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REVIEW

Redox Regulation of Cellular Functions

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Abstract—Maintenance of normal intracellular redox status plays an important role in such processes as DNA synthesis, gene expression, enzymatic activity, and others. In addition, it is clear that changes in the redox status of intracellular content and individual molecules, resulting from stress or intrinsic cellular activity, are involved in the regulation of different processes in cells. Small changes in intracellular levels of reactive oxygen species participate in intracellular signaling. Thiol-containing molecules, such as glutathione, thioredoxins, glutaredoxins, and peroxiredoxins, also play an important role in maintaining redox homeostasis and redox regulation. This review attempts to summarize the current knowledge about redox regulation in different cell types.

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While the medium surrounding living organisms is usually characterized by oxidative conditions, intracellular redox state is maintained in the range of reducing values. Such redox homeostasis is very important for vital cellular functions, and its disruption can be accompanied by increase in the level of reactive oxygen species (ROS), resulting in oxidative damage of lipids, DNA, and proteins. Maintaining normal cellular redox state plays a significant role in such processes as DNA synthesis, gene expression, enzymatic activity, etc. In addition, it is evident that changes in the redox state of intracellular content and individual molecules, resulting from stress or intrinsic cellular activity, are involved in the regulation of different processes in cells.

In connection with this, it is reasonable to give a definition of redox state. There is no strict and generally acknowledged definition of the redox state for complex biological systems. Historically, the term redox state is used for describing the ratio of interconverting oxidized and reduced forms of a specific redox pair. In recent years, this term is used in a broader sense for describing the redox state of cells and other complex biological systems. Thus, the term is less well defined than in the case when it is used for the description of one particular redox pair. In a situation requiring the description of complex systems, the term redox environment is suggested. Redox environment, formed by interconnected redox pairs in biological fluids, organelles, cells, or tissues, is determined by the sum of reduction potential and reduction capacity of these redox pairs. Respectively, the redox state of a redox pair is determined by a half-cell reduction potential (Ehc) and reduction capacity of the pair [1]. The cited work also shows how the Nernst equation can be used for determination of redox state, reviews the most important biological redox pairs determining the intracellular redox environment, and demonstrates the unique role of glutathione in this process.

ROS AS REDOX SIGNALING MOLECULES

The sources of reactive oxygen species, such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), and others, can be either of exogenous or endogenous origin. Generation of ROS involves lysosomes, NADPH oxidases, 5-lipoxygenase, xanthine oxidase, and cytochrome P450. The effect of heavy metal ions, γ-radiation, and UV light is also accompanied by the formation of ROS. One of the main sources of ROS in many cell types is mitochondria. There are several functional ROS producing complexes found in these

Abbreviations: Grxs) glutaredoxins; GSH) reduced form of glutathione; GSSG) oxidized form of glutathione; MAP) mitogen-activated protein kinases; Prxs) peroxiredoxins or thioredoxin peroxidases; Ras) small G-protein; ROS) reactive oxygen species; TR) thioredoxin reductase; Trx) thioredoxin.

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organelles, but the components of the initial and middle segments of the respiratory chain are more active in this respect [2-6].

Due to high reactivity, ROS can damage any macromolecule (proteins, DNA, and lipids); however, the presence of an antioxidant defense system under normal conditions maintains intracellular concentration of antioxidants at a safe level. Under certain conditions, ROS production rate exceeds the rate of its detoxication, resulting in cell damage and death due to oxidative stress. At the same time, at low ROS concentrations participate in the regulation of different functions in eukaryotic cells, such as proliferation, hormone biosynthesis, chemotaxis, oxidative burst, aggregation, apoptosis, and others [7]. Along with the toxic effect, ROS regulatory function is a significant part of a number of pathological conditions [8]. Upon the effect of different cytokines and hormones, eukaryotic cells can stimulate the formation of ROS, using them as messengers for transducing regulatory signals from intercellular signaling molecules and their membrane receptors to intracellular regulating systems involved in the control of gene expression [7, 9-12].

