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Forum Review

Zinc Center as Redox Switch—New Function for an Old Motif

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ABSTRACT

Oxidative stress affects a wide variety of different cellular processes. Now, an increasing number of proteins have been identified that use the presence of reactive oxygen species or alterations in the cellular thiol–disulfide state as regulators of their protein function. This review focuses on two members of this growing group of redox-regulated proteins that utilize a cysteine-containing zinc center as the redox switch: Hsp33, the first molecular chaperone, whose ability to protect cells against stress-induced protein unfolding depends on the presence of reactive oxygen species and RsrA, the first anti-sigma factor that uses a cysteine-containing zinc center to sense and respond to cellular disulfide stress. *Antioxid. Redox Signal.* 8, 835–846.

REACTIVE OXYGEN SPECIES—FRIENDS AND FOES

Oxidative stress

AEROBIC METABOLISM IS AN INEVITABLE SOURCE of reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO•) (4, 37). These compounds are capable of modifying most cellular components including nucleic acids, lipids, and proteins. In proteins, ROS can cause irreversible side chain and backbone modifications and can lead to protein unfolding and aggregation, as well as to their premature degradation (37). While protein cofactors like the Fe–S clusters of aconitase and fumarase are particularly sensitive to oxidation by $O_2^{\bullet -}$ (20, 58), H_2O_2 preferentially oxidizes methionine residues and the thiol groups of cysteine residues (75). Hydroxyl radicals, on the other hand, which are described as the most reactive oxygen species known in biology, are less specific and damage most biomolecules as soon as they come into contact (28, 37, 73, 76). To counteract these reactive oxygen species, all aerobically growing organisms express a set of proteins and synthesize various small molecules (e.g., glutathione) that eliminate ROS and reverse some of the oxidative protein modifications (5, 71). Superoxide dismutase, for instance, converts superoxide anions into hydrogen peroxide and oxygen, whereas catalase, glutathione peroxidase, and peroxiredoxins reduce and detoxify peroxides into alcohols or H_2O. Disulfide reductases like thioredoxin and glutaredoxin, on the other hand, reduce unwanted disulfide bonds in cytosolic proteins (for review, see Refs. 13, 34, 70), while methionine sulfoxide reductases reduce methionine sulfoxides (62). All of these proteins, while constitutively expressed, can be upregulated in response to different kinds of oxidative stress (78).

Over the past few years, an increasing number of physiological and pathological conditions such as aging, neurodegenerative diseases, diabetes, and cancer have been found to be linked to the cellular accumulation of reactive oxygen species (2, 19, 49, 52). This condition, generally termed oxidative stress, occurs when the level of ROS present inside the cells become so high that the cellular antioxidative defense mechanisms become overwhelmed and the damaging effects of ROS begin to take over. It is defined as "a disturbance in the prooxidant–antioxidant balance in favor of the prooxidant, leading to potential damage" (73). This balance can be modified either by exposure to exogenous compounds or by mutations in one of the antioxidant systems. Oxidative stress is related and partially overlapping with disulfide stress, a stress condition which occurs upon changes in the cytoplasmic thiol–disulfide redox balance (5). Disulfide stress, which is induced by redox cycling reagents and antibiotics (32, 48)

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also causes the formation of nonnative disulfide bonds and can lead to enzyme inactivation, protein aggregation, and degradation.

To prevent the severe oxidative damage that the accumulation of ROS can cause on cellular macromolecules, cells have developed additional strategies to combat the increased levels of ROS (for review, see Refs. 57 and 66). These strategies involve redox-regulated proteins that sense the presence of high ROS concentrations and adjust their functional activities accordingly. Some of the known redox-regulated proteins include the oxidative stress-sensing transcription factors in *Escherichia coli* and *Saccharomyces cerevisiae*, which either become directly activated by oxidative stress (e.g., OxyR) or modify their cellular localization in response to oxidative stress (e.g., Yap1p) (46, 51, 87). Both strategies lead to the specific induction of antioxidant gene transcription (17, 54, 77). Metabolic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GapDH) (16) have been shown to be redox-regulated. In response to elevated ROS levels, GapDH becomes reversibly oxidized and inactivated. This causes the rapid downregulation of glycolysis upon exposure to oxidative stress in both prokaryotes and eukaryotes (14, 74). In our laboratory, we have discovered that oxidative stress leads to the specific activation of the molecular chaperone Hsp33, which effectively protects proteins against stress-induced unfolding and aggregation processes (40, 84). More recently, the function of several kinases (e.g., protein kinase C) and phosphatases (e.g., PTEN) has been shown to be redox-sensitive (18, 24). The functional diversity of the identified redox-regulated proteins reflects the many different fundamental cellular functions that are either directly or indirectly influenced by oxidative stress. This might provide an explanation for the finding that at least 10% of the yeast transcriptome is altered by oxidative stress (11).

Cysteine—The amino acid of choice to sense oxidative stress in vivo

How do redox-regulated proteins sense ROS and react to their presence by altering their functional activity? Although it is now clear that the mechanisms of redox-regulation are highly diverse, one feature that most redox-regulated proteins have in common is the presence of one or more highly reactive cysteine residues, which become reversibly modified upon exposure to oxidative stress.

The amino acid cysteine confers exceptional versatility to proteins due to the unique biochemistry of its thiol group (22, 23). Cysteines are able to stabilize proteins by forming disulfide bonds, to coordinate metal and to undergo redox reactions. The amino acids cysteine and serine differ only by the substitution of sulfur for oxygen, yet cysteine can participate in many reactions that serine cannot. This is due to the chemical differences between the two Group 16 elements sulfur and oxygen. Thiol groups are more easily deprotonated than alcohols ($pK_a^{Cys} = 8.3$, $pK_a^{Ser} > 14$) and strongly bind to Lewis acids, including transition metals like zinc. The presence of nearby positively charged groups (i.e., metals or basic amino acyl side chains) further decrease the pK_a of a cysteine residue, thus enhancing its reactivity. These properties, which make cysteines such versatile building blocks in proteins, also make them highly vulnerable to oxidation by reactive oxygen species. Depending on the local environment and therefore the reactivity of the thiol group in the protein structure, cysteines can either undergo reversible thiol modifications like disulfide bond, sulfenic acid and disulfide-S-oxide formation, or form irreversible oxidation products such as sulfinic and sulfonic acids (for review, see Refs. 15 and 21, and Leichert and Jakob, this issue, pp. 763–772). Mechanistic analysis of redox-regulated proteins revealed that most of the known reversible thiol modifications have found use as redox sensors in one protein or another (8, 57). The modification of thiol groups often leads to major conformational changes in redox-regulated proteins. Depending on the affected proteins, this can cause alterations in the affinity to partner proteins (e.g., RsrA) or DNA promoter sequences (e.g., OxyR, OhrR), can lead to changes in the oligomerization state of the protein (e.g., Hsp33), or can alter the reactivity towards effector molecules (e.g., protein kinase C) or the accessibility of subcellular targeting sequences (e.g., Yap1p) (24, 47, 57, 66).

Zinc centers as redox switches

Many redox-regulated proteins such as GapDH, OxyR, Yap1p, and p53 simply use the presence of one or more reactive cysteine residues as redox sensors, while others such as SoxR and FNR use the redox-sensitive metal iron as the redox sensor (44, 79). In the first case, the redox chemistry occurs at the sulfur atoms of cysteine residues, whereas in the second case the metal will undergo oxidation/reduction reactions. In this review, we will focus on a subclass of redox-regulated proteins that utilize a cysteine-coordinating zinc center as the redox switch. The fact that zinc is a redox-inert metal ensures that all the redox chemistry occurs at the coordinating cysteines, rather than the metal itself; therefore, unwanted side reactions with water or oxygen to create additional ROS are minimized.

Zinc coordination, which is in most cases tetrahedral in geometry (29), has originally been shown to either play a catalytic or a structural role in proteins. In catalytic zinc sites as found for instance in hydrolases, two or three zinc ligands (most often His or Glu/Asp) are provided by the protein while the remaining coordination sites are occupied by either water or substrate (60). In structural zinc sites, on the other hand, all four coordination sites are occupied by side chains of the protein (usually Cys or His) leading to the exclusion of the solvent (60). Based on the observation that the stabilization energy provided by structural zinc sites is very similar to the stabilization energy that is provided by covalent disulfide bonds (81), structural zinc sites have also been termed "metal cross-links" (9).

