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Microbial H_2O_2 sensors as archetypical redox signaling modules

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The *Saccharomyces cerevisiae* Orp1–Yap1 sensor and its prokaryotic counterpart OxyR are master regulators of cellular H_2O_2 homeostasis. These sensors share a stereotypical mechanism, involving the reaction of a unique cysteine with H_2O_2 and a physiology coupled to metabolism of H_2O_2 and thiol reduction–oxidation (redox). However they differ fundamentally in their core mechanism. Whereas OxyR carries out both sensing and transcriptional regulatory functions, these functions are dissociated in yeast, where Orp1 senses H_2O_2 and converts this signal into a cysteine-based redox cascade that culminates in oxidation of the Yap1 regulator. We propose a model in which H_2O_2 sensors are viewed as archetypical receptor-initiated redox signaling modules and suggest, on the basis of the high specificity of the H_2O_2-sensing mechanism, that kinetic factors rather than thermodynamics define the cellular pathways of cysteine residue oxidation.

The intracellular concentration of peroxide (H_2O_2 and organic peroxides) in microorganisms is tightly maintained at very low levels in the face of permanent endogenous production by cellular metabolism and large extracellular variations. Oxidant-specific sensors regulate this concentration by 'measuring' cellular peroxide and setting the expression of oxidant-scavenger genes in proportion to this concentration. These sensors are thought to detect very low nontoxic levels of H_2O_2 that are otherwise not seen by the cell. This type of regulation, which is meant to prevent cellular damage induced by oxidative stress, is essential for aerobic life and has the hallmarks of homeostatic control.

Studies of microbial H_2O_2 sensing have shown that the underlying mechanisms involve direct protein oxidation by H_2O_2 at unique cysteine residues, thereby illustrating how a potential cellular toxicant such as H_2O_2 can engage in very specific cellular regulation and establishing the concept of H_2O_2-initiated cysteine-based redox signaling [1]. These studies have also shown that H_2O_2 sensors are tightly coupled to both cellular H_2O_2 metabolism and control of thiol redox by the glutathione (GSH) and thioredoxin pathways (Box 1), thereby providing a powerful tool with which to study these cellular responses.

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Here, we compare the eukaryotic *Saccharomyces cerevisiae* Orp1–Yap1 H_2O_2 sensor with its prokaryotic counterpart, the *Escherichia coli* OxyR H_2O_2 sensor. Orp1–Yap1 is a redox relay system, comprising the actual H_2O_2 sensor Orp1, which surprisingly has been identified as a homolog of the glutathione peroxidase (GPx) family of H_2O_2 scavengers, and the downstream transcriptional regulator Yap1, which is activated by Orp1-mediated oxidation. By contrast, OxyR carries out both sensing and regulatory functions.

On the basis of mechanistic principles learned from microbial H_2O_2 sensors, we propose a model of the interaction of H_2O_2 with cellular cysteine residues. In particular, this model suggests that, given the specificity of the effect of H_2O_2, only a very limited set of cellular cysteine residues, which we call 'peroxide receptors', should be affected by this oxidant. These principles should help us to understand the mechanism of cysteine-based redox signaling by H_2O_2 in metazoans.

Constitution of the yeast H_2O_2 sensor

Oxidant receptor peroxidase 1 (Orp1; also known as Hyr1 or Gpx3) and yeast AP-1 protein (Yap1) constitute the *S. cerevisiae* H_2O_2 sensor relay (Figure 1). A third component, Yap1-binding protein (Ybp1), is a Yap1-associated protein that is crucial for the mechanism of the redox relay [2,3].

Orp1 is a protein of 20 kDa with sequence homology to the GPx family of peroxidases [4,5]. Orp1 carries three cysteine residues, of which Cys36 is the conserved

Box 1. Cellular control of thiol redox by the glutathione and thioredoxin pathways

Thiol reductases comprise the glutathione (GSH) and thioredoxin pathways, which maintain reduced thiol residues in the cytoplasm by assisting enzymes that have a thiol-reducing step in their catalytic cycle, such as ribonucleotide reductase, glutathione peroxidase (GPx) family enzymes for the GSH pathway and peroxiredoxin (Prx) family enzymes for the thioredoxin pathway. GSH reductase, GSH and glutaredoxins constitute the GSH pathway, whereas thioredoxin reductase and thioredoxin comprise the thioredoxin pathway [35].

Glutaredoxin and thioredoxin are the ultimate reductants because they act as thiol disulfide transferases. The two pathways are considered to be 'electron flow' systems, because there is a gradient in redox potential along each pathway that directs electrons from one molecule to the next. Ultimately, the electron source for both pathways is NADPH, which has an extremely low redox potential of −315 mV. Kinetic factors are also important here, however, because the two pathways operate independently [50].