Hydrogen peroxide plays a special role in the regulation of cellular activity. This is associated with relative stability of H_2O_2, which is generated in cells as a metabolic byproduct, and can easily penetrate across biological membranes, thus participating in both intracellular and intercellular signaling. At the same time, the use of H_2O_2 as a signaling molecule by cells involves a number of problems [13]. One of the ways for H_2O_2, as well as for other ROS, to participate in regulation is to change intracellular redox state due to oxidation of molecules (for instance, glutathione). Potentially, these changes can nonspecifically influence a large number of metabolic reactions. However, the capacity of redox buffer involving glutathione (GSH) is very high, and a large amount of oxidant is required to substantially decrease the GSH/GSSG ratio. At these concentrations, damage to a large number of macromolecules is inevitable, and it is, per se, the state of oxidative stress. Intracellular H_2O_2 concentration above 1 μM is toxic to cells [14] and cannot be used for regulatory purposes. On the other hand, at low concentrations this oxidant should react rapidly and specifically with its target with a rate exceeding the rate of its reduction by the components of the antioxidant system (catalases, peroxidases, GSH). From these considerations and from experimental data it follows that, first, H_2O_2 concentrations used by cells for regulatory purposes are within a narrow range (1-700 nM), second, H_2O_2 interaction with target is not accompanied by dramatic changes in cell redox state, and third, redox-regulated pathways should include sensor molecules containing sites that are very sensitive to low ROS concentrations. It is also important that the oxidized state in this sensor site be maintained during a time that is sufficient for signal transduction to other molecules [13, 14].

Recently a model of the interaction of H_2O_2 with cellular thiol residues has been suggested, according to which thiol-containing H_2O_2 sensors are considered as archetypal redox signaling modules, where kinetic (not thermodynamic) factors control the redox reactions *in vivo*, determining the pathways for oxidation of cysteine residues and enabling high sensitivity of redox sensors to H_2O_2 [15].

Available data indicate that high rates of interaction between sensor molecules and H_2O_2 are provided by the presence of sensor sites containing a residue that is able to react with antioxidant and a residue that forms a specific environment around the first one. A significant role in redox regulation belongs to cysteine residues (Cys-SH) in proteins. During the interaction with $NO^{\cdot}$ and H_2O_2, they can undergo stepwise oxidation to cysteine sulfenic (Cys-SOH), cysteine sulfinic (Cys-SO_2H), and cysteine sulfonic (Cys-SO_3H) acids. It is now known that such modification of cysteine residues plays an important role in cell signaling. The rate of interaction of protein thiols with oxidants significantly increases upon the ionization of the former, since thiolates (Cys-S^-) are more nucleophilic than the respective protonated forms. The degree of thiol ionization, its sensitivity to oxidation, and specificity to different oxidants are largely determined by amino acid environment. The importance of environment is evidenced by the fact that the rate of reaction of thiolate anions with H_2O_2 varies in different proteins from ~10 to ~10^6 $M^{-1}\cdot sec^{-1}$ [16]. The formation of sulfenate is of special significance. Depending on the conditions and a particular protein, sulfenate can be not only further oxidized to sulfinate, but also reduced back to thiol, can participate in the formation of intra- and intermolecular bonds including glutathionylation, or remain in the stable state. This provides broad possibilities for participation of reactions, associated with the formation of sulfenate and its conversions in redox regulation. The role of protein oxidative modification is illustrated below in more detail by way of the example of peroxiredoxins.

ADAPTATION TO HYPOXIA AND O_2 SENSORS

In aerobic organisms, oxygen is necessary for efficient ATP synthesis during the process of oxidative phosphorylation; on the other hand, the ability of cells and tissues to maintain oxygen homeostasis is tightly linked to ROS generation. Paradoxically, during hypoxia (~1.5% O_2) a dramatic increase in ROS production by mitochondria is observed. Prolonged oxygen starvation can result in energy crisis, irreversible destruction of mitochondria, and cell death. Hypoxia is one of the inducers of apoptosis. It is not surprising that the ability to sense the changes in oxygen content and getting adapted to them is critical for vital functions of different cell types in norm and pathology [17, 18]. The mechanisms involved in sensing

the changes in oxygen content in animal, yeast, and bacterial cells are briefly reviewed below. The role of these mechanisms in plant cells is discussed in a recent review [18].

One of the key factors involved in sensing O_2 changes and elucidating an adaptive response to the decrease of its content in animal cells is a transcriptional regulator HIF-1 (hypoxia-inducible factor) [17, 19]. HIF-1 is a heterodimer consisting of the oxygen-sensitive α-subunit (HIF-1α) and constitutively expressed β-subunit (HIF-1β). Under normal oxygen content, the HIF-1α protein is constitutively expressed, but is rapidly cleaved by proteasomes. HIF-1α cleavage is stimulated by hydroxylation of a specific proline residue in this protein by enzymes belonging to the family of O_2- and oxoglutarate-dependent dioxygenases, followed by polyubiquitination. Prolyl hydroxylase activity is inhibited during hypoxia, which subsequently leads to stabilization of HIF-1α, its accumulation in the nucleus, dimerization of HIF-1β, and activation as a transcriptional factor, controlling the expression of over 70 genes. HIF regulation also includes hydroxylation of asparagine residue in its N-terminal domain with involvement of other oxoglutarate-dependent dioxygenase; the consequence of this is weakened interaction between HIF-1 and transcription co-activators. An important property of HIF-1α prolyl hydroxylases is their ability to utilize molecular oxygen as a substrate during hydroxylation of proline residues and high K_m for O_2 (230-250 μM). Consequently, oxygen level is a factor limiting the rate of reaction catalyzed by HIF-1α prolyl hydroxylases, which allows these enzymes to function as O_2 sensors during hypoxia response [17, 19].