In a number of zinc-binding proteins including Hsp33 (25), RsrA (65), and protein kinase C (24), structural zinc sites have now been found to play an additional role. These proteins use their thiol-coordinated zinc sites as redox switches, to sense and react to high levels of ROS and/or disulfide stress *in vivo*. This might be possible because zinc acts as Lewis acid and accepts electron pairs from the thiol groups (31). This lowers the pK_a of the thiol groups and maintains them in their nucleophilic and highly reactive deprotonated thiolate anion state at neutral pH. Zinc binding, therefore,

appears to prime the cysteine ligands for high reactivity with electrophilic ROS (38). At the same time, and by an unknown mechanism, zinc binding appears to protect cysteines in RsrA and Hsp33 from nonspecific air oxidation (55; unpublished observations). Upon exposure to strong oxidants like ROS or diamide, however, the cysteines are quickly oxidized, and zinc is released. This causes the destruction of the structural zinc site with all its conformational consequences, which explains how the oxidative modification of only a few amino acids can lead to such dramatic functional changes in an entire protein.

HSP33, A REDOX-REGULATED MOLECULAR CHAPERONE

Hsp33—member of a large redox-regulated chaperone family

Hsp33 is a member of a new family of redox-regulated chaperones, which is regulated on the transcriptional level by heat shock and on the posttranslational level by the presence of ROS (40). Database searches revealed Hsp33 homologues in nearly every prokaryotic species sequenced to date. Eukaryotic Hsp33 homologues have been identified in *Chlamydomonas reinhardtii*, *Dictyostelium discoideum,* and in three different species of the *Trypanosomatidae* family. While the prokaryotic homologues are localized to the reducing environment of the cytoplasm, the five eukaryotic Hsp33 homologues are predicted to encode putative N-terminal mitochondrial targeting signals (http://psort.nibb.ac.jp). Multiple sequence alignments of all these proteins revealed a nearly absolutely conserved cysteine-containing motif $CXCX_{(27–32)}$ CXXC in the C-terminal domain of the protein, which plays an essential role for Hsp33's functional regulation.

Escherichia coli Hsp33, which is encoded by the gene *hslO*, was first discovered by Chuang and Blattner in 1993 as part of their global analysis of the *E. coli* heat-shock regulon (12). Hsp33 is a 32.8 kDa protein that is constitutively expressed under nonstress conditions and whose protein amount increases up to twofold (3 μ*M*) upon shift to heat-shock temperature. Early on, Hsp33 was identified to function as a highly potent molecular chaperone in *E. coli* (40). What makes Hsp33 so unique among molecular chaperones is its functional regulation. Most known chaperones including GroEL and DnaK continuously cycle between low and high affinity binding states depending on their nucleotide binding status (86). For example, DnaK switches from a low affinity binding state in the presence of ATP to a high affinity binding state upon ATP hydrolysis. Hsp33, however, appears to be permanently in a low affinity binding state during nonstress conditions. When cells are exposed to ROS at elevated temperatures, however, the chaperone function of Hsp33 is specifically activated to protect cells against the otherwise lethal consequences of oxidative stress (40, 84).

Using a zinc center to "switch off" a chaperone

Purification of overexpressed Hsp33 from nonstressed *E. coli* cells yields high concentrations of reduced, chaperone-inactive and monomeric protein, even in the absence of reducing conditions in the buffer (40). Biochemical analysis of the purified protein revealed that the four highly conserved cysteines in the $C^{232}XC^{234}X_{(27–32)}C^{265}XXC^{268}$ motif of Hsp33's C-terminal region are involved in the high affinity binding of one zinc(II) ion ($K_a > 10^{17}\ M^{-1}$, 25ºC at pH 7.5) (39). This significantly stabilizes the reduced protein and maintains Hsp33 in an inactive state (Fig. 1). These findings were confirmed by the recently solved NMR structure of the isolated C-terminal zinc-binding domain of Hsp33 from *E. coli* (85) as well as by the crystal structures of the full length reduced Hsp33 dimer from *Bacillus subtilis* (41) and *Thermotoga maritima* (42). These structures showed that the zinc binding site of Hsp33 forms a very compact folding unit, in which zinc binds to the four conserved cysteines of Hsp33 in tetrahedral geometry. Although the structure of the reduced Hsp33 monomer has yet to be solved, mutational studies on Hsp33 suggest that the zinc binding domain blocks both the substrate-binding site as well as the dimerization interface of Hsp33 (26). This explains why reduced and zinc coordinated Hsp33 is monomeric and inactive in the reducing environment of the *E. coli* cytosol under nonstress conditions.

Zinc center meets ROS–activation of a molecular chaperone

Upon exposure of Hsp33 to oxidants such as H_2O_2 or $HO^{\bullet}$, zinc is rapidly released (27, 40) (Fig. 1). This is probably caused by the oxidative modification of the coordinating cysteines, which form two intramolecular disulfide bonds between the nearby cysteines, connecting C^{232} with C^{234}, and C^{265} with C^{268} (7). These four highly conserved cysteines appear to be the primary targets of ROS and their rapid oxidation forms the basis of the redox-regulation of Hsp33. How does disulfide bond formation and zinc release lead to the activation of Hsp33's chaperone function? We found that the oxidation of Hsp33's zinc center induces the unfolding of the C-terminal redox switch domain of Hsp33 (Fig. 1). This unfolding appears to unmask the substrate binding site and dimerization interface in Hsp33 and allows Hsp33 to switch from low affinity monomers to high affinity dimers (26). The structure of the reduced form of the C-terminal domain of Hsp33 is stabilized by the high affinity coordination of zinc to two pairs of cysteine residues that are far apart in primary sequence. This brings together distant regions of the redox domain, much like a button fastens together two sides of a shirt. Release of the zinc due to oxidative stress induced disulfide bond formation unfastens this button, and causes the unfolding of this domain.

The finding that disulfide bond formation in Hsp33 causes domain unfolding was very much in contrast to the common belief that disulfide bonds are stabilizing and promote structure formation (83). However, structural analysis of Hsp33's zinc binding domain revealed a weak hydrophobic core with α-helices that are further destabilized by β-branched valines and isoleucines (85). This explained why the oxidation-induced loss of the stabilization energy provided by zinc binding leads to the destabilization of the secondary and tertiary structure of this domain and causes its unfolding. In addition, any stabilizing long-range interac-