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peroxidase active-site cysteine in the GPx family and is a selenocysteine residue in most other species. The GPx catalytic cycle involves the reduction of peroxides by their reaction with the active-site residue, which is first oxidized to a selenic (Cys-SeOH) or sulfenic acid (Cys-SOH) and then reduced by GSH [6].

Orp1 has *in vitro* peroxidase activity operated by a mechanism involving a Cys36–Cys82 catalytic disulfide, that is distinct from those of classical GPx peroxidases [7] (Figure 1a). Furthermore, Orp1 is recycled by thioredoxin and not by GSH. Although the Orp1 catalytic disulfide forms in cells exposed to H_2O_2, Orp1 contributes to H_2O_2 tolerance only as sensor and not as a peroxidase, as indicated by the lack of phenotype shown by an Orp1 mutant lacking Cys82, a residue required only for the peroxidative cycle. Orp1 has a low cellular abundance that is sufficient for its sensing or regulatory function and might explain its inefficient peroxidase function *in vivo*.

Yap1 is a basic leucine zipper (bZip) trancription factor that, in response to H_2O_2, activates genes encoding most yeast antioxidants, including proteins in the thioredoxin and GSH systems and other stress and detoxification proteins [8,9]. Its importance in the metabolism of peroxide is reflected by the inability of cells lacking Yap1 to survive in the presence of increased concentrations of these oxidants [10]. Yap1 is activated by oxidation in response to H_2O_2 [11]. This activation occurs rapidly (within 1 min) and transiently (lasting ~30–45 min), with half-maximal oxidation at a concentration of 100 μM H_2O_2. Yap1 redox regulation by H_2O_2 involves two cysteine-rich domains (CRDs) that each carry a three-cysteine repeat motif. These domains are located in the N and C terminus of Yap1 and are referred to as n-CRD and c-CRD, respectively.

Oxidation triggers the nuclear accumulation of Yap1 from its mainly cytoplasmic localization by inhibiting its nuclear export by Crm1 (also known as Xpo1) [12,13]. This inhibition is the consequence of the oxidation of Yap1, which causes the formation of an intramolecular disulfide bond between the n-CRD (Cys303) and c-CRD (Cys598) domains (Figure 1b), which masks a Crm1-cognate nuclear export signal encoded in the c-CRD [11–13]. A second intramolecular disulfide bond between Cys310 and Cys629 has been recently identified in purified and air-oxidized Yap1 [14]. Although its *in vivo* relevance and mechanism have not been established, this second disulfide could add stability by locking the protein in its oxidized active conformation.

Operating mechanism of the Orp1–Yap1 sensor

The Orp1–Yap1 H_2O_2 sensor has been elucidated as a redox relay system (Figure 1b) [7,11]. The unresponsiveness of Yap1 to H_2O_2 in cells lacking Orp1 established the role of this protein as a sensor and signal transducer of the H_2O_2 signal and also showed that Yap1 oxidation does not take place directly [7]. The H_2O_2 signal is sensed by Cys36 of Orp1, which oxidizes to Cys36-SOH (Figure 1b). Oxidized Orp1 transduces this signal to Yap1 by engaging the latter in a Cys36–Cys598 intermolecular disulfide bond, which is then converted to the intramolecular Cys303–Cys598 disulfide bond in active Yap1. Once formed, the nascent Cys36 sulfenic acid of Orp1 is poised to react with Cys82 of Orp1 to complete the peroxidative cycle (Figure 1a), but it can also react with Cys598 of Yap1