A model according to which the increase in ROS production during hypoxia can contribute to stabilization of HIF-1α, and, respectively, to HIF-1α-dependent activation of gene expression has also been suggested. In this model, mitochondria play a role of O_2 sensor [20, 21]. According to another model, hypoxic response is mediated by O_2-sensitive ion channels with involvement of ROS produced by mitochondria or NADPH oxidase, which are postulated to be O_2 sensors [22]. It should be noted that there is still no agreement regarding these models, as well as some others, and thus the problem of O_2 sensors still remains open [17, 21, 22].

In yeast, O_2 sensors and mechanisms coupling them to signaling pathways are arranged in a slightly different way. During their lifecycle, yeast cells, in particular *Saccharomyces cerevisiae*, can pass through such physiological conditions as aerobic enzymatic growth, anaerobic enzymatic growth, and aerobic growth with respiratory metabolism (respiratory phase). This requires the presence of genetic programs in yeast that coordinate the expression of a large number of genes in response to changing conditions, including changes in oxygen concentration. A key role in functioning of the O_2 sensor in *S. cerevisiae* belongs to heme [23, 24]. Activity of transcription factors in response to changes in oxygen concentration in the medium is not regulated through direct interaction with oxygen, but is mediated by the interaction with heme, the concentration of which is regulated in a redox-independent manner and is proportional to oxygen concentration above 0.1 mM. Heme concentration is regulated by the rate of its synthesis by several consecutive mitochondrial enzymes, including enzymes that use O_2 as a substrate. The most significant of these is ferrochelatase, catalyzing the last step in heme biosynthesis. It is not clear how this enzyme is controlled by oxygen, but it is likely to be performed indirectly, since O_2 is not the substrate [25]. Changes in oxygen concentration result in a significant change in the level of heme, playing the role of activator of Hap1 transcription factor, which in turn activates the transcription of multiple genes necessary for adaptation to aerobic conditions and hypoxia. On the other hand, decrease in Hap1 activity decreases the production of another transcription factor, Rox1, suppressing the genes necessary for anaerobic metabolism [23, 24].

Interestingly, distinct cycles are observed at high cell density in *S. cerevisiae* growing in continuous culture with forced aeration; in each of them respiratory burst, characterized by maximal oxygen consumption rates and ATP production, is succeeded by a reduction phase with dramatic decrease in respiration intensity (despite the aeration of the culture) [26]. These oscillations occur independently of glycolysis and cell cycle, and they are coupled to periodic increase in H_2S level, inhibiting the respiratory chain [27]. Simultaneously with respiratory oscillation, phase changes in the concentrations of NADPH and GSH were observed. Addition of GSH or GSSG disrupts the oscillations and inhibits respiration, which indicates the important role of intracellular redox state in the regulation of oscillations [28]. Studies using microchips revealed that synchronically with metabolic events there are oscillations in transcription of a large number of genes, organized in three clusters, two of which exhibit maximal expression during the reduction phase, and one during the respiratory phase of the cycle. It was also found that processes associated with DNA replication occur, mostly, in the reduction phase. Therefore, DNA synthesis, being sensitive to oxidative damage, is separated from ROS producing processes not only spatially, but also temporally [29, 30].

One of the main transcriptional regulators in the bacterium *Escherichia coli*, controlling the switch between aerobic and anaerobic metabolism, is the cytoplasmic protein FNR consisting of a sensory and DNA binding domains. Under anaerobic conditions, the signal from the sensory domain to the DNA binding domain is transduced due to conformational changes resulting in the formation of homodimers. Being in this state, FNR binds to DNA and suppresses transcription of the genes involved in anaerobic electron transport and metabolism

[31]. The sensor domain in the active (anaerobic) state contains an [4Fe-4S] cluster. In the presence of oxygen, FNR inactivation takes place, resulting from direct interaction of oxygen with the cluster and its conversion into [2Fe-2S] state. This process occurred even at very low intracellular O_2 concentrations (1 μM). In this state, FNR is not capable of dimerization and is not active. It was reported that reactivation of FNR can involve GSH as a reducing agent [32].