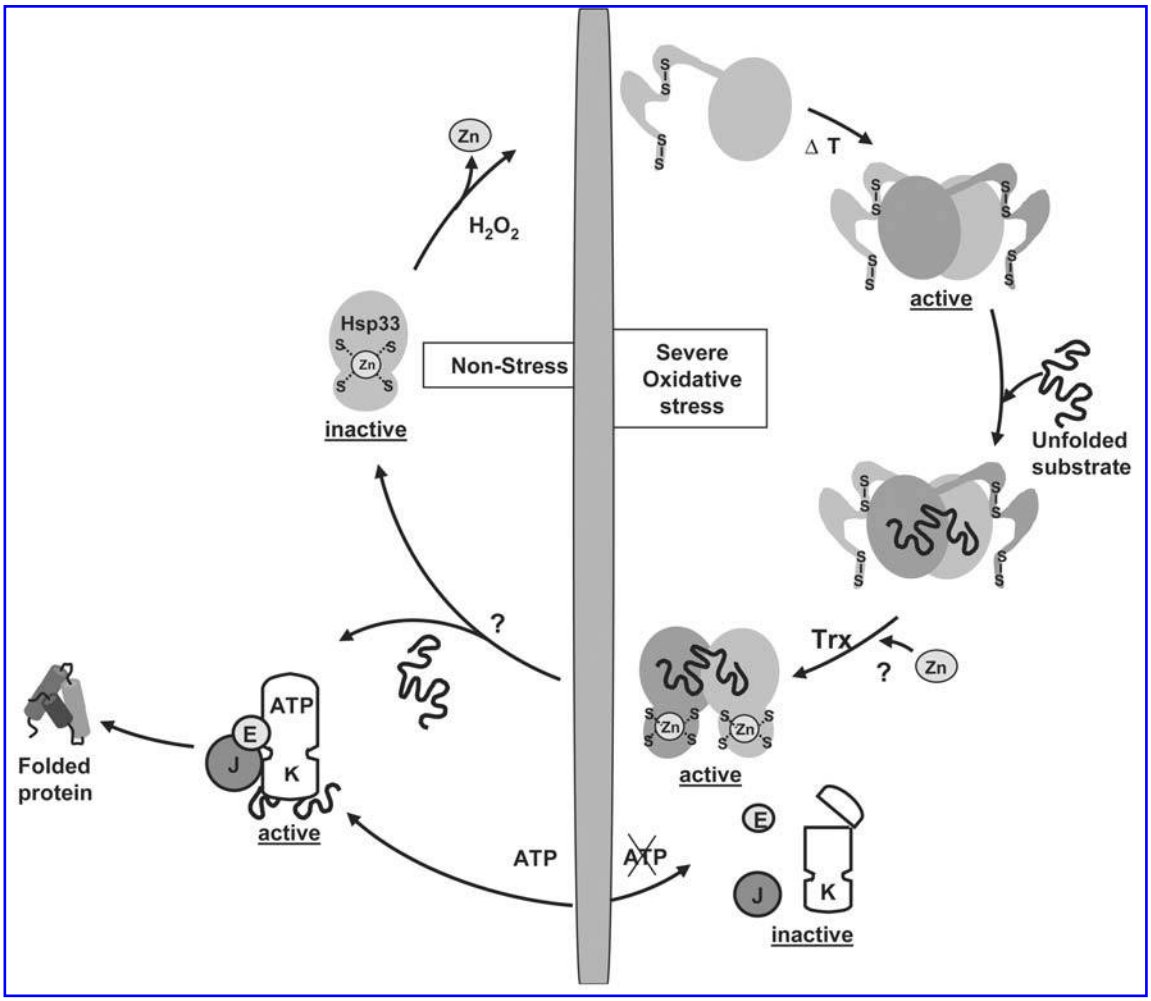

FIG. 1. Hsp33: central player of a redox-regulated chaperone network. Under nonstress conditions, Hsp33 is inactive. The C-terminal redox switch domain is in its off-position. All four absolutely conserved cysteines in this domain coordinate zinc. This apparently blocks the substrate binding site and dimerization interface of Hsp33. Upon exposure to oxidative stress (e.g., H_2O_2) conditions, the redox switch domain of Hsp33 turns into its on-position. Two intramolecular disulfide bonds form and zinc is released. This induces important conformational changes in Hsp33 and lead to the unfolding of Hsp33's redox switch domain. Unfolding of the redox switch domain in combination with elevated temperature and increased *in vivo* concentration of Hsp33 supports the formation of highly active Hsp33 dimers. Activated Hsp33 dimers work independently of ATP and are able to bind to a large number of unfolded proteins and to maintain them in a folding competent state until nonstress conditions are restored. Upon return to reducing conditions, the cysteines in Hsp33 are reduced and Hsp33 forms reduced chaperone-active Hsp33 dimers. Only reduced Hsp33 dimers are able to transfer the substrate proteins onto the DnaK/DnaJ/GrpE system. While Hsp33 is specifically activated by oxidative stress conditions at elevated temperatures, DnaK is specifically and reversibly inactivated. This is caused by the rapid decrease in cellular ATP levels, which leaves DnaK's N-terminus thermolabile and prone to unfolding. Upon return to nonstress conditions, ATP levels are restored. This induces the rapid reactivation of DnaK, which can then accept the substrate proteins from Hsp33 and uses ATP to support their refolding to the native state. The exact mode of substrate transfer and Hsp33 inactivation is still under investigation.

tions that are provided by metal binding are also being lost upon zinc release.

One consequence of the tetrahedral arrangement of cysteines in zinc centers is that the sulfur atoms are approximately equidistant from each other. Each cysteine should, therefore, be equally likely to form a disulfide bond with all three other cysteines. Such long-range disulfide bonds would be expected to confer a similar stabilization to the C-terminal domain as zinc coordination itself. This, however, might not lead to the unfolding of Hsp33's C-terminal domain, which is the necessary prerequisite for Hsp33's activation. To prevent the formation of such stabilizing long-range disulfide bonds in Hsp33, it was therefore postulated that unfolding of the redox domain must occur before disulfide bond formation, thus ensuring that disulfide bonds form solely between the cysteines nearby in primary sequence (85). This suggested, however, that oxidative modifications other than disulfide bond formation would cause zinc release and the unfolding of the C-terminus, and would only later be resolved into disulfide bonds. One possible initial modification could be

sulfenic acid formation, which has been implicated to be the first oxidation step in OxyR and which later leads to disulfide bond formation with a nearby cysteine (53). So far, however, we have been unable to detect any sulfenic acid formation in either wild type or single cysteine mutants of Hsp33 upon treatment with H_2O_2 (Graf and Jakob, unpublished observations). Thus, it remains to be elucidated how H_2O_2 causes the formation of two disulfide bonds that exclusively link the nearby cysteines and allow for the unfolding of the C-terminus of Hsp33.

After zinc release, disulfide bond formation and the unfolding of the C-terminal redox switch domain, two oxidized Hsp33 monomers associate to form highly chaperone active Hsp33 dimers (27) (Fig. 1). The existence of Hsp33 dimers was first revealed when the crystal structures of two C-terminally truncated *E. coli* Hsp33 fragments (1–255 and 1–235, respectively) were solved (45, 82). The two crystal structures showed Hsp33 as a domain swapped dimer, in which the polypeptide of one subunit crosses over after Pro178 to the other subunit. There, it forms three α-helices that pack against the outer surface of the other surface. It has yet to be determined whether domain swapping also occurs in solution. However, the finding that this largely hydrophobic interface confers most of the dimerization energy as it buries about 80% of the total 4900 Å^2 accessible surface area that are buried per protomer, suggests that it also occurs in solution. The rest of the dimerization interface is constituted by a number of highly conserved amino acids of the Hsp33 core domain, whose deletion renders the protein constitutively monomeric (45, 82). *In vitro* studies show that dimerization plays an important part of Hsp33's activation process. It generates active Hsp33 dimers, which are able to prevent the aggregation of numerous unfolding substrate proteins *in vitro* (27). Recently, the existence of higher oligomeric species of Hsp33 with increased affinity to unfolding substrate proteins were discovered *in vitro* (1). Formation of these higher oligomers was dependent on high salt or osmolytes. Both the nature of the oligomers and their physiological significance remain to be investigated.

Noteworthy, dimer formation of Hsp33 using hydroxyl radicals as oxidant was found to be both Hsp33 concentration- and temperature-dependent *in vitro* (27). This is in contrast to disulfide bond formation and zinc release, which are both Hsp33 concentration- and temperature-independent processes (27). It therefore appears that additional conformational changes beyond those induced by zinc release are required for the dimerization of Hsp33. These *in vitro* results, which showed that the activation of Hsp33 is most efficient upon oxidative stress at elevated temperatures, are in excellent agreement with phenotypical studies that showed that Hsp33 protects cells particularly against a combination of H_2O_2 treatment at elevated temperatures (84).

Hold me—hold me tight: the promiscuous chaperone holdase Hsp33

Oxidized and activated Hsp33 functions as an efficient chaperone holdase both *in vitro* and *in vivo* (27, 40, 84). Chaperone holdases, which also include small heat shock proteins (sHsps), Hsp40 (DnaJ), and Hsp110, bind their substrate proteins in an ATP-independent manner and maintain them in a folding-competent state but do not support their refolding. Upon return to nonstress conditions, they usually transfer their substrate proteins to chaperone foldases such as Hsp70 (DnaK) family members, which support the refolding of the substrates using ATP hydrolysis to drive the folding process. As a holdase, Hsp33 was shown to form apparently stable complexes with its substrate proteins to prevent their aggregation. Even long-term incubation of Hsp33-substrate complexes at heat shock temperatures did not lead to any detectable release of the substrate proteins (33). *In vivo*, Hsp33 was found to protect more than 80% of all aggregation-sensitive proteins in *E. coli* against irreversible aggregation under oxidative stress conditions at elevated temperatures (84). These substrate proteins include numerous metabolic enzymes as well as proteins involved in transcription and cell division (84).

The binding site of Hsp33 for its substrate proteins remains yet to be identified. It has been shown that oxidation of Hsp33 significantly increases the extent of hydrophobic surfaces in the protein (26, 69). These hydrophobic sites could potentially represent the substrate binding site(s) of Hsp33. In the case of other general chaperones such as DnaK or GroEL, the binding sites are also characterized by a number of highly conserved hydrophobic residues, which interact with hydrophobic amino acids that are solvent exposed in the partially unfolded substrate protein (35, 72). Analysis of Hsp33's crystal structure revealed two potential substrate binding sites, which extend over and involve conserved hydrophobic residues of both subunits (45, 82). Both sites are in the N-terminal domain of Hsp33. This agrees with functional studies that showed that a C-terminally truncated mutant (1–235) of Hsp33 exhibits chaperone activity *in vitro* (45).