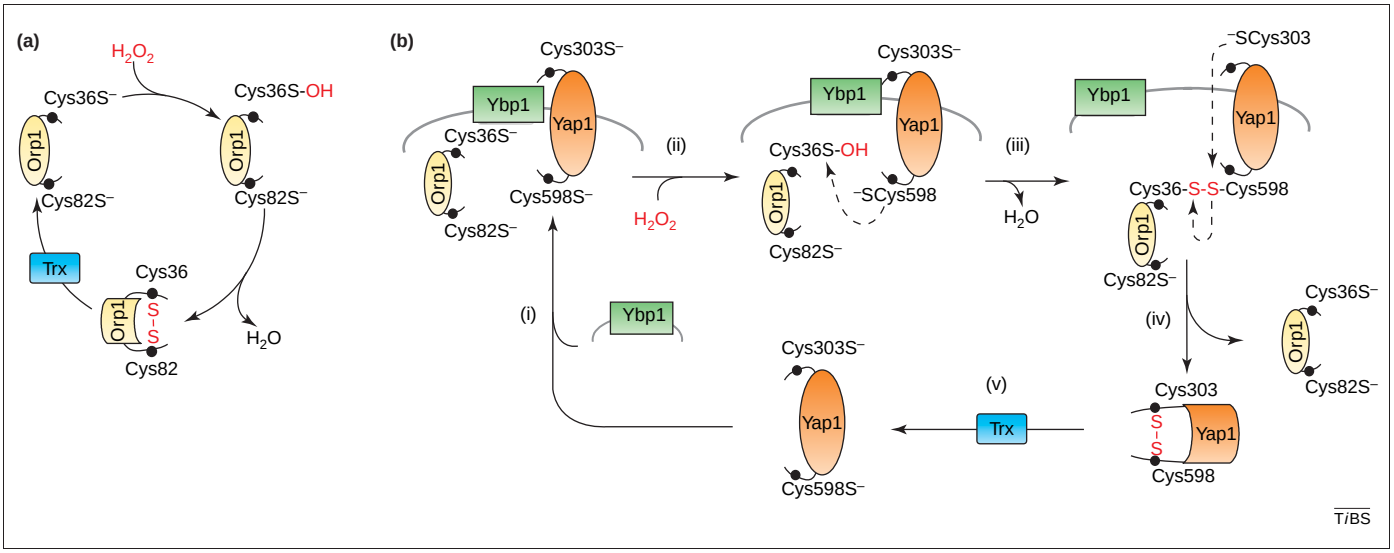

Figure 1. Model of the Orp1–Yap1 H_2O_2-sensing mechanism showing the dual function of Orp1. In response to H_2O_2, Orp1 can either enter into a peroxidative cycle or initiate Yap1 activation. **(a)** The Orp1 peroxidative cycle involves reduction of H_2O_2 by Cys36, which first oxidizes to Cys-SOH and then condenses with Cys82 to form an intramolecular disulfide bond that is subsequently reduced by thioredoxin (Trx). **(b)** Orp1–mediated oxidation of Yap1 by H_2O_2 is a highly controlled process. (i) Orp1 associates with Yap1, which is already bound to Ybp1 in a pre-complex. Ybp1, which is required to operate the redox relay, has been identified as a cytoplasmic protein [2], indicating that the relay takes place in this compartment. (ii) The cycle of the redox relay involves the initial reaction of H_2O_2 with Cys36 of Orp1, which leads to reduction of H_2O_2 and formation of a sulfenic acid form (Cys36-SOH). (iii) Cys36-SOH then condenses with Cys598 of Yap1 to form a Orp1–Yap1 transient disulfide linkage. (iv) A thiol-disulfide exchange reaction between Cys36 of Orp1 and Cys303 of Yap1 converts the Orp1–Yap1 disulfide bond to a Cys303-Cys598 intramolecular disulfide bond in Yap1, representing the active form of this regulator, accompanied by the recycling of reduced Orp1. (v) Oxidized Yap1 is recycled by thioredoxin (Trx). This model is based mainly on the identification of the transient Orp1–Yap1 mixed-disulfide linkage in cells exposed to H_2O_2 and its stabilization on the substitution of Cys303 of Yap1 [7]. In the oxidized conformation of Yap1, the nuclear export signal is masked, promoting the nuclear accumulation of this protein. Mutations in either Orp1 or Ybp1 reproduce the phenotype of the Yap1 mutation, namely, the inability of the cell to adapt to increased concentrations of H_2O_2.

(Figure 1b), because both Orp1 intra- and intermolecular disulfides are observed in cells treated with H_2O_2 [7].

Ybp1 is required for signal transduction from Orp1 to Yap1 [2] because the intermolecular Orp1–Yap1 disulfide cannot form in its absence. Although its molecular function has not been elucidated, Ybp1 could act as a scaffold, bringing Orp1 and Yap1 together in a non-redox complex. Ybp1 could also chaperone the formation of mixed disulfide bonds by guiding Cys36-SOH of Orp1 towards Cys598 of Yap1 and/or by preventing the formation of the competing Cys36–Cys82 disulfide bond of Orp1. Deactivation of Yap1 proceeds through recycling of its reduced form via the thioredoxin pathway (Refs [11,15,16] and see below).