Another system controlling gene expression under decreased oxygen pressure in *E. coli* is the ArcAB system. It includes membrane-bound ubiquitin-dependent ArcB kinase and ArcA protein. Under O_2 deficiency, oxygen-sensitive ArcB kinase undergoes autophosphorylation and then phosphorylates ArcA, conferring the ability to bind DNA and regulate a large number of operons controlling carbon metabolism and intracellular redox state [33]. ArcB kinase activity is inhibited under aerobic conditions. ArcB sensitivity to oxygen is not coupled to direct interaction of protein with O_2, but results from response to the decreased electron flow through the respiratory chain [34]. The molecular mechanism underlying the link between the respiratory chain and inhibition of ArcB kinase activity under aerobic conditions includes the formation of intermolecular disulfide bonds due to specific oxidation of redox active cysteine residues in the sensor by quinones [35]. Performing regulatory functions, FNR and ArcB interact with each other in a complex way. It should be noted that ArcB, like the above-mentioned HIF-1, belongs to a large protein group containing so-called PAS domains. As a rule, these proteins are involved in signal transduction upon response of different cell types to changes in redox state, oxygen concentration, energy metabolism, and so on [36].

GLUTATHIONE, THIOREDOXINS, GLUTAREDOXINS

It now becomes increasingly clear that the major role in redox regulation is played by modification of sulfhydryl groups in signal proteins, which, from one hand, involves ROS, and from the other such thiol-containing molecules as glutathione (GSH), glutaredoxins (Grxs), thioredoxins (Trxs), and peroxiredoxins (Prxs).

Glutathione. GSH is widespread in various types of cells; it is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine), a specific feature of which is the presence of a γ-glutamyl bond and a free sulfhydryl group (SH-). Sulfhydryl group can serve as an electron donor, conferring reducing properties and ability to remove free radicals to glutathione. Due to the high glutathione level (up to 10 mM) and the fact that the concentration of reduced form (GSH) is 1-2 orders of magnitude higher than the concentration of the oxidized form (GSSG), glutathione is the major redox buffer in cells [37]. Glutathione level in cells is mainly determined by the rate of its synthesis (on one hand) and the rate of export and conjugation (on the other hand). The reduction of GSSG is catalyzed by a flavoenzyme, glutathione reductase. An important role in metabolism is played by γ-glutamyl transpeptidase catalyzing the initial step in glutathione degradation. As an antioxidant, GSH plays a key role in protecting eukaryotic cells from oxidative stress, being an electron donor for the enzyme glutathione peroxidase. In most cell types, GSH is involved in detoxication of foreign compounds, catalyzed by GS-S-transferases. Paradoxically, under certain conditions GSH can act as a prooxidant [38, 39].

One of the functions of glutathione in redox regulation is associated with the formation of mixed disulfides between protein thiol groups and glutathione. This process is called glutathionylation, contrary to a broader term S-thiolation, implying the formation of mixed protein disulfides with GSH, cysteine, and other thiols including non-physiological ones [40, 41]. The process is initiated by oxidation of protein SH-groups to sulfenate by ROS, followed by formation of mixed disulfide in the presence of glutathione. It is assumed that one of the functions of glutathionylation is the protection of protein SH-groups from further irreversible oxidation. The biological importance of glutathionylation is confirmed by the fact that in aging organisms with decreased level of antioxidants the intensity of irreversible oxidation of sulfhydryl groups in proteins is increased [42]. Many proteins undergoing glutathionylation in the presence of ROS also undergo glutathionylation in the presence of NO, indicating the integrative role of glutathionylation in the association of two independent signaling pathways, linked to ROS or NO, in a common functional response to different regulatory signals [40].

Dethiolation of glutathionylated proteins can occur either non-enzymatically or enzymatically with the involvement of thioredoxin, glutaredoxin, or protein disulfide isomerase [43, 44]. There is evidence proving that in the latter case the enzymes are able to shift equilibrium between GSH and mixed disulfides in both directions [45]. Recently is has been discovered that Cys72 in thioredoxin can undergo glutathionylation, which influences its enzymatic activity and functions [46]. Thioredoxin glutathionylation is of special interest also as an example of structural interaction between glutathione and thioredoxin redox systems of a cell. Several metabolic pathways and regulatory factors have been revealed, whose activity is modulated by glutathionylation. They include transcription factors AP-1 and NF-κB, ubiquitin-dependent proteolytic protein degradation, cAMP, cAMP-dependent protein kinase, etc. [43, 47-50].

Thioredoxins. Thioredoxins (Trxs) are a family of small (11-12 kD) thermostable proteins containing redox active region Cys-X1-X2-Cys, which can be reversibly oxidized and reduced. The ability of thioredoxin to reduce protein disulfides is important for its role in redox

regulation. Oxidized thioredoxin is reduced in a NADPH-dependent reaction catalyzed by flavoenzyme thioredoxin reductase (TR). One of the main functions of the Trx/TR redox system is its participation in DNA synthesis. Thioredoxin was first discovered as an electron donor for ribonucleotide reductase, the enzyme that catalyzes the reduction of ribonucleotides to deoxyribonucleotides. This reaction is the first step in DNA synthesis and an important step in cellular proliferation [51].