Closing the circle: inactivation of Hsp33

The inactivation of Hsp33 was found to require the return to nonstress conditions, which included both reducing conditions as well as nonstress temperatures (33). It was originally hypothesized that reduction of Hsp33 should be sufficient for the inactivation of Hsp33 and the release of substrate proteins (80). However, *in vitro* reduction studies demonstrated that reducing conditions alone are insufficient for substrate release (33). Fluorescence anisotropy measurements showed that the reduction of oxidized Hsp33 dimers significantly precedes their dissociation and revealed the existence of a new species of Hsp33, the kinetically stable reduced Hsp33 dimer (33). While substrate-free reduced Hsp33 dimers were found to eventually dissociate into the inactive reduced Hsp33 monomers, substrate-bound Hsp33 dimers do not dissociate unless the substrate is released (33). This mechanism apparently allows Hsp33 to retain its full chaperone activity even after reducing conditions are restored and prevents premature substrate release *in vivo* under reducing, yet nonpermissive folding conditions.

What causes Hsp33 to release its substrate proteins? *In vitro* experiments demonstrated that the presence of the active DnaK/DnaJ/GrpE system (DnaK system) is required to release the substrate proteins from reduced Hsp33 dimers and to refold the substrate proteins (33) (Fig. 1). The exact *modus operandi* of substrate transfer and Hsp33 inactivation is still unclear. As oxidized and reduced Hsp33 dimers ap-

pear to have very similar affinities for their substrate proteins (33), a simple competition between Hsp33 and the DnaK system for the substrate proteins seems unlikely. It appears more likely that reduction-induced conformational changes in Hsp33 allow the DnaK system to access the substrate proteins once Hsp33 is reduced. Alternatively, the DnaK system might actively participate in the dissociation of substrate-bound reduced Hsp33 dimers. This would return Hsp33 into its monomeric, inactive state and cause the substrate to be released.

It also remains to be investigated at what point during the reduction process of Hsp33 the C-terminal zinc-binding domain refolds. It is safe to assume that this occurs upon zinc reassociation and should be accompanied by major conformational rearrangements. These conformational changes induced by disulfide reduction and zinc-reassociation might involve the "un-swapping" of the C-terminal domains as suggested by the recently solved crystal structures of full length Hsp33 from *B. subtilis* (41) and *T. maritima* (42). In both structures, Hsp33 is found in a reduced dimeric conformation with properly folded zinc binding domains and no apparent domain swapping. As previously described, active Hsp33 crystallized as a C-terminally domain swapped dimer (45, 82). A dimer without domain swapping would presumably be less stable than a domain swapped dimer. As such, it would dissociate more easily in the absence of outside stabilization (i.e., substrate binding), thus perhaps allowing the DnaK system to actively dissociate reduced, but not oxidized, Hsp33 dimers. It remains to be determined to what extent the DnaK system is involved in these conformational rearrangements prior to dimer dissociation and whether domain unswapping is indeed the necessary prerequisite for substrate release and/or the inactivation of Hsp33 (41).

Hsp33's activation complements DnaK's inactivation

Hsp33 prevents the aggregation of a large variety of different proteins that unfold under extreme conditions of oxidative stress at elevated temperatures (84). Interestingly, a significant number of Hsp33's substrate proteins have previously been shown to be substrate proteins of the DnaK system under heat shock conditions (84). This raised the question why would cells require the presence of such a highly sophisticated chaperone like Hsp33 when the DnaK system is able to protect the same set of proteins against stress-induced unfolding? Comparative analysis of the chaperone function of Hsp33 and the DnaK system *in vivo* revealed that the very conditions that specifically activate Hsp33 lead to the transient inactivation of the DnaK system (Fig. 1). The inactivation of DnaK is triggered by the rapid decrease in intracellular ATP concentration, which is the result of oxidative stress induced inactivation of GapDH, one of the key enzymes of glycolysis (64, 84). This renders the N-terminal nucleotide binding domain largely nucleotide free *in vivo* and prone to unfold at elevated temperatures (61, 84) (Fig. 1). The ATP-independent chaperone holdase Hsp33, which is activated by these very conditions, now binds the proteins that are usually protected by the DnaK system. Once reducing, nonstress conditions are restored, and the original ATP levels have returned to normal (84), the DnaK system reactivates, and Hsp33's cysteines are reduced. As outlined above, reduction of Hsp33 primes this chaperone holdase to release its substrate proteins to the chaperone foldase DnaK (Fig. 1). Although substrate transfer between Hsp33 and the DnaK system has only been shown using *in vitro* substrates, the significant overlap in substrate proteins between Hsp33 and the DnaK system suggests that Hsp33 transfers a substantial number of its *in vivo* substrate proteins after oxidative stress onto the DnaK system for their successful refolding (84).

RSRA, A REDOX-REGULATED ANTI-SIGMA FACTOR

RsrA, the Regulator of Sigma R activity is a member of a growing family of zinc containing anti-sigma factors. This family, which includes ChrR from *Rhodobacter sphaeroides* (36), is characterized by the presence of a highly conserved HX_3CX_2C motif that is involved in zinc binding. RsrA was first discovered in *Streptomyces coelicolor* A3(2), a Gram-positive organism of the *actinomycetes* group (43). It was found to be involved in the redox-dependent regulation of sigma factor R (σ^R) activity in response to cellular disulfide stress. Similar to Hsp33, RsrA appears to sense these cellular redox variations through a cysteine containing zinc center.

Sigma R—a cell's response to disulfide stress

Sigma R belongs to a large family of variable extracytoplasmic function (ECF) sigma factors, whose activation in response to environmental variations leads to the induction of specific target genes (30). σ^R was found to be involved in the protection of *S. coelicolor* cells against the thiol-specific oxidant diamide (67). Diamide treatment of cells causes rapid changes in the cytoplasmic thiol–disulfide redox balance, a condition termed disulfide stress (5). This leads to σ^R activation and to the induction of the σ^R regulon, which includes the *trxBA* operon, *trxC*, *sigR* itself and 27 other recently identified genes that are necessary to restore the original redox status of the cell (67, 68).

RsrA: an inhibitor of sigma R activity under nonstress conditions

Sigma R is specifically activated by disulfide stress *in vivo* but does not contain any cysteines in its amino acid sequence. This immediately raises the question how σ^R senses cellular disulfide stress *in vivo*? Analysis of the DNA sequence downstream of the *sigR* gene revealed a gene *rsrA*, which is translationally coupled to *sigR* and encodes for the seven cysteines containing protein RsrA. Functional analysis of the RsrA protein *in vitro* and *in vivo* revealed that it acts as the disulfide stress redox sensor in *S. coelicolor* that regulates σ^R activity in the cell (43).

Under nonstress conditions, RsrA binds to σ^R and functions as its anti-sigma factor (43) (Fig. 2). The interaction between RsrA and σ^R, which was demonstrated *in vitro* using native PAGE and surface plasmon resonance, effectively pre-

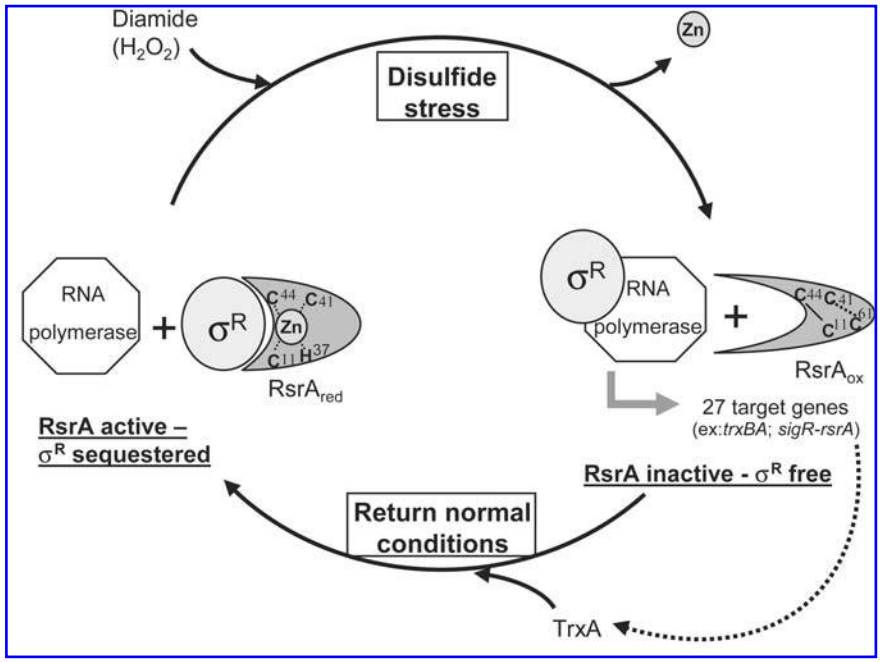

FIG. 2. RsrA—a redox regulated anti-sigma factor. During nonstress conditions, RsrA has an N-terminal zinc center, which is essential for the association with its sigma factor σ^R. This stable RsrA/σ^R interaction prevents binding of σ^R to core RNA polymerase. Upon disulfide stress, at least one and possibly two disulfide bonds form and zinc is released. These events cause conformational rearrangements in RsrA, which lead to the dissociation of σ^R. The sigma factor is then free to interact with core RNA polymerase and to induce the expression of its target genes. One of the target genes encodes for TrxA, which can reduce RsrA, thereby creating a feedback loop.

vents σ^R from binding to RNA core polymerase and from inducing target gene expression. RsrA was shown to interact with the N-terminus of σ^R (56). This domain has a three-dimensional structure, which is very similar to the structure of the RNA polymerase binding domain of the *E. coli* σ^{70} family (56). It was then demonstrated that RsrA competes with core RNA polymerase for binding to σ^R (56) suggesting that the anti-sigma factor activity of RsrA is based on RsrA's ability to effectively out-compete RNA polymerase in binding to σ^R.

Sensing disulfide stress in vivo means letting go of σ^R

The interaction between RsrA and σ^R binding was found to be strongly dependent on the redox state of RsrA (43). Under nonstress conditions, RsrA was shown to bind to σ^R, thereby inhibiting its transcriptional activity. The presence of the disulfide-generating oxidant diamide or H_2O_2, however, leads to the rapid dissociation of the RsrA/σ^R complex (6) (Fig. 2). Once free, σ^R is then able to bind to core RNA polymerase and to induce the expression of its target genes (43). This was shown using *in vitro* transcription assays with the purified components and agreed with phenotypical studies of the *rsrA* deletion strain, which shows high levels of σ^R activity and constitutively overexpresses its target genes, including the thioredoxin system (65).

The redox sensitivity of the RsrA/σ^R interaction strongly suggested the involvement of cysteine residues in the regulation. In contrast to σ^R, which does not contain any cysteine residues, RsrA contains seven cysteines in its sequence of 105 amino acids. The substitution of each individual cysteine residue for serine or alanine was carried out to first define the residues that are involved in the anti-sigma factor function of RsrA. This study revealed that each of the three highly conserved cysteines C^{11}, C^{41}, and C^{44} (Fig. 3) play an essential role in the anti-sigma factor function of RsrA and that they also are involved in the redox regulation (65). Individual substitution of each of these residues led to RsrA variants that were no longer able to associate with σ^R. These results suggested that the cysteines play a major role in the RsrA/σ^R interaction and provided an explanation as to how disulfide stress-induced modification of these cysteines could cause the dissociation of the RsrA/σ^R complex and the activation of the disulfide stress response.

To further investigate the role of these cysteine residues in RsrA, it was first tested whether disulfide stress conditions might cause the formation of disulfide bonds in RsrA. These experiments revealed that the *in vitro* oxidation of RsrA leads to the formation of different RsrA species with up to three disulfide bonds, with the most abundant subpopulation of RsrA containing two disulfide bonds (43). The observation that only three of the seven cysteines in RsrA were absolutely essential for the anti-sigma factor activity of RsrA *in vitro* and *in vivo* suggested that only one disulfide bond might play an indispensable role *in vivo* (65). This was confirmed by the analysis of an RsrA mutant protein, whose four nonessential cysteines were replaced by alanines. This mutant protein was found to be still redox regulated, suggesting the involvement of only one disulfide bond. (65). To identify this critical disulfide bond in RsrA, Li *et al.* exposed purified RsrA to air oxidation in the presence of EDTA (55). This RsrA preparation, which was inactive as an anti-sigma factor, contained only one disulfide bond. Mass spectrometric analysis revealed that this disulfide bond was formed between C^{11} and either C^{41} or C^{44}, with the C^{11}–C^{44} subpopulation being three times more prominent than the C^{11}–C^{41} subpopulation (55). At the same time, Bae *et al*. used MALDI-TOF analysis to

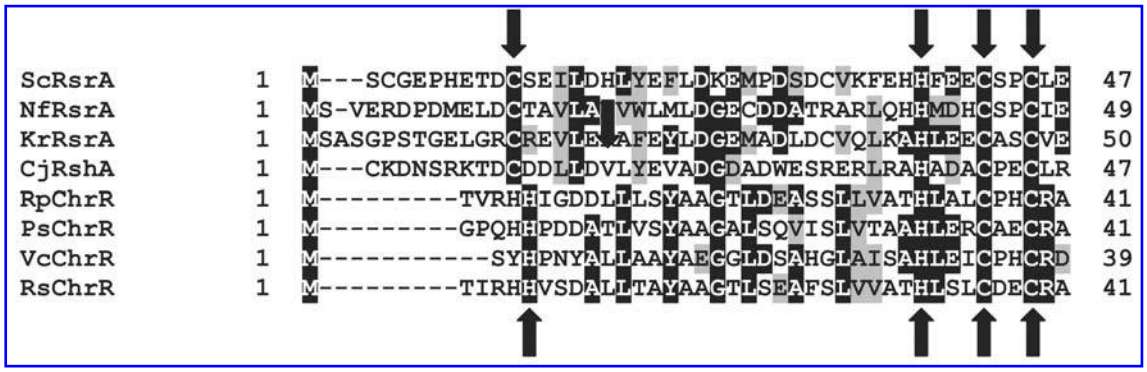

FIG. 3. N-terminal sequence alignment of zinc containing anti-sigma factors. The HX_3CX_2C motif is conserved in all members of the zinc containing anti-sigma factor family. C^{11} is conserved in RsrA subfamily while the equivalent H^6 is conserved in ChrR subfamily. The zinc-binding residues are indicated with *arrows*. Sc, *Streptomyces coelicolor* RsrA, CAB94602; Nf, *Nocardia farcinica* IFM 10152 putative RsrA homologue, YP_120798; Kr, *Kineococcus radiotolerans* SRS30216 putative RsrA homologue, EAM75201; Cj, *Corynebacterium jeikeium* K411 RshA, CAI37796; Rs, *Rhodobacter sphaeroides* ChrR, ZP_00004392; Rp, *Rhodopseudomonas palustris* CGA009 ChrR, NP_945902; Ps, *Pseudomonas syringae pv. syringae* B728a ChrR, YP_233987; VC2301,

show the formation of two disulfide bonds in RsrA, the first one between C^{11} and C^{44}, confirming the findings of Li *et al.* (55), and the second, probably less important disulfide bond, connecting C^{41} and the less conserved C^{61} (6). These studies suggested that the formation of one disulfide bond most likely between C^{11}–C^{44} is sufficient to induce the necessary conformational changes in RsrA that lead to its inactivation and to the release of σ^R (55). The formation of a degenerate disulfide bond between C^{11} and $C^{41/44}$ might be caused by nonspecific air oxidation or disulfide rearrangements of zinc-free RsrA before the thiol trapping with iodoacetamide was complete (6, 55).

Upon disulfide stress-induced σ^R release, σ^R associates with core RNA polymerase and induces the transcription of more than 27 different genes, including its own *sigR* gene (43) (Fig. 2). What turns the σ^R- response off? *In vitro* experiments revealed that oxidized RsrA is a substrate of purified thioredoxin, the product of the σ^R-regulated *trxA* gene (43). This finding suggests that thioredoxin-mediated reduction and reactivation of RsrA creates a simple feedback loop in which the σ^R regulon is deactivated in response to the restoration of the thiol–disulfide redox balance in the cell. However, despite the fact that σ^R positively regulates its own synthesis through the σ^R promoter, the constitutive level of transcription of σ^R target promoters in an *rsrA* null mutant is not higher than the level in the wild-type strain after 15 min of exposure to diamide (65). This implies that additional mechanism such as proteolysis are involved in σ^R regulation.

The redox switch in RsrA—a zinc center

How does the redox switch work in RsrA? The existence of near stoichiometric amounts of zinc in RsrA was first discovered using inductively coupled plasma atomic emission spectroscopy (ICP–AES) (65). Later it was shown that zinc binds with extremely high affinity to RsrA ($1.0 \times 10^{17}\ M^{-1}$ at 23ºC, pH 7.6), suggesting that zinc plays a structural rather than a catalytic role in RsrA (6). Sequence comparison of members of the zinc containing anti-sigma factors family, including the RsrA and ChrR subfamilies, suggested that the absolutely conserved $H^{37}X_3C^{41}X_2C^{44}$ motif might constitute part of the zinc-binding site in RsrA (Fig. 3)(65). Recently, extended X-ray absorption fine structure (EXAFS) analysis of purified wild type RsrA, RsrA-H37A, and RrsA with all four nonconserved cysteines C^3, C^{31}, C^{61}, and C^{62} replaced by alanine was conducted and showed that zinc is coordinated by C^{11}, H^{37}, C^{41}, and C^{44} (K. Zdanowsk, C. Kleanthous, M. Paget and M. Buttner, personal communication). This zinc binding motif, $C^{11}X_{25}H^{37}X_3C^{41}X_2C^{44}$, which is conserved in all RsrA members of the family of zinc coordinating anti-sigma factors (Fig. 3), differs from the motif $C^3X_3H^7X_{33}C^{41}X_2C^{44}$, which has previously been suggested to be involved in the zinc coordination of RsrA by Bae and coworkers (6). This binding motif was suggested based on zinc binding studies of purified, zinc reconstituted cysteine and histidine mutants of RsrA. Although the $C^3X_3H^7$ motif is characteristic of a canonical Zn binding loop (29), C^3 and H^7 are not present in any other zinc coordinating anti-sigma factors except RsrA of *S. coelicolor* (Fig. 3), making it rather unlikely that it represents the general zinc binding motif of this protein subfamily.

Zinc confers considerable stability to RsrA under nonstress conditions, and is crucial for preventing the spontaneous air oxidation of RsrA's cysteines (55). Upon exposure of RsrA to disulfide stress and formation of disulfide bond(s), zinc is rapidly released (55). Similar to Hsp33, this leads to significant conformational rearrangements in RsrA. Secondary structure analysis of RsrA revealed that reduced RsrA contains ~20% α-helix, ~30% β-sheet, whereas oxidized RsrA is ~30% α-helical and ~20% β-sheet (55). These conformational changes appear to lower the affinity for the sigma factor and are the likely trigger of the σ^R response.

Interestingly, while C^{11}, which plays the leading role in RsrA's redox regulation, is highly conserved within the RsrA subfamily, in ChrR from *Rhodobacter sphaeroides* the fourth zinc ligand is a histidine (H^6) (Fig. 3). Mutational analysis of ChrR from *R. sphaeroides* together with the recently solved

crystal structure of ChrR in complex with its cognate sigma factor RpoE, revealed that H^6 indeed takes the place of C^{11} in ChrR and coordinates zinc together with the absolutely conserved HX_3CX_2C motif (E. Campbell, S. Darst, R. Greenwell and T. Donohue, personal communication). In contrast to RsrA, however, ChrR does not seem to be regulated by oxidative stress (H_2O_2) (63), agreeing with the notion that it lacks the critical C^{11}. However, it was recently shown that ChrR senses and responds to singlet oxygen (3). Thus, it is conceivable that zinc coordinating anti-sigma factors that contain C^{11} might be regulated by disulfide stress while those with histidine as the fourth zinc ligand respond to different oxidants such as singlet oxygen or other unknown signals (3). This very attractive model remains to be experimentally verified.

REDOX-REGULATED ZINC PROTEINS: A COMMON MECHANISM

This review focuses on two proteins, whose activity is regulated by the oxidation of critical cysteine residues that form a zinc center under nonstress conditions. Common and central to this new form of functional regulation is the conformational change that accompanies the oxidative stress induced release of zinc and which alters the protein function. The individual conformational changes that Hsp33 and RsrA undergo upon the oxidation of their zinc centers are, however, rather specific and quite intriguing. Even though both proteins lose the structural stabilization otherwise provided by zinc binding, the C-terminal redox domain of Hsp33 completely loses its structure upon oxidation and zinc release (26), whereas RsrA "simply" reorganizes its secondary structure (55). One possible explanation is that the disulfide bonds in Hsp33 are close in range and do not compensate for loss of the metal cross-link, whereas the disulfide bond(s) in RsrA are far apart in the primary sequence and thus bring together distant regions of the protein in a new way that may compensate for the loss of the metal cross-link. However, as there are currently no structural data available for RsrA in either redox conformation, it is difficult to speculate exactly how the structure changes upon oxidation and reduction.

What are the other advantages of using zinc as part of a redox switch domain apart from its potential to amplify conformational changes significantly? It has been suggested that zinc binding enhances the reactivity of the cysteine to which it is bound by stabilizing the negative charge on the thiolate anion, thereby lowering its pK_a value and making the cysteine more reactive to H_2O_2 at near neutral pH (31, 59). Because zinc(II) itself is a redox-inert metal, this arrangement would also guarantee that the redox chemistry is limited to the coordinating cysteines and does not involve the metal. This would prevent unwanted side reactions of the metal with water or oxygen that could potentially create additional ROS. Also, because oxidative modification of the coordinating cysteines causes metal release, Fenton chemistry would further exacerbate the oxidative stress inside the cell if the coordinated metals were redox active (50). Last but not least, it has been speculated that the released zinc might have additional significant biological relevance as cellular antioxidant (10).

The discovery that an increasing number of prokaryotic and eukaryotic proteins involved in many different pathways use their structural zinc sites as redox-regulatory devices to adjust their protein activity to the redox conditions of the environment, suggests that this form of redox signaling might represent one of the central events in redox biology.

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ABBREVIATIONS

2D, two dimensional; ECF, extracytoplasmic function sigma factors; EXAFS, extended X-ray absorption fine structure; ICP–AES, inductively coupled plasma atomic emission spectroscopy; MALDI–TOF, matrix assisted laser desorption/ionization–time of flight; ROS, reactive oxygen species;

REFERENCES

1. Akhtar MW, Srinivas V, Raman B, Ramakrishna T, Inobe T, Maki K, Arai M, Kuwajima K, and Rao Ch M. Oligomeric Hsp33 with enhanced chaperone activity: gel filtration, cross-linking, and small angle x-ray scattering (SAXS) analysis. *J Biol Chem* 279: 55760–55769, 2004.
2. Aliev G, Smith MA, Seyidov D, Neal ML, Lamb BT, Nunomura A, Gasimov EK, Vinters HV, Perry G, LaManna JC, and Friedland RP. The role of oxidative stress in the pathophysiology of cerebrovascular lesions in Alzheimer's disease. *Brain Pathol* 12: 21–35, 2002.
3. Anthony JR, Newman JD, and Donohue TJ. Interactions between the *Rhodobacter sphaeroides* ECF sigma factor, sigma(E), and its anti-sigma factor, ChrR. *J Mol Biol* 341: 345–360, 2004.
4. Apel K and Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55: 373–399, 2004.
5. Aslund F and Beckwith J. Bridge over troubled waters: sensing stress by disulfide bond formation. *Cell* 96: 751–753, 1999.
6. Bae JB, Park JH, Hahn MY, Kim MS, and Roe JH. Redox-dependent changes in RsrA, an anti-sigma factor in *Streptomyces coelicolor*: zinc release and disulfide bond formation. *J Mol Biol* 335: 425–435, 2004.
7. Barbirz S, Jakob U, and Glocker MO. Mass spectrometry unravels disulfide bond formation as the mechanism that activates a molecular chaperone. *J Biol Chem* 275: 18759–18766, 2000.
8. Barford D. The role of cysteine residues as redox-sensitive regulatory switches. *Curr Opin Struct Biol* 14: 679–686, 2004.

9. Berg JM. Zinc fingers and other metal-binding domains. Elements for interactions between macromolecules. *J Biol Chem* 265: 6513–6516, 1990.
10. Bray TM and Bettger WJ. The physiological role of zinc as an antioxidant. *Free Radic Biol Med* 8: 281–291, 1990.
11. Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, and Young RA. Remodeling of yeast genome expression in response to environmental changes. *Mol Biol Cell* 12: 323–337, 2001.
12. Chuang SE and Blattner FR. Characterization of twenty-six new heat shock genes of *Escherichia coli*. *J Bacteriol* 175: 5242–5252, 1993.
13. Collet JF and Bardwell JC. Oxidative protein folding in bacteria. *Mol Microbiol* 44: 1–8, 2002.
14. Colussi C, Albertini MC, Coppola S, Rovidati S, Galli F, and Ghibelli L. H2O2-induced block of glycolysis as an active ADP-ribosylation reaction protecting cells from apoptosis. *FASEB J* 14: 2266–2276, 2000.
15. Cooper CE, Patel RP, Brookes PS, and Darley-Usmar VM. Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem Sci* 27: 489–492, 2002.
16. Cotgreave IA, Gerdes R, Schuppe-Koistinen I, and Lind C. S-glutathionylation of glyceraldehyde-3-phosphate dehydrogenase: role of thiol oxidation and catalysis by glutaredoxin. *Methods Enzymol* 348: 175–182, 2002.
17. Delaunay A, Isnard AD, and Toledano MB. H2O2 sensing through oxidation of the Yap1 transcription factor. *EMBO J* 19: 5157–5166, 2000.
18. Downes CP, Walker S, McConnachie G, Lindsay Y, Batty IH, and Leslie NR. Acute regulation of the tumour suppressor phosphatase, PTEN, by anionic lipids and reactive oxygen species. *Biochem Soc Trans* 32: 338–342, 2004.
19. Finkel T and Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239–247, 2000.
20. Gardner PR and Fridovich I. Superoxide sensitivity of the *Escherichia coli* aconitase. *J Biol Chem* 266: 19328–19333, 1991.
21. Giles GI and Jacob C. Reactive sulfur species: an emerging concept in oxidative stress. *Biol Chem* 383: 375–388, 2002.
22. Giles NM, Giles GI, and Jacob C., Multiple roles of cysteine in biocatalysis. *Biochem Biophys Res Commun* 300: 1–4, 2003.
23. Giles NM, Watts AB, Giles GI, Fry FH, Littlechild JA, and Jacob C. Metal and redox modulation of cysteine protein function. *Chem Biol* 10: 677–693, 2003.
24. Gopalakrishna R and Jaken S. Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* 28: 1349–1361, 2000.
25. Graf PC and Jakob U. Redox-regulated molecular chaperones. *Cell Mol Life Sci* 59: 1624–1631, 2002.
26. Graf PC, Martinez-Yamout M, VanHaerents S, Lilie H, Dyson HJ, and Jakob U. Activation of the redox-regulated chaperone Hsp33 by domain unfolding. *J Biol Chem* 279: 20529–20538, 2004.
27. Graumann J, Lilie H, Tang X, Tucker KA, Hoffmann JH, Vijayalakshmi J, Saper M, Bardwell JC, and Jakob U. Activation of the redox-regulated molecular chaperone Hsp33—a two-step mechanism. *Structure (Camb)* 9: 377–387, 2001.
28. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med* 91: 14S–22S, 1991.
29. Harding MM. The architecture of metal coordination groups in proteins. *Acta Crystallogr D Biol Crystallogr* 60: 849–859, 2004.
30. Helmann JD. The extracytoplasmic function (ECF) sigma factors. *Adv Microb Physiol* 46: 47–110, 2002.
31. Hightower KE and Fierke CA. Zinc-catalyzed sulfur alkylation: insights from protein farnesyltransferase. *Curr Opin Chem Biol* 3: 176–181, 1999.
32. Hochgrafe F, Mostertz J, Albrecht D, and Hecker M. Fluorescence thiol modification assay: oxidatively modified proteins in *Bacillus subtilis*. *Mol Microbiol* 58: 409–425, 2005.
33. Hoffmann JH, Linke K, Graf PC, Lilie H, and Jakob U. Identification of a redox-regulated chaperone network. *EMBO J* 23: 160–168, 2004.
34. Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. *Antioxid Redox Signal* 2: 811–820, 2000.
35. Houry WA, Frishman D, Eckerskorn C, Lottspeich F, and Hartl FU. Identification of *in vivo* substrates of the chaperonin GroEL. *Nature* 402: 147–154, 1999.
36. Hughes KT and Mathee K. The anti-sigma factors. *Annu Rev Microbiol* 52: 231–286, 1998.
37. Imlay JA. Pathways of oxidative damage. *Annu Rev Microbiol* 57: 395–418, 2003.
38. Jacob C, Giles GI, Giles NM, and Sies H. Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew Chem Int Ed Engl* 42: 4742–4758, 2003.
39. Jakob U, Eser M, and Bardwell JC. Redox switch of hsp33 has a novel zinc-binding motif. *J Biol Chem* 275: 38302–38310, 2000.
40. Jakob U, Muse W, Eser M, and Bardwell JC. Chaperone activity with a redox switch. *Cell* 96: 341–352, 1999.
41. Janda I, Devedjiev Y, Derewenda U, Dauter Z, Bielnicki J, Cooper DR, Graf PC, Joachimiak A, Jakob U, and Derewenda ZS. The crystal structure of the reduced, Zn2+-bound form of the *B. subtilis* Hsp33 chaperone and its implications for the activation mechanism. *Structure (Camb)* 12: 1901–1907, 2004.
42. Jaroszewski L, Schwarzenbacher R, McMullan D, Abdubek P, Agarwalla S, Ambing E, Axelrod H, Biorac T, Canaves J, Chiu H, Deacon A, Didonato M, Elsliger M, Godzik A, Grittini C, Grzechnik S, Hale J, Hampton E, Han G, Haugen J, Hornsby M, Klock H, Koesema E, Kreusch A, Kuhn P, Lesley S, Miller M, Moy K, Nigoghossian E, Paulsen J, Quijano K, Reyes R, Rife C, Spraggon G, Stevens R, van den Bedem H, Velasquez J, Vincent J, White A, Wolf G, Xu Q, Hodgson K, Wooley J, and Wilson I. Crystal structure of Hsp33 chaperone (TM1394) from *Thermotoga maritima* at 2.20 Å resolution. *Proteins Struc Func Bioinform* 61: 669–673, 2005.
43. Kang JG, Paget MS, Seok YJ, Hahn MY, Bae JB, Hahn JS, Kleanthous C, Buttner MJ and Roe JH. RsrA, an anti-sigma factor regulated by redox change. *EMBO J* 18: 4292–4298, 1999.
44. Kiley PJ and Beinert H. The role of Fe–S proteins in sensing and regulation in bacteria. *Curr Opin Microbiol* 6: 181–185, 2003.

45. Kim SJ, Jeong DG, Chi SW, Lee JS, and Ryu SE. Crystal structure of proteolytic fragments of the redox-sensitive Hsp33 with constitutive chaperone activity. *Nat Struct Biol* 8: 459–466, 2001.
46. Kim SO, Merchant K, Nudelman R, Beyer WF, Jr., Keng T, DeAngelo J, Hausladen A, and Stamler JS. OxyR: a molecular code for redox-related signaling. *Cell* 109: 383–396, 2002.
47. Knapp LT and Klann E. Superoxide-induced stimulation of protein kinase C via thiol modification and modulation of zinc content. *J Biol Chem* 275: 24136–24145, 2000.
48. Kosower NS and Kosower EM. Diamide: an oxidant probe for thiols. *Methods Enzymol* 251: 123–133, 1995.
49. Kovacic P and Jacintho JD. Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. *Curr Med Chem* 8: 773–796, 2001.
50. Kruszewski M. Labile iron pool: the main determinant of cellular response to oxidative stress. *Mutat Res* 531: 81–92, 2003.
51. Kuge S, Arita M, Murayama A, Maeta K, Izawa S, Inoue Y, and Nomoto A. Regulation of the yeast Yap1p nuclear export signal is mediated by redox signal-induced reversible disulfide bond formation. *Mol Cell Biol* 21: 6139–6150, 2001.
52. Kyselova P, Zourek M, Rusavy Z, Trefil L, and Racek J. Hyperinsulinemia and oxidative stress. *Physiol Res* 51: 591–595, 2002.
53. Lee C, Lee SM, Mukhopadhyay P, Kim SJ, Lee SC, Ahn WS, Yu MH, Storz G, and Ryu SE. Redox regulation of OxyR requires specific disulfide bond formation involving a rapid kinetic reaction path. *Nat Struct Mol Biol* 11: 1179–1185, 2004.
54. Lee J, Spector D, Godon C, Labarre J, and Toledano MB. A new antioxidant with alkyl hydroperoxide defense properties in yeast. *J Biol Chem* 274: 4537–4544, 1999.
55. Li W, Bottrill AR, Bibb MJ, Buttner MJ, Paget MS, and Kleanthous C. The role of zinc in the disulphide stress-regulated anti-sigma factor RsrA from *Streptomyces coelicolor*. *J Mol Biol* 333: 461–472, 2003.
56. Li W, Stevenson CE, Burton N, Jakimowicz P, Paget MS, Buttner MJ, Lawson DM, and Kleanthous C. Identification and structure of the anti-sigma factor-binding domain of the disulphide-stress regulated sigma factor sigma(R) from *Streptomyces coelicolor*. *J Mol Biol* 323: 225–236, 2002.
57. Linke K and Jakob U. Not every disulfide lasts forever: disulfide bond formation as a redox switch. *Antioxid Redox Signal* 5: 425–434, 2003.
58. Liochev SI and Fridovich I. Modulation of the fumarases of *Escherichia coli* in response to oxidative stress. *Arch Biochem Biophys* 301: 379–384, 1993.
59. Maret W and Vallee BL. Thiolate ligands in metallothionein confer redox activity on zinc clusters. *Proc Natl Acad Sci U S A* 95: 3478–3482, 1998.
60. McCall KA, Huang C, and Fierke CA. Function and mechanism of zinc metalloenzymes. *J Nutr* 130: 1437S–1446S, 2000.
61. Montgomery D, Jordan R, McMacken R, and Freire E. Thermodynamic and structural analysis of the folding/unfolding transitions of the *Escherichia coli* molecular chaperone DnaK. *J Mol Biol* 232: 680–692, 1993.
62. Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, and Stadtman ER. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci USA* 98: 12920–12925, 2001.
63. Newman JD, Anthony JR, and Donohue TJ. The importance of zinc-binding to the function of *Rhodobacter sphaeroides* ChrR as an anti-sigma factor. *J Mol Biol* 313: 485–499, 2001.
64. Osorio H, Carvalho E, del Valle M, Gunther Sillero MA, Moradas-Ferreira P, and Sillero A. H2O2, but not menadione, provokes a decrease in the ATP and an increase in the inosine levels in *Saccharomyces cerevisiae*. An experimental and theoretical approach. *Eur J Biochem* 270: 1578–1589, 2003.
65. Paget MS, Bae JB, Hahn MY, Li W, Kleanthous C, Roe JH, and Buttner MJ. Mutational analysis of RsrA, a zinc-binding anti-sigma factor with a thiol-disulphide redox switch. *Mol Microbiol* 39: 1036–1047, 2001.
66. Paget MS and Buttner MJ. Thiol-based regulatory switches. *Annu Rev Genet* 37: 91–121, 2003.
67. Paget MS, Kang JG, Roe JH, and Buttner MJ. SigmaR, an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). *EMBO J* 17: 5776–5782, 1998.
68. Paget MS, Molle V, Cohen G, Aharonowitz Y, and Buttner MJ. Defining the disulphide stress response in *Streptomyces coelicolor* A3(2): identification of the sigmaR regulon. *Mol Microbiol* 42: 1007–1020, 2001.
69. Raman B, Siva Kumar LV, Ramakrishna T, and Mohan Rao C. Redox-regulated chaperone function and conformational changes of *Escherichia coli* Hsp33. *FEBS Lett* 489: 19–24, 2001.
70. Ritz D and Beckwith J. Roles of thiol-redox pathways in bacteria. *Annu Rev Microbiol* 55: 21–48, 2001.
71. Rojkind M, Dominguez-Rosales JA, Nieto N, and Greenwel P. Role of hydrogen peroxide and oxidative stress in healing responses. *Cell Mol Life Sci* 59: 1872–1891, 2002.
72. Rudiger S, Buchberger A, and Bukau B. Interaction of Hsp70 chaperones with substrates. *Nat Struct Biol* 4: 342–349, 1997.
73. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med* 91: 31S–38S, 1991.
74. Spragg RG, Hinshaw DB, Hyslop PA, Schraufstatter IU, and Cochrane CG. Alterations in adenosine triphosphate and energy charge in cultured endothelial and P388D1 cells after oxidant injury. *J Clin Invest* 76: 1471–1476, 1985.
75. Stadtman ER, Van Remmen H, Richardson A, Wehr NB, and Levine RL. Methionine oxidation and aging. *Biochim Biophys Acta* 1703: 135–140, 2005.
76. Storz G and Imlay JA. Oxidative stress. *Curr Opin Microbiol* 2: 188–194, 1999.
77. Storz G, Tartaglia LA, and Ames BN. The OxyR regulon. *Antonie Van Leeuwenhoek* 58: 157–161, 1990.
78. Storz G and Toledano MB. Regulation of bacterial gene expression in response to oxidative stress. *Methods Enzymol* 236: 196–207, 1994.
79. Touati D. Sensing and protecting against superoxide stress in *Escherichia coli*–how many ways are there to trigger *soxRS* response? *Redox Rep* 5: 287–293, 2000.

80. Tsai B, Rodighiero C, Lencer WI, and Rapoport TA. Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. *Cell* 104: 937–948, 2001.
81. Vallee BL and Auld DS. Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* 29: 5647–5659, 1990.
82. Vijayalakshmi J, Mukhergee MK, Graumann J, Jakob U, and Saper MA. The 2.2 A crystal structure of Hsp33: a heat shock protein with redox-regulated chaperone activity. *Structure (Camb)* 9: 367–375, 2001.
83. Wedemeyer WJ, Welker E, Narayan M, and Scheraga HA. Disulfide bonds and protein folding. *Biochemistry* 39: 4207–4216, 2000.
84. Winter J, Linke K, Jatzek A, and Jakob U. Severe oxidative stress causes inactivation of DnaK and activation of the redox-regulated chaperone Hsp33. *Mol Cell* 17: 381–392, 2005.
85. Won HS, Low LY, Guzman RD, Martines–Yamout M, Jakob U, and Dyson HJ. The zinc-dependent redox switch domain of the chaperone Hsp33 has a novel fold. *J Mol Biol* 341: 893–899, 2004.
86. Zhang X, Beuron F, and Freemont PS. Machinery of protein folding and unfolding. *Curr Opin Struct Biol* 12: 231–238, 2002.
87. Zheng M, Aslund F, and Storz G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* 279: 1718–1721, 1998.

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1. Olivier Sénèque, Estelle Bonnet, France Lyne Joumas, Jean-Marc Latour. 2009. Cooperative Metal Binding and Helical Folding in Model Peptides of Treble-Clef Zinc Fingers. *Chemistry - A European Journal* **15**:19, 4798-4810. [CrossRef]
2. Florence Luttringer, Etienne Mulliez, Bernard Dublet, David Lemaire, Marc Fontecave. 2009. The Zn center of the anaerobic ribonucleotide reductase from E. coli. *JBIC Journal of Biological Inorganic Chemistry* . [CrossRef]
3. Klaus-D. Kröncke , Lars-Oliver Klotz . 2009. Zinc Fingers as Biologic Redox Switches?Zinc Fingers as Biologic Redox Switches?. *Antioxidants & Redox Signaling* **11**:5, 1015-1027. [Abstract] [PDF] [PDF Plus]
4. Stephen G. Bell, Bert L. Vallee. 2009. The Metallothionein/Thionein System: An Oxidoreductive Metabolic Zinc Link. *ChemBioChem* **10**:1, 55-62. [CrossRef]
5. Manuel Covarrubias, Aditya Bhattacharji, Jose A. Santiago-Castillo, Kevin Dougherty, Yuri A. Kaulin, Thanawath Ratanadilok Na-Phuket, Guangyu Wang. 2008. The Neuronal Kv4 Channel Complex. *Neurochemical Research* **33**:8, 1558-1567. [CrossRef]
6. Benoît D'Autréaux, Michel B. Toledano. 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nature Reviews Molecular Cell Biology* **8**:10, 813-824. [CrossRef]
7. Koreaki Ito . 2006. Redox Control of Protein Processing: From Electrons to CellsRedox Control of Protein Processing: From Electrons to Cells. *Antioxidants & Redox Signaling* **8**:5-6, 729-730. [Citation] [PDF] [PDF Plus]