The *E. coli* OxyR H_2O_2 sensor

Pioneering studies of the prokaryotic transcription factor OxyR by Storz and coworkers [17–19] have established the first principles of peroxide sensing. In response to H_2O_2, OxyR activates the OxyR regulon, which includes genes involved in H_2O_2 metabolism and cytoplasmic thiol redox control [20]. OxyR is also activated by oxidation [21], with half-maximal oxidization *in vitro* at H_2O_2 concentrations as low as 0.05–0.2 μM [22]. Thus, given the predicted intracellular H_2O_2 concentration (<1 μM [23]), OxyR should be activated at concentrations that just exceed physiological intracellular levels but that are below the threshold toxicity level (estimated at >1 μM). Higher concentrations of exogenous H_2O_2 are needed to oxidize OxyR *in vivo* (5 μM), which is consistent with the membrane-permeability coefficient of H_2O_2 [23]. After cell exposure to an optimal H_2O_2 concentration (200 μM), OxyR oxidation is very rapid – occurring after 30 s – and transient – lasting ~5 min [22].

In *E. coli*, H_2O_2 sensing only involves OxyR, a protein of 34 kDa that in its reduced and oxidized forms binds as a tetramer to two different classes of promoter DNA-binding sites that specify transcription repression and activation, respectively [24]. Reduced OxyR represses its own expression, whereas the oxidized form activates the OxyR regulon. Activation of OxyR by H_2O_2 proceeds by its direct oxidation, which changes its DNA-binding specificity and converts it from a repressor to an activator of transcription. Of its six cysteines, OxyR relies for its sensing function on Cys199, which first reacts with H_2O_2 to form an SOH moiety, which then condenses with Cys208 to form the Cys199–Cys208 intramolecular disulfide bond of oxidized active OxyR [1,22,25]. OxyR is deactivated via its reduction by the GSH pathway enzyme glutaredoxin [1].

Three-dimensional studies have revealed how disulfide bond formation affects the structure and function of OxyR [26]. In the reduced protein, Cys208 is positioned 17 Å away from Cys199 and thus disulfide bond formation involves substantial remodeling of the protein. This structural change, which has been termed redox 'fold editing', is coupled to a change in the geometry of the tetramer that is thought to be central to the OxyR DNA-binding specificity switch.

Stamler and coworkers [27] have challenged this model, however, because they failed to detect the Cys199–Cys208 intramolecular disulfide bond, but instead identified modification of the OxyR Cys199 thiol either to sulfenic acid (-SOH) in air-oxidized protein or by glutathionylation (-SSG) in cells exposed to H_2O_2. These data do not exclude formation of the Cys199–Cys208 disulfide bond of OxyR, which has been rigorously identified by mass spectrometry and thiol–disulfide titration *in vitro* [1] and by two different cysteine derivatization techniques *in vivo* [22,25], but rather they expand the range of Cys199 modifications that can activate OxyR [17,18,28–30].

Stamler's group [27,31] also identified a nitrosylated form (-SNO) of OxyR Cys199 in both protein reacted with nitrosoglutathione and in cells exposed to *S*-nitrosocysteine. Because the different OxyR redox forms had distinct overall conformations, DNA-binding affinities and transcriptional potentials, these researchers proposed that OxyR could process different redox-related signals into distinct transcriptional responses. They also suggested that the response to a given signal might be graded by the relative amount of alternative OxyR redox conformers produced at different concentrations of the elicitor. This model, which contrasts with the model of a bimodal on–off switch mechanism ensuing from the OxyR intramolecular disulfide, will have to be tested. Recent genome-wide studies have indicated that the transcriptional responses of *E. coli* to H_2O_2 [20] and to nitrosative stress [32] are indeed different. But although they confirmed that OxyR has a major role in regulating the H_2O_2 response, these studies observed only a minor contribution of this regulator to the response to nitrosative stress.

Sensors functionalities and specificities

The prokaryotic and eukaryotic systems sense H_2O_2 in a stereotypical manner, but their distinctive constitution and components impose important differences in sensor functionality and specificity.

Owing to its two-component make-up, the Orp1–Yap1 response is restricted to an on–off switch mechanism because only one redox exchange reaction can possibly proceed between H_2O_2-oxidized Orp1 and reduced Yap1 (Figure 1b). This on–off switch mechanism is fully adapted to inhibit the nuclear export of Yap1 and contrasts with the OxyR model of a graded transcriptional response resulting from distinct activated states of OxyR generated either by increased concentrations of peroxide or by multiple elicitors. It should be noted that when oxidized to its intramolecular disulfide form Orp1 cannot signal to Yap1, which suggests that this pathway could become 'desensitized' at increased concentrations of H_2O_2.

Another important difference between the *E. coli* and yeast sensors is their specificity. In contrast to OxyR, which responds through a unique residue (Cys199) to peroxide, nitrosothiols [27], the thiol oxidant diamide [33] and 'disulfide stress' [22,34], Orp1–Yap1 responds only to peroxide. This absolute specificity of Orp1–Yap1 is also the result of its two-component architecture. Redox transduction from Orp1 to Yap1 can proceed only in the presence of the SOH form of Cys36 of Orp1, which in turn is known to form only on the reaction of this cysteine with peroxide. In fact, Yap1 is also activated by diamide, heavy metals, several electrophiles [10,35–38] and nitrosothiols;

however, these responses are independent of Orp1 and involve the direct modification of Yap1 cysteines in the c-CRD by oxidation, alkylation or noncovalent binding [36,39]. Actually, Cys36 of Orp1 is presumably modified by these same redox-based signals, but these modifications would not be conveyed to Yap1.

The Orp1–Yap1 two-component architecture might have another advantage in facilitating both optimal peroxide reactivity and disulfide-switch stability – two features that could require distinct physicochemical properties of cysteine residues. The yeast sensor might thus operate a functional specialization, in which Orp1 confers peroxide reactivity and the Yap1 disulfide provides the stability of the active conformation that is needed for the kinetics of the yeast H_2O_2 response. Bacteria may bypass this functional specialization because of their shorter peroxide response.

Receptor-initiated redox signaling modules

H_2O_2 is considered to be an important signaling molecule in the regulation of metazoan cell growth and differentiation [40]. Although this effect of H_2O_2 is probably mediated through cysteine redox modifications, its molecular mechanism is still obscure. The Orp1–Yap1 peroxide sensor, viewed here as a 'receptor-initiated redox relay' system in which Orp1 acts as a 'peroxide receptor' and 'redox transducer' that funnels the 'ligand'-oxidizing equivalents to Yap1, could represent an archetypical redox signaling module used by metazoans. Such a module should be fit for H_2O_2 signaling by providing high response specificity, signaling specificity and stability of redox-dependent conformations – three important criteria of signal transduction.

The Orp1–Yap1 redox relay is actually built on two unique features of Orp1. First, owing to its presumed high chemical reactivity, the reaction of Orp1 Cys36 with H_2O_2 seems to be almost exclusive relative to all cellular cysteine residues, rather like the binding of a ligand to its receptor. Indeed, within the whole Orp1–Yap1 relay, Cys36 of Orp1 is the only residue out of four cysteines known to engage in redox reactions (Cys36, Cys82, Cys303 and Cys598; Figure 1) that actually reacts with peroxide, and within yeast cells mostly thiol-based peroxidases – especially the peroxiredoxin (Prx) family – are capable of reacting with peroxide in a manner similar to that of Orp1.

Second, redox signal transmission is based on the specific thiol oxidase function of Orp1, namely, its ability with the help of Ybp1 to oxidize specifically cysteine residues of Yap1 or by default the cysteine residues of its cognate reducing system thioredoxin. Classes of protein that share these two unique features of Orp1 and could also act as peroxide receptors and redox transducers in other organisms include the selenocysteine- and cysteine-based peroxidases of the GPx and Prx families, respectively. In *Schizosaccharomyces pombe*, a two-cysteine Prx functions similarly to Orp1 in the peroxide-induced activation of Pap1, the structural and functional homolog of Yap1 (E. Hidalgo *et al.*, pers. commun.).

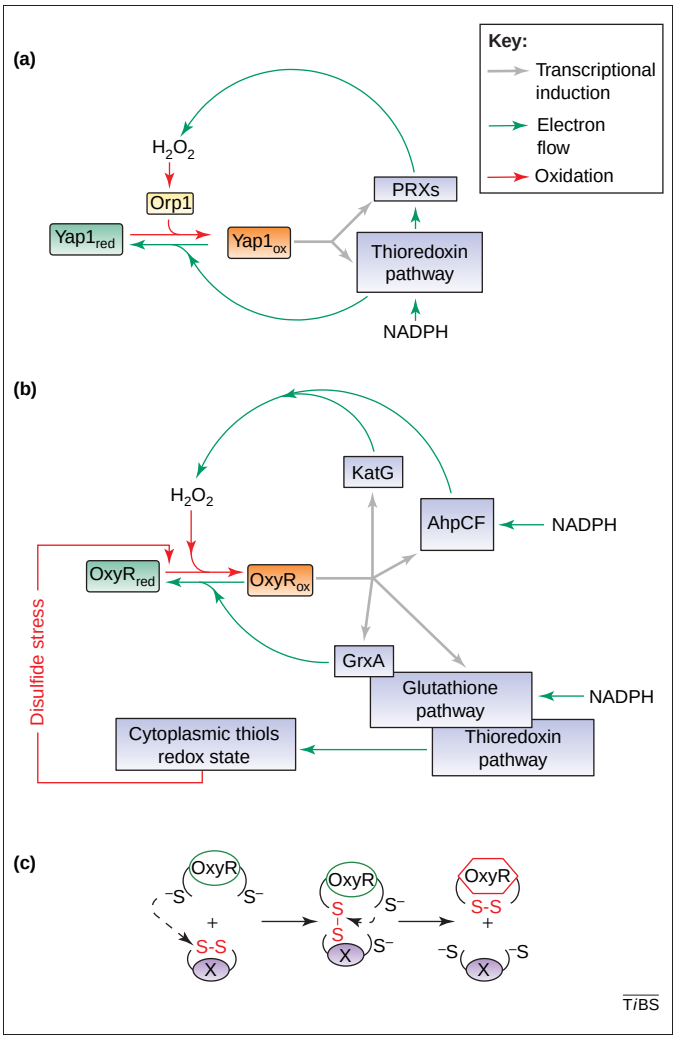

Figure 2. Feedback control autoregulation of Yap1 and OxyR. **(a,b)** In both *S. cerevisiae* (a) and *E. coli* (b), autoregulation originates from a feedback control of both sensor activation and sensor deactivation by sensor target peroxidases and thiol reductase systems (see Box 1). The main peroxide scavengers are the peroxiredoxin (Prx) family enzymes Tsa1, Tsa2 and Ahp1 in *S. cerevisiae* (a), and the *katG*-encoded catalase (hydroperoxidase I) and the Prx family AhpC in *E. coli* (b). AhpCF comprises the Prx AhpC and its specific reductase, NADPH-dependent AhpF. GrxA is glutaredoxin. H_2O_2 keeps the sensor activated until its concentration has been corrected by peroxidases. Deactivation follows by means of sensor reduction by a sensor-specific reductase, which terminates the response. Sensor activity is thus a measure of not only intracellular H_2O_2 concentration but also the NADPH-dependent thiol reducing power, which can be affected by chronic oxidative overload, scarce metabolic conditions or mutations. In yeast, the thioredoxin pathway affects both sensor deactivation by reducing oxidized Yap1 and sensor activation by means of Prx enzymes. Furthermore, this system seems to be the unique electron donor for reduction of H_2O_2 in *S. cerevisiae*. In *E. coli*, by contrast, glutaredoxin affects only sensor deactivation, because *E. coli* bypasses both the glutathione (GSH) and thioredoxin pathways to scavenge peroxide through either AhpC, which carries its own reductase AhpF, or heme-containing catalase, which does not require any reducing system [14,23,47]. **(c)** The thiol-disulfide exchange reaction that is thought to activate OxyR under 'disulfide stress' conditions.

Redox sensing is coupled to both H_2O_2 and thiol redox homeostasis

One of the most striking features of Yap1 activation is its dynamic and highly autoregulated nature, which reflects the permanent adjustment of intracellular concentrations of H_2O_2 (Figure 2a). Autoregulation originates from a feedback control of both sensor activation and deactivation by sensor gene target peroxidases and thiol reductase systems.

In yeast strains lacking cytoplasmic thioredoxin activity [5,11,16], Yap1 is constitutively active. This

deregulation embodies synergistic alterations in both activation and deactivation processes of Yap1. As the reducing system for Prx enzymes, thioredoxin contributes to peroxide reduction. Thioredoxin deficiency thus leads to Yap1 constitutive activation by causing the accumulation of endogenous peroxide. In addition, thioredoxin represents the Yap1 disulfide reducing system, although evidence of a direct effect of this reductant on the reduction of Yap1 *in vivo* is lacking [11,16]. As such, inactivation of thioredoxin prevents the recycling of endogenous peroxide-oxidized Yap1. Transcriptional regulation of both the thioredoxin pathway and Prx enzymes by Yap1 establishes the autoregulatory feedback control of the pathway.

The GSH pathway does not seem to interfere with Yap1 regulation, as attested by the lack of a Yap1 phenotype in mutants in which GSH reductase or both dithiol glutaredoxins are inactivated [11,15]. However, depletion of GSH via the inactivation of γ-glutamylcysteine synthetase, the GSH biosynthesis-limiting enzyme, has been observed by some [41], but not others [11,15], to result to some extent in the activation of Yap1; this discrepancy is due either to the paucity of the effect or to the difficulty of achieving total cellular depletion of GSH, an essential molecule in yeast.

OxyR controls transcriptional expression of the two principal *E. coli* peroxide scavengers, *katG*-encoded catalase and the Prx family alkyl hydroperoxide reductase AhpC, as well as transcriptional expression of its specific reductase glutaredoxin, producing, like Yap1, an autoregulatory system [1,42,43] (Figure 2b). Thus, in contrast to the yeast system, the GSH and not the thioredoxin pathway is the *E. coli* sensor reducing system. Furthermore, glutaredoxin affects only the deactivation of OxyR because this enzyme does not actively contribute to peroxide scavenging (Figure 2b).

Are redox sensors activated by 'disulfide stress'?

Storz and coworkers [22] have interpreted the OxyR-constitutive phenotype of *E. coli* strains, coupled with the simultaneous inactivation of the thioredoxin and GSH systems, to be a consequence of OxyR oxidation by oxidized thiols in the cytoplasm of these strains (Figure 2b, c). They proposed that OxyR can sense H_2O_2 and 'disulfide stress' by distinct mechanisms. OxyR activation induced by disulfide stress would restore the reducing conditions of the cytoplasm by triggering the expression of its target genes encoding disulfide reductases.

Although this model is appealing, an alternative interpretation of these data lies in the consequence of inactivating the thiol-reducing system on the recycling of endogenous peroxide-oxidized OxyR. Disulfide stress has been defined as a cytoplasmic accumulation of disulfides resulting from environmental oxidative stress, exposure to diamide or defective control of thiol redox [22,34,44]. Although diamide potently activates Yap1 [8,10], there is no indication that Yap1 senses disulfide stress: the strongest evidence against this function is the wild-type Yap1 status of a strain carrying a ratio of oxidized GSH to GSSG of 1 (rather than the normal ratio of >10) as a result of the inactivation of GSH reductase [11].

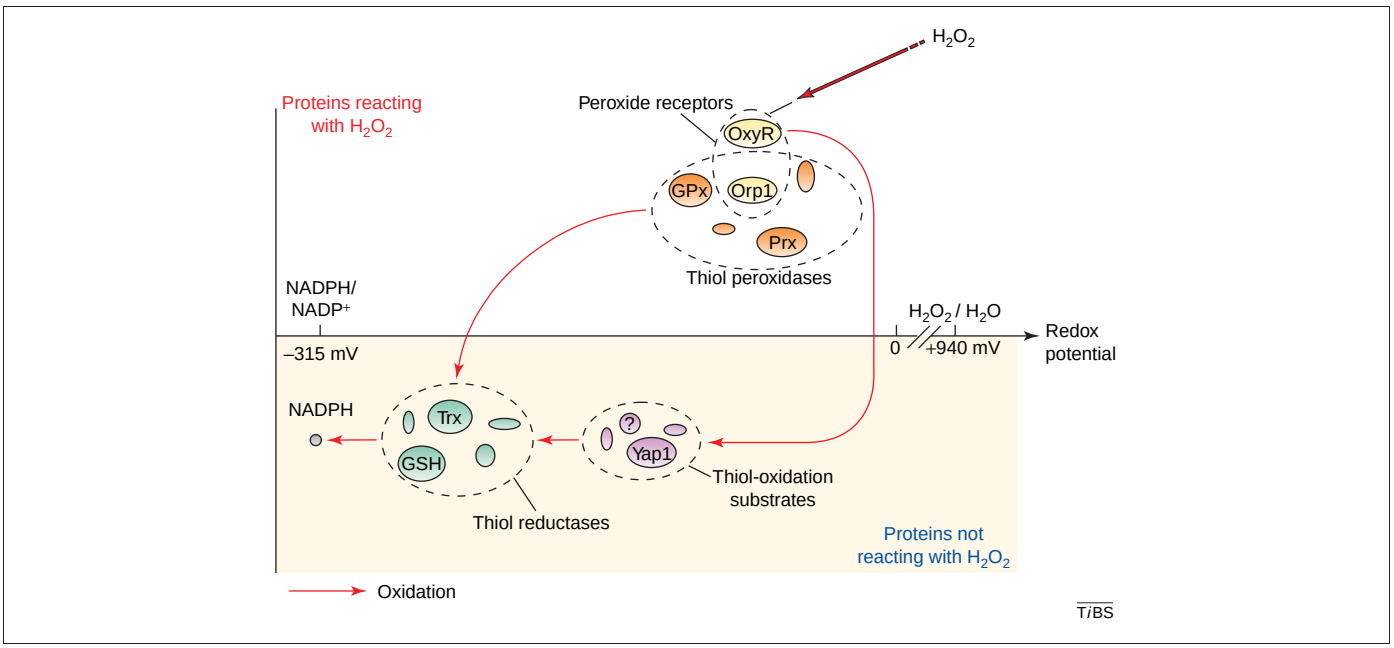

Figure 3. Model of the interaction of H_2O_2 with cellular thiol residues. Red arrows represent the flow of H_2O_2-oxidizing equivalents from 'receptor' peroxidases to NADPH. The reverse flow of electrons from NADPH to H_2O_2 is not indicated. Thiol redox proteins are plotted on an arbitrary two-dimensional scale that correlates reactivity to H_2O_2 with redox potential (see Box 1). The two-disulfide reductase systems are shown in green (see Box 1); peroxiredoxin (Prx) and glutathione peroxidase (GPx) family cysteine-based peroxidases in red; Orp1 and OxyR, considered here as 'H_2O_2 receptors', and specific thiol oxidases in yellow; and Yap1 and other unknown thiol-oxidation H_2O_2 receptor substrates distinct from the disulfide reductases in purple. The redox potential is known only for a few couples [1,48,49]: NADPH, red/ox −315 mV; *E. coli* thioredoxin (Trx), −280 mV; glutathione (GSH), −280 mV; OxyR, −185 mV; H_2O_2, +940 mV (off scale). An arbitrary redox potential has been attributed to the other proteins. An arbitrary threshold of the reactivity of a cysteine residue for H_2O_2 has been drawn on the basis of empirical observations, because this reactivity, expressed as the rate constant of the reaction kinetics, is not established in most cases. This reactivity threshold predicts that only a few cytoplasmic thiol proteins – the thiol peroxidases and H_2O_2 receptors – can react with H_2O_2 *in vivo*. General properties that confer H_2O_2 reactivity on a cysteine residue have been described and include a high nucleophilicity and the ability to stabilize the peroxide substrate RO^- leaving group by a proton-donating group [17]. This model indicates that, as previously suggested by Danon [45], kinetics rather than thermodynamic factors govern redox reactions *in vivo*.

The conundrum that Yap1 is not sensitive to 'disulfide stress' but is activated by diamide, which almost completely reverts the GSH/GSSG ratio, is explained by the fact that this chemical directly modifies, in an Orp1-independent fashion, C-terminal cysteines of Yap1 through either disulfide bond formation or covalent modification [36,39]. The *Streptomyces coelicolor* σ^R-cognate anti-sigma factor RsrA further exemplifies the molecular and physiological differences between peroxide and disulfide stress responses [18]. RsrA is a proposed sensor of disulfide but not peroxide stress that controls expression of the thioredoxin operon but not peroxide scavengers.

A model of the oxidation of cellular cysteine residues by H_2O_2

We have presented the *S. cerevisiae* Orp1–Yap1 and *E. coli* OxyR sensors in a manner that highlights the built-in exquisite specificity of the H_2O_2-sensing phenomenon and shows that its physiology is integral to the cellular systems of H_2O_2 and thiol redox metabolism. We now propose a model that summarizes this view and also integrates the knowledge gained from the two-component nature of the yeast sensor (Figure 3). This model, which indicates that kinetic aspects rather than thermodynamics govern *in vivo* redox reactions [45], predicts that only H_2O_2 sensors (viewed here as 'receptors'), thiol- or selenothiol-based peroxidases and a few other proteins can react with H_2O_2, thereby imposing a hierarchy in the oxidation of cytoplasmic thiols by H_2O_2.

This hierarchical H_2O_2-induced oxidation of thiols challenges the concept of thiol-based 'disulfide stress' sensing, which integrates the notion of a thermodynamic redox balance. Indeed, if disulfide stress emanates from thiol oxidation by peroxides, then oxidation of cysteine residues in 'receptors' should precede the oxidation of other cysteine residues. Furthermore, by suggesting such a high specificity in the interaction of H_2O_2 with cysteines, our model also challenges the view that cysteines can process different redox signals into distinct responses [27]. Yap1 best illustrates this specificity, by recruiting different redox-active cysteines for different redox signals [36].

Concluding remarks

The principles of nitric oxide signaling are now well established [46]. Further validating the principles of cellular thiol oxidation by H_2O_2 that have been developed here should now help to decipher the mechanisms that operate H_2O_2 signaling in higher eukaryotes. In particular, it will be important to identify the proteins with H_2O_2-reactive cysteine residues that we have likened to peroxide 'receptors' and to evaluate their ability to act as redox transducers. Proteins of the GPx and Prx families are the best candidates for such a function.

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