiol to form an S-nitrosothiol (SNO), has emerged as a prototypic redox-based post-translational modification (Stamler et al. 2001, Wang et al. 2006(a)). The temporal and spatial regulation of S-nitrosylation / de-nitrosylation, confer specificity to NO-based signalling. This contrasts with global regulatory mechanisms that function through changes in cellular redox tone.

S-nitrosylation is regulated by factors that control both the addition and removal of an NO from a Cys thiol (Hess et al. 2001, Feechan et al. 2005, Benhar et al. 2008). These might reflect changes in proximity of the target thiol to the source of NO or transition metals (Kim et al. 2005, Bosworth et al. 2009), hydrophobicity (Hess et al. 2001), electrostatic environment or alterations in protein-protein interactions (Stamler et al. 1997, Fang et al. 2000). Sources of NO synthesis, such as nitric oxide synthase (NOS) isoforms in animals, are also key players in the control of S-nitrosylation (Palmer 1993, Kim et al. 2005). Thus, receptor-mediated activation of NO production in response to appropriate cues has been demonstrated to correlate with increased SNO formation in both plants and animals (Wang et al. 2006(b), Wang et al. 2009).

S-nitrosylation can be driven by NO or higher order NO oxides, nitrite, metal-NO complexes or SNOs (Foster et al. 2003). While an enzyme that functions specifically to S-nitrosylate proteins has not been identified to date, a number of proteins have been shown to promote SNO formation in some contexts. For example, ceruloplasmin drives the S-nitrosylation of the proteoglycan, glypican (Mani et al. 2004). Transnitrosylation, the transfer of an NO group between thiols has also been reported. S-nitrosylated haemoglobin, S-nitrosohaemoglobin, can transfer an NO group to an adjacent thiol embedded within an interacting protein, Band-3 (Pawloski et al. 2001).

The tripeptide glutathione (glutamyl-cysteinyl-glycine) (GSH) is the most abundant and important low-molecular-mass thiol within both plant and animal cells and is also a key antioxidant. NO can react with GSH to form S-nitroso glutathione (GSNO) in both kingdoms (Gaston et al., 1993, Feechan et al. 2005). This molecule can then function in transnitrosylation reactions passing on its NO group to reactive Cys
thiols within target proteins. Therefore GSNO can function as a source of NO to support protein S-nitrosylation. As GSNO is a relatively stable molecule, it might also operate in NO storage and transfer.

The emerging evidence suggests that S–nitrosylation is an important and widespread regulator of protein function in plant biology. Application of proteomics-based strategies utilising the biotin-switch procedure, which identifies S-nitrosylated Cys in vivo (Jaffrey et al. 2001), has uncovered an increasing number of protein targets for this modification (Lindermayr et al. 2005, Romero Puertas, 2007, Wang et al. 2009). One recent example is the S-nitrosylation of Arabidopsis thaliana salicylic acid binding protein 3 (AtSABP3) during the plant defence response (Wang et al. 2009). NO is a conspicuous feature of plants responding to attempted microbial infection and this redox-based regulator underpins the development of disease resistance (Hong et al. 2007; Delledonne et al. 1998, Durner et al. 1998), although the associated molecular mechanisms remain to be defined. Recognition of avirulent isolates of the bacterial pathogen Pseudomonas syringae pv tomato triggers a strong nitrosative burst, leading to the S-nitrosylation of AtSABP3 (Wang et al., 2009). Site-directed mutagenesis and mass spectrometry studies identified the site of SNO formation as Cys280. Further, S-nitrosylation suppressed both binding of the plant immune activator, salicylic acid (SA) and the carbonic anhydrase (CA) activity of this protein. The CA function of AtSABP3 was required for full resistance against bacterial infection. Thus, inhibition of AtSABP3 function by S-nitrosylation could contribute to a negative feedback loop that modulates the plant defence response. AtSABP3 is one of the first targets in plants for which the in vivo biological function of this post-translational modification has been demonstrated. Further, these findings provide a molecular link between increases in NO concentration activated by pathogen challenge and the expression of disease resistance.

Signal turnover is a key feature underpinning the dynamic nature of cell signalling systems. In this context, denitrosylation, the removal of an NO group from a S-nitrosylated Cys thiol constitutes an important but less well understood aspect of NO signalling. While cellular redox tone is a significant factor in SNO homeostasis, significant insights into enzymatic mechanisms integral to SNO turnover in plants have now begun to emerge. An A. thaliana GSNO reductase (AtGSNOR1) has been identified that governs the global levels of both basal and pathogen-induced S-nitrosylation in this reference species (Feechan et al. 2005). Loss-of-function mutations in AtGSNOR1 resulted in heightened basal and pathogen triggered SNO concentrations. Furthermore, these increased cellular SNO levels disabled plant defence responses conferred by distinct resistance (R) protein subclasses and also basal and non-host resistance. Alterations in total cellular S-nitrosylation were found to regulate both SA synthesis and signalling (Feechan et al. 2005). Recent experiments have now revealed that the function of Non-expressor of PR
genes 1 (NPR1), a key regulator of SA responses, is controlled through the S-nitrosylation / de-nitrosylation of Cys156 (Tada et al. 2008).

Sulphenic acid formation
Protein tyrosine phosphatases (PTPs), which catalyse the dephosphorylation of tyrosine residues, are unique in that they possess a reactive cysteine in their catalytic domain that is integral to activity (Barford et al. 1995). Due to the chemical environment surrounding this cysteine, its pKₐ (~5.4) is extremely low relative to that of a typical Cys residue (~8.5) (Lohse et al. 1997). This residue is extremely reactive but this property renders it susceptible to oxidation. In this context, it has been shown that treatment of mammalian cells with H₂O₂ leads to oxidation (S-hydroxylation) of the active site Cys of PTPs to sulphenic acid (S-OH) (Denu et al. 1998). This modification results in inhibition of activity because this residue can no longer function as a phosphate acceptor in the first step of PTP catalysis. Importantly, oxidation of this Cys residue to sulphenic acid is reversible and thus may function as a molecular switch to regulate PTP function (Meng et al. 2002). Epidermal growth factor (EGF) triggered activation of the EGF receptor drives the production of ROIs and it has been proposed that this burst of oxidants may S-hydroxylate the reactive Cys residues of PTPs, blunting their activity and thus promoting a surge in phosphorylation (Lee et al. 1998). Mitogen-activated protein kinase phosphatases (MKPs) also possess a reactive Cys, required for catalytic activity. S-hydroxylation of this residue leads to inhibition of protein activity and consequently this may promote increased activation of mitogen-activated protein kinases (MAPKs) (Kamata et al. 2005).

Plants also possess PTPs which harbour a Cys at the active site involved in the formation of the phosphoenzyme reaction intermediate and consequently this residue is also required for enzyme activity. Exogenous treatment of the Arabidopsis PTP, AtPTP1, with H₂O₂ blunted AtPTP1 activity without impacting upon the stability of this protein (Gupta and Luan, 2003). Further, H₂O₂ exposure attenuated the AtPTP1-mediated inhibition of MAPK6 activity. These findings complemented an earlier report that implied ROIs potentiated MAPK activity during the establishment of plant disease resistance (Grant et al. 2000). Thus, the regulation of plant PTP function by the reversible formation of sulphenic acid at the active site Cys may be an important point of control for a broad range of plant signalling networks.

Generation of disulphide bridges
Sulphenic acid formation often drives the subsequent formation of disulphides (-S-S-), that can be either intra or inter-molecular in nature. Classically, this level of Cys oxidation controls protein multimerisation, such as the dimerisation of various heat-shock factors or chaperone proteins or the self-assembly of
dynamin (D’Autréaux and Toledano 2007). As outlined above, NPR1 is a global co-activator of SA-dependent defence gene expression and its activity underpins the establishment of plant immunity. NPR1 exists in a dynamic equilibrium between monomer and oligomeric forms, with NPR1 oligomer predominant within the cytoplasm (Mou et al. 2003). Oligomer assembly is supported by intermolecular disulphide bonds and site-directed mutagenesis has suggested that Cys82 and Cys216 are critical for oligomer formation. Following attempted pathogen infection there is a burst of ROIs synthesized by NADPH oxidases (Grant and Loake, 2000, Torres et al. 2002). Subsequently, there may be an over compensation of both enzymatic and non-enzymatic antioxidant mechanisms to guard against oxidative damage. This more reductive environment might favour reduction of the intermolecular disulphide bonds of NPR1, releasing monomers from NPR1 oligomers. These monomers are then thought to be translocated to the nucleus where they promote SA-dependent gene expression leading to the development of immunity (Mou et al. 2003). Redox-regulated disulphide formation may therefore provide another molecular toggle which can be deployed to control protein function.

The accumulating evidence suggests that in some cases disulphide formation can be reversed by the action of thioredoxins (TRXs), NADPH-dependent reductases. For example, NPR1 has recently been shown to interact with either plant TRX-h5 or TRX-h3, which may reduce the intermolecular disulphides of NPR1 oligomers, driving the formation of NPR1 monomers and the subsequent activation of SA-dependent gene expression (Tada et al. 2008). Further, loss-of-function mutations in either TRX-h5 or TRX-h3 compromised the development of SAR leading to increased pathogen growth. Thus, TRX proteins appear to function as key positive regulators of SAR by promoting NPR1 monomer formation. Interestingly, TRX function may be opposed by S-nitrosylation, because SNO formation at Cys156 appears to encourage NPR1 oligomer formation (Tada et al. 2008). Plant immunity may therefore be controlled in the cytoplasm by the opposing actions of two distinct redox-based post-translational modifications.

**S-glutathionylation**

S-glutathionylation is the modification of a reactive protein Cys thiol by the addition of GSH. The GSH-dependent detoxification of ROIs generates glutathione disulphide (GSSG), which can be reduced back to GSH by NADPH-dependent enzyme GSSG reductase (Lillig et al. 2003). Alternatively, GSSG can be exported from the cell. Traditionally, S-glutathionylation was widely assumed to represent a by-product of either oxidative or nitrosative stress rather than a molecular switch controlling protein function. However, the emerging evidence implies that at least some of the criteria required for S-gluthionylation to represent a bona fide regulatory event have been fulfilled. Proteomics-based screens have identified an increasing number of proteins that are targets for S-glutathionylation (Fratelli et al. 2003). However, there are only a
few reports of S-glutathionylation-dependent changes in protein activity within a physiological context (Chen et al. 2007, Rinna et al. 2006). Nevertheless, these findings have already implicated this post-translational modification in human disease (Shelton and Mieyal, 2008). S-glutathionylation may also protect sensitive thiols from further oxidation to irreversible sulphenic and sulphonic acids (Fiaschi et al. 2006). Further, it may even constitute a mechanism with which to store GSH under extreme oxidative conditions, as GSSG would otherwise be exported from the cell.

Oxidation of reactive Cys residues can lead to the formation of inter and intra-molecular disulphide bonds (as described above). TRXf is a key redox regulator of chloroplastic carbon fixation enzymes, for example fructose-1,6-bisphosphatase. Typically, these enzymes are largely inactive in the dark, due to the formation of intramolecular disulphide bridges. Upon illumination TRX reduces these disulphides and restores the activity of these chloroplastic carbon fixation enzymes (Meyer et al. 2007). A conserved Cys residue, Cys60, outwith the active site of TRXf was found to be specifically S-glutathionylated by GSSG or GSH plus oxidants (Casagrande et al. 2002). Other classes of chloroplastic TRX proteins were not modified in this fashion. S-glutathionylation of TRXf blunted its ability to be reduced by ferridoxin-thioredoxin reductase. Further, this modification impaired the ability of TRXf to reduce carbon fixation enzymes and restore their activity (Michelet et al. 2005). Thus, glutathionylation may regulate carbon-fixation in the chloroplast in response to oxidative stress.

The glycolytic enzyme, GAPDH, has a primary role in energy production. In addition to a cytoplasmic GAPDH, plants also contain a chloroplastic isoform that participate in the Calvin cycle. All GAPDH proteins share a common catalytic mechanism that is based on Cys149, a highly reactive Cys that is thiolated by an interaction with His176. The chloroplastic A4-GAPDH isoform has also been shown to be specifically S-glutathionylated on its catalytic Cys149 residue decreasing its activity (Zaffagnini et al. 2007). In contrast, the An-Bn-GAPDH isoforms, the most abundant in the chloroplast, are not substantially S-glutathionylated, despite being sensitive to oxidation. It is noteworthy that in addition to GAPDH, two other enzymes integral to sugar metabolism, aldolase and triphosphate isomerase are both targeted with this post-translational modification, which suppresses their activity (Ito et al. 2003). Therefore, S-glutathionylation might protect key enzymes metabolic enzymes under conditions of oxidative or nitrosative stress.

**Sulphinic acid and sulphonic acid formation**

Cysteine thiols oxidized to sulphenic acid are often relatively unstable and consequently either form a disulphide with an adjacent thiol or are oxidized further to a stable sulphinic acid (SO$_2$). Under conditions
of extreme oxidative stress this Cys modification and can be further oxidized to a sulphonic acid (SO$_3^-$). SNOs can be reversed by the action of GSH and GSNOR and sulphenic acids and disulphides can be reduced by GSH or thioredoxin in biological systems. However, sulphinic and sulphonic acid formation was until recently thought to be irreversible (Biteau et al. 2003). Consequently, these redox-based Cys modifications were not thought to undertake regulatory roles.

Peroxiredoxins (Prx) along with catalase and glutathione peroxidase are important enzymes in H$_2$O$_2$ turnover. Three classes of Prx have been described: 2 Cys, atypical 2-Cys and 1-Cys, which form homodimers and are located in a variety of cellular compartments (Rhee et al. 2005). Over-expression of Prx reduces H$_2$O$_2$ tone and diminishes platelet derived growth factor or ceramide signalling in mammals, implying the function of Prx in the signal transduction is to control H$_2$O$_2$ levels (Rhee et al. 2005).

The peroxidatic Cys of one subunit of a head-to-tail homodimer is oxidized by H$_2$O$_2$ to generate sulphenic acid, a resulting conformation change then supports attack of the resolving Cys forming a intersubunit disulphide bond. Reduced homodimer is then re-generated by the action of the disulphide reductase, Trx. In the case of eukaryotic Prx, a C-terminal protein domain can stabilize the sulphenic acid once formed and thus facilitate further oxidation of the peroxidatic Cys to generate a sulphinic acid. This over-oxidation disrupts the characteristic catalytic cycle because disulphide bond formation between the peroxidatic and resolving Cys is blocked (Wood et al. 2003). These events are routinely manifested during relatively high levels of oxidative stress and in this scenario the ability of the Trx system to re-cycle oxidized Prx is overwhelmed.

Until recently the formation of a sulphinic acid at a reactive Cys was thought to constitute an irreversible protein modification. However, a protein was identified from Saccharomyces cerevisiae, termed sulphiredoxin (Srx), that reduced the Cys-sulphenic acid in the yeast Prx, Tsa1 (Biteau et al. 2003). The catalytic mechanism of this Cys-sulphinic acid reductase required ATP hydrolysis and magnesium and a conserved active site Cys which formed a transient disulphide linkage with Tsa1. Thus, Srx is an important player in the re-cycling of Prx. Significantly, a plethora of other proteins are likely to be over-oxidised and conceivably Srx may be integral to the reversal of these modifications. Further, Srx is evolutionary conserved and may therefore function in redox regulation across kingdoms. Arabidopsis possesses a single Srx, which displays sulphinic acid reductase activity (Rey et al. 2007) and is located within chloroplasts. Counter-intuitively, however, loss-of-function mutations in this Srx resulted in slightly enhanced resistance to photooxidative stress.
Sulphinic acid modification may also be integral to some redox signalling networks. The *Schizosaccharomyces pombe* transcription factor, Pap1, engages antioxidant gene expression in response to H$_2$O$_2$ stress (Vivancos et al. 2005). Pap1 activation occurs at low but not high H$_2$O$_2$ tone, which triggers Sty1 MAPK signalling. At low H$_2$O$_2$ levels the Prx, Tpx1, conveys an activating redox signal to Pap1. Conversely, at high H$_2$O$_2$ concentrations, a sulphinic acid is formed at the catalytic Cys of Tpx1, inhibiting its redox activation of Pap1. This elevated H$_2$O$_2$ tone, however, engages Sty1 signalling which results in the accumulation of the *S. pombe* sulphirexoin (Srx1). Subsequently, Srx1 reverses Tpx1 sulphinic acid formation re-cycling this redox sensor (Vivancos et al. 2005). Therefore, Tpx1 oxidation to the Cys-sulphinic acid and its subsequent reversion by Srx1 represents a key node in H$_2$O$_2$ signalling in *S. pombe*, which restricts Pap1 activation to within a narrow range of H$_2$O$_2$ levels.

**A redox-based code for Cys modifications**

There are a large variety of potential oxidants and nitrosants and as outlined, a series of potential Cys modifications that can occur in response to these redox-active agents. Intriguingly, the emerging data implies that, at least in some cases, Cys-based redox switches are able to discriminate between distinct chemical inputs such as ROIs, RNIs or thiol-specific oxidants. Furthermore, these switches may also have the capability to produce specific biological outputs that might fine-tune the cellular response to a given redox-active compound. Studies on the *Escherichia coli* transcriptional regulator OxyR have established that distinct redox-active agents may modify the same reactive Cys but generate different biological outcomes (Kim et al. 2002). OxyR function is activated in response to ROIs, RNIs, a decrease in the GSH:GSSG ratio or following exposure to diamide, a thiol-specific oxidizing agent (Aslan et al. 1999, Hausladen et al. 1996). While OxyR has six Cys residues the highly reactive Cys199 is pivotal in the response of this transcriptional regulator to redox cues and its differing functional outputs. Following exposure to H$_2$O$_2$, the RNIs GSNO or S-nitrocysteine or GSSG the stable Cys reaction products formed were sulphenic acid, an S-nitrosothiol or a mixed disulphide, respectively. Informatively, these three distinct oxidation forms plus the reduction product of OxyR, each possess a unique protein conformation and further differential binding properties to the OxyR DNA target motif. The helical content of these OxyR forms, as measured by circular dichroism, ranged from 52% for the S-nitrosylated product, 65% for the reduced form, 71% for the sulphenic acid derivative and 78% for the glutathionylated product. These OxyR derivatives all exhibit significant differences in DNA binding affinity, in addition to cooperativity at promoters containing OxyR binding sites. Thus, a variety of structural rearrangements must occur in the chemical environment into which Cys199 is embedded to effect the stabilization of these different redox forms within OxyR. While an attractive model, the relative significance of these different modifications at
Cys199 in vivo remains to be rigorously tested and this is not straightforward due to the challenges in quantifying these distinct forms of OxyR.

An alternative strategy whereby a protein could respond differentially to distinct redox cues is through the action of a receptor protein, which could perceive a given redox-active compound and via a protein-protein interaction, pass-on the given signal. Such a mechanism has been demonstrated for the S. cerevisiae transcription factor, Yap1, a functional homolog of OxyR. This regulatory protein orchestrates the expression of a variety of genes in response to H$_2$O$_2$ (Kuge and Jones, 1994). Yap1 is not directly oxidised by H$_2$O$_2$, rather this ROI oxidizes a glutathione peroxidase-like enzyme Gpx3, which functions as a H$_2$O$_2$ sensor and transducer (Delaunay et al. 2002). When oxidized by H$_2$O$_2$, Cys36 of Gpx3 forms an intermolecular disulphide with Cys598 of Yap1. Subsequently, this bond is resolved into an intramolecular disulphide bond within Yap1, activating this transcription factor. Yap1 function is blunted by the action of Trx, which reduces both Yap1 and the interacting Gpx3.

**Perspectives**

RNIs such as NO are relatively mild oxidants and their impact on key regulatory Cys residues reversible, hence they are well suited to a cell signalling role. Consequently, S-nitrosylation has emerged as the prototypic redox-based post-translational modification in animals. In contrast, ROI function has largely been associated with stress responses, although there are increasing examples of a regulatory function for these redox-active molecules out with microbial reference systems.

In plants, redox regulation of protein function by disulphide bridge formation is now well established, exemplified by the control of FBPase and related chloroplastic enzymes (Meyer et al. 2007). However, examples of the control of protein activity in planta by other redox-based Cys modifications, such as S-nitrosylation, are only just beginning to appear (Wang et al. 2009, Tada et al. 2008). There is a large body of evidence highlighting key roles for RNIs and ROIs in both plant immune function (Feechan et al. 2005, Durner et al. 1998, Delledonne et al. 1998, Grant and Loake, 2000) responses to the environment (Lee et al. 2008) and also during plant growth and development (Lamattina et al. 2003). Furthermore, new technologies can now provide opportunities, for the first time, to uncover sites of redox-based Cys modifications within proteins, which may convey physiological effects (Jaffrey et al. 2001). Therefore, a significant leap in our understanding of how redox-based regulatory mechanisms can shape the life of plants is eagerly anticipated. Further, these insights may provide novel strategies for future crop design and the development of new varieties by plant breeding.
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Figure legends

Figure 1. Schematic overview of redox-based Cys modifications. Cys thiol (SH), S-nitrosylation (SNO), sulphenic acid (SOH), disulphide (S-S), S-glutathionylation (SSG), sulphinic acid (SO$_2$H) and irreversible sulphonic acid formation (SO$_3$H).

Figure 2. Regulation of SA signalling by protein S-nitrosylation. SNO-NPR1 and SNO-AtSABP3 formation, coupled to the S-nitrosylation of at least another protein upstream of SA synthesis blunts the establishment of disease resistance. AtGSNOR1 activity reduces total cellular SNO levels thus reversing the effects of S-nitrosylation on SA signalling.

Figure 3. Redox regulation of PAP1 during the antioxidant response in *S. pombe*. At low concentrations of H$_2$O$_2$ a sulphenic acid is formed within the peroxiredoxin, Tpx1. This oxidative signal is passed from Tpx1 to the transcriptional regulator, Pap1, resulting in the formation of an intramolecular disulphide bond between Cys278 and either Cys501 or Cys532. This Cys modification activates Pap1 which drives the expression of antioxidant genes. At high H$_2$O$_2$ concentration, however, the redox-relay between Tpx1 and Pap1 is inhibited because its catalytic cysteine is oxidized to a sulphinic acid. These levels of H$_2$O$_2$, however, activates Sty1, which induces the expression of the sulphiredoxin, Srx1, that can re-cycle Tpx1 by reducing its sulphinic acid modification.