In contrast to glutathione, intracellular concentration of thioredoxin is very low. At the same time, its reducing activity toward transcription factors is approximately 1000 times higher than in the case of GSH, thus explaining why thioredoxin is more specific as a reductant for redox-regulated cascades [52]. It is interesting that *E. coli* thioredoxin 1, exported into the periplasm, acquires the properties of thiol oxidase [53]. Many peroxiredoxins, catalyzing the decomposition of hydrogen peroxide, are reduced by thioredoxin [54].

Glutaredoxins (Grxs) are similar to thioredoxins in structure and size; they also contain a redox active Cys-X1-X2-Cys sequence. Contrary to the Trx/TR system, glutaredoxin lacks a specific reductase for reduction of its oxidized form; this role belongs to the GSH/glutathione reductase system. An important property of glutaredoxins is their ability to reduce mixed disulfides. In this connection, glutaredoxin reduces the mixed disulfide (protein-SSG), forming Grx-GSH intermediate, which is subsequently reduced to the second glutathione molecule [51]. Involvement of glutaredoxin in reversible protein-S-glutathionylation makes this protein an important component of redox regulation of cellular activity [43].

PEROXIREDOXINS

In the recent years, it has been demonstrated that peroxiredoxins play an important role in redox regulation. Peroxiredoxins or thioredoxin peroxidases (Prxs) include a large family of multifunctional thiol-containing peroxidases found in a wide range of living organisms from bacteria to mammals [55, 56]. Prxs are antioxidant enzymes, the main function of which is reduction and detoxication of H_2O_2, organic hydroperoxides, and peroxynitrite. Being antioxidant enzymes, peroxiredoxins perform the same function as catalases and glutathione-dependent peroxidases; however, their catalytic activity is significantly lower than that of the latter. Nevertheless, a number of data provide evidence about the important role of Prxs in antioxidant defense of different cell types [57, 58].

To be able to perform peroxide reduction, Prxs contain redox active cysteine residues in their catalytic sites. By the number and location of the redox active cysteines, Prxs can be divided in three classes: typical 2-Cys, atypical 2-Cys, and 1-Cys peroxiredoxins. For all three classes, in the first step of the catalytic cycle the "peroxidative" cysteine of the catalytic site (Cys-S_pH) located at the N-terminus of the protein attacks peroxide and becomes oxidized to cysteinesulfenic acid (Cys-S_pOH). In 2-Cys peroxiredoxins, there is a second "resolving" redox active cysteine residue (Cys-S_rH) located at the C-terminus. Typical 2-Cys peroxiredoxins are obligate homodimers containing two identical active sites. In the second step of the peroxidase reaction, Cys-S_pOH from one subunit interacts with Cys-S_rH cysteine from the other subunit, forming a stable intersubunit disulfide bond. The catalytic cycle is completed after reduction of this bond with the involvement of one of the disulfide reductases (thioredoxin, AhpF, AhpD, and others) [56]. In atypical 2-Cys peroxiredoxins, the peroxidative and resolving cysteines are included in the same polypeptide; in the course of the peroxidase reaction, they form, respectively, intermolecular disulfide bond, reduced by thioredoxin [59]. 1-Cys peroxiredoxins contain only peroxidative cysteine, whose oxidation by peroxide also results in the formation of sulfenate, reduced by a thiol-containing donor. This donor can be GSH, lipoic acid, or cyclophilin. An important feature of many typical 2-Cys Prxs is their ability for redox-sensitive oligomerization, where the oxidized and reduced enzyme forms exist predominantly as decamers (whereas the disulfide-containing forms exist as dimers). It should be noted that the formation of sulfenic acid upon the effect of H_2O_2 is typical not only for Prxs, but also to many other proteins localized in different cell compartments [60, 61].

Regulation of peroxiredoxin activity is performed on the level of gene expression and by posttranslational protein modification [58]. In the first case, the expression of genes encoding Prxs is stimulated by different factors, inducing oxidative damage. Besides redox-dependent oligomerization, posttranslational mechanisms for the regulation of peroxiredoxin activity include protein phosphorylation, specific proteolysis, and effects of ligands [56, 58].

In the recent years, it has been determined that in addition to antioxidant function, peroxiredoxins play an important role in redox signaling in eukaryotic cells. The important feature, allowing some peroxiredoxins to participate in redox regulation of cell activity, is the ability of cysteine thiols in active sites to overoxidation. As a consequence of the fact that sulfur atoms in cysteines able to form disulfide bond (see above) are located relatively far from each other (~13 Å), the formation of disulfide occurs slowly, and sulfenic intermediate can under certain conditions be further oxidized to cysteine-sulfinic (Cys-SO_2H), and even to sulfonic (Cys-SO_3H) acid, leading to the inhibition of peroxidase enzyme activity, and, respectively, to the increase in intracellular H_2O_2 level [56]. The sulfination reaction is reversible, and there are ATP-dependent enzymes catalyzing the reduction of sulfinic acid in yeast and mammals. The *Saccharomyces*

cerevisiae enzyme, reducing sulfinite in Tsa1 peroxiredoxin, was called sulfiredoxin (Srx1) [62]. In mammals, proteins with similar functions are involved in the reduction of Cys-SO_2H in 2-Cys peroxiredoxin [63-65]. In human cells, cysteine sulfinyl reductase activity is exhibited by sestrins. These proteins participate in cell defense against ROS and are regulated by p53 factor—a tumor suppressor and apoptosis inducer. Sestrins contain a domain homologous to the enzyme AhpD, catalyzing the reduction of disulfide bonds in bacterial peroxiredoxin AhpC [63].

It is supposed that sulfenylation and reversible sulfinylation of peroxiredoxins in eukaryotes can modulate the level of H_2O_2 in cells and tissues, which allows these proteins to play a role of the flood gate, preventing accumulation of toxic oxidant concentrations under normal conditions. Upon the increase in oxidant concentration above a certain level, protein inactivation results in the closure of the sluice gate, and, respectively, in the increase in H_2O_2 concentration up to the level necessary for functioning as a secondary messenger in H_2O_2-dependent signaling pathways [56, 60, 66]. Taken together, such features of peroxiredoxins as relatively high rate of interaction with H_2O_2, reversibility of redox transformations, and capability of redox-sensitive conformational changes determine the ability of these proteins to perform antioxidant and signaling functions simultaneously. The latter function was acquired by eukaryotic peroxiredoxins in the course of evolution, since in bacteria they play only the antioxidant role, participating in reduction of endogenous H_2O_2 [57].

EXAMPLES OF REDOX REGULATION IN EUKARYOTES

Protein kinases and phosphatases. A large number of signaling pathways regulated by ROS are known. Among these, the pathways based on phosphorylation and dephosphorylation of proteins by specific kinases and phosphatases, triggering the activation of transcription factors, are the most studied [11, 67]. Redox regulation can be performed at different levels of signaling pathways from a receptor to the nucleus [10]. The role of ROS is best shown for mitogenic stimulation of cells. Growth factors and cytokines initiate multiple signaling pathways involving phospholipase C, different families of tyrosine kinases, and mitogen activated protein (MAP) kinases. These pathways are controlled by the small G-protein Ras. Ras plays an important role in ROS generation; cells overexpressing Ras start producing ROS constitutively and are able to pass through the cell cycle in the absence of growth factors [68]. Many growth factor receptors possess tyrosine kinase activity and can be activated by ROS [10]. The link between the effect of ROS and phosphorylation is well established for the family of serine/threonine protein kinases (protein kinases B and C) and serine/threonine and tyrosine phosphatases [69]. The most detailed studied is the mechanism of activation of MAP kinases, mediated by redox-sensitive regulation of ASK1 kinase [70]. ASK1 is inhibited after interaction with thioredoxin [70, 71]. The increase in ROS concentration leads to oxidation of SH-groups in thioredoxin and its dissociation from the complex with ASK1, which is then multimerized with the formation of active form, stimulating apoptosis. This case is interesting since the interaction between thioredoxin and ASK1 occurs without the involvement of protein kinase cysteines, and, respectively, without changes in thioredoxin redox state. Associated thioredoxin acts in this case as a regulatory prosthetic group of ASK1–Trx complex, functioning as a redox sensor and molecular switch [67].

MAP kinases at the next levels of regulation, for instance JNK, also can be activated by ROS. Due to the formation of dimeric complex with redox-sensitive glutathione-S-transferase (GSTp), JNK activity is maintained at a low level even in the presence of high concentrations of growth factors. Upon treatment with H_2O_2 or UV irradiation, the GSTp/JNK complex is dissociated with the formation of GSTp dimer and JNK active form [72]. Finally, downstream transcription factors (AP-1 and NF-κB) at the end of these signaling pathways undergo redox regulation themselves [47, 48].

Transcription factors. Several dozens of redox-regulated transcription factors are now known [73]. NF-κB controls the expression of many dozens of genes, whose effect is directed toward increasing cell resistance against stress conditions, suppression of apoptosis, and regulation of immunity [74]. The latent form of NF-κB (the most widespread kind of which is a heterodimer of two subunits, p50 and p65) is located in the cytoplasm and is associated with IκB-α inhibitor. During activation by different effectors, IκB-α becomes phosphorylated, and dissociates from the complex with NF-κB. The free NF-κB factor is transferred into the nucleus and activates the expression of genes containing regulatory κB elements. Activation of NF-κB is facilitated by oxidative conditions in the cytoplasm; increase in intracellular concentrations of GSH and reduced thioredoxin prevents this process [75]. However, reducing conditions in the nucleus are required for NF-κB binding to DNA. It was demonstrated in *in vitro* experiments that oxidation of Cys62 in the DNA-binding domain of p50 subunit is accompanied by the formation of mixed disulfide with GSH and inhibition of DNA binding activity [48]. Regulation of NF-κB redox status in the nucleus involves thioredoxin and redox factor 1 (Ref-1, a unique molecule for the nucleus; its reduction is coupled to thioredoxin) [76].

Transcription factor AP-1, consisting of the two subunits c-Fos and Jun, controls the expression of many genes implicated in cellular proliferation. Oxidative conditions in the cytoplasm facilitate the increase in its activity due to enhancement of *c-fos* and *c-jun* transcription,

resulting from ROS-sensitive activation of JNK and ERK protein kinases [77]. The increase in intracellular GSH inhibits the expression of *c-fos* and *c-jun*, as well as the activation of AP-1 by different stimuli. As in the case of NF-κB, binding of AP-1 to DNA requires oxidative conditions in the cell nucleus. Redox sensitivity is due to the presence of a single cysteine residue in the DNA-binding domain of the c-Jun protein, forming a disulfide with GSH upon the decreased ratio of GSH/GSSG, thus preventing its binding to DNA [47]. The redox state of AP-1 is regulated by Ref-1 and thioredoxin [78]. Oxidative inactivation of transcription factors AP-1 and NF-κB occurs under higher level of ROS than the one enabling the activation of these factors by MAP kinases. Besides AP-1 and NF-κB, a number of transcription factors lose their DNA binding activity after the oxidation of crucial cysteine residues in DNA-binding domains. These proteins include p53, NF-1, Sp1, USF, and others [79, 80]. Since the products of many genes regulated by these transcription factors are involved in defense against stress, one can expect that cell defense systems become weakened starting from a certain threshold ROS level, whereas apoptosis-inducing reactions become increasingly stimulated [11].

There is now a large body of data indicating the importance of intracellular redox state in the regulation of such physiological processes as proliferation, differentiation, cell aging, and apoptosis [81-84]. Based on the literature, there is a summary given in the study [1] providing evidence of a correlation between reduction potential of the GSH/GSSG pair and biological cell status: thus, Eh ≈ −240 mV corresponds to proliferation; Eh ≈ −200 mV to differentiation, and Eh ≈ −170 mV to apoptosis. The link between intracellular redox state and phases of the mitotic cycle has been found a relatively long time ago [1]; a number of recent investigations confirms the assumption that changes in the levels of endogenous ROS and thiol-disulfide status can play a significant role in mitogenic stimulation [85-87]. This is proved by data on cyclic changes in thiol-disulfide status in synchronously dividing cultures of different cell types [1, 88, 89].

Data indicating the possibility of modulating the proliferation of both normal and tumor cells by artificial change in intracellular redox state are of special interest. It was reported that the Eh value in normal proliferating fibroblasts and in proliferating fibrosarcoma cells are noticeably different [90]. It is supposed that by optimizing the dose of an exogenous agent able to shift intracellular Eh, one can inhibit the proliferation of tumor cells with no effect on normal cells. It is probable that some inhibitors of tumor cell growth act according to just this mechanism [91-93]. At present, the search for specific regulatory molecules that can act as redox regulation targets during the normal cell cycle is ongoing. These targets may include cyclin D1, p27, Rb, and other regulatory proteins in which the cysteine residues can participate in thiol-dependent intra- and intermolecular reactions [86].

Despite the fact that the correlation between intracellular redox state and phases of the cell cycle is reliably determined, the cause and effect relationship between the redox state and activity of the regulatory factors, determining the progression of the cell cycle *in vivo*, remain mostly uncertain. This problem also remains unsolved for many other processes, where the changes in thiol-disulfide status, ROS levels, and the entire intracellular redox state are observed. Many cellular processes regulated by ROS are simultaneously accompanied by the modulation of concentration and redox state of such thiol-containing molecules as glutathione and thioredoxin. On the other hand, artificial changes in intracellular redox state upon the depletion of glutathione or addition of reductants can modulate the activity of transcription factors, proliferation, and apoptosis [10]. Now it is evident that ROS and cell redox systems are two sides of the same process, two equal participants during transduction of regulatory signals, both of which can be actively changed by the cells themselves.

Apoptosis. Apoptosis is one of the most important processes in living cells and serves as a demonstrative example, being controlled with involvement of redox regulation [52, 94-96]. One of the most important components in the reaction cascade leading to apoptosis is mitochondria. A large amount of data has accumulated indicating the existence of a relation between ROS generation by mitochondria and apoptosis. Mitochondria can be either targets for regulatory molecules in the reaction cascades, leading to apoptosis, or generators of ROS used as signaling molecules in these cascades [3, 4]. In mammals, one of the key factors involved in the regulation of ROS production and apoptosis is p66Shc protein. The p66 is controlled by p53 protein [97, 98], one of the multiple functions of which is the induction of apoptosis. Data elucidating the mechanisms mediating p66 involvement in ROS generation by mitochondria have been published recently [99]. According to these data, p66 is a redox enzyme, which (after activation by proapoptotic signals) generates mitochondrial ROS as signaling molecules for apoptosis, using reducing equivalents from the mitochondrial electron transport chain. In the course of these reactions, p66 oxidizes cytochrome *c* and catalyzes the reduction of O_2 to H_2O_2, which, in turn, induces opening of nonspecific channels and apoptosis.

Mouse $p66^-$ mutants are more resistant to apoptosis induced by such factors as H_2O_2, UV irradiation, ischemia, and others. The most striking feature of these mutants is an increased lifetime compared to wild type species [97]. It is also noteworthy that p66 is involved in negative regulation of proteins belonging to the family of Forkhead transcription factors (FOXO), which, like p66, participate in the regulation of life span. It was shown that the activity of FKHRL1 factor in $p66^-$ cells is increased, and redox-sensitive Forkhead inactivation is decreased

[100]. One of the known functions of Forkhead factors is linked to the activation of Mn-superoxide dismutase expression, making these factors play an important role in antioxidant cell defense. These results demonstrate the interaction between three different elements related to aging—Forkhead and p66 factors and ROS [100]. Generally, these data indicate the key role of ROS, and mitochondria as the main ROS producers, in such fundamental processes as apoptosis and aging [101].

The role of ROS redox counterparts, antioxidant systems, in apoptosis is not less important. One of the early events during apoptosis is the efflux of reduced glutathione from cells. GSH efflux occurs across the intact cytoplasmic membrane through specific channels, and it can be blocked by addition of GSH transport inhibitors. Decrease in glutathione efflux results in a lower degree of apoptosis [95, 102]. GSH efflux causes the disruption of redox balance and can ultimately lead to oxidative stress that is not directly associated with ROS production. This, in turn, can induce changes in the activity of proteases implicated in apoptosis. It is known that the activity of some proteases can be modulated by oxidation or glutathionylation of a cysteine residue [103, 104].

Stimulation of apoptosis by three different inducers in experiments with HepG2 cells was accompanied by release of GSH into the medium during the time between activation of proteases and nonspecific cell damage typical for apoptosis. The authors concluded that the recovery of normal GSH level after stimulation of apoptosis has no effect on caspase activity and nuclear morphology and, because of this, cannot protect the cells from apoptosis. At the same time, prevention of GSH release can have a protective effect on the plasma membrane [105]. An alternative mechanism for redox regulation of apoptosis can involve the effect of GSH on the multi-ion thiol-sensitive mitochondrial channel [106].

It has been demonstrated that apoptosis of HIV-infected cells is accompanied by weakening of antioxidant defense, and increase in intracellular GSH level can block the apoptosis [107]. Special interest is aroused by the discovery of a connection between anti-apoptotic BCL-2 protein and glutathione metabolism [52, 108]. BCL-2 possesses a unique ability to block the apoptosis induced by different cytotoxic agents. It is assumed that BCL-2 acts as a regulator of activity of many channels and pores; in particular, its association with multi-ion channel in mitochondria and nuclear pore in nuclei has been shown [108]. The studies have indicated that BCL-2 control is mediated by GSH, since the depletion of GSH in BCL-2-overexpressing cells restored their sensitivity to apoptosis [109]. BCL-2 expression increased the intracellular GSH level, inhibiting its release from cells [110], and had an effect on redistribution of GSH between the cytoplasm and nucleus, increasing the concentration of GSH in the nucleus [111]. The increase in GSH concentration in the nucleus has a considerable physiological importance, since, as mentioned above, many transcription factors are sensitive to redox changes due to redox regulation of their DNA-binding domains. In this connection, it should be pointed out that another reductant, thioredoxin, is transferred from the cytosol into the nucleus in response to oxidative stress, and it regulates gene expression through the Ref-1 redox protein [112]. It has been demonstrated that submicromolar concentrations of thioredoxin are able to block apoptosis, probably by inducing BCL-2 [113]. Therefore, it is not only the ROS level being modulated during the redox regulation of apoptosis, but also the concentration of intracellular reductants.

Of special interest are data regarding the role of ROS and thiol status in the induction of apoptosis [14]. The authors showed that H_2O_2-induced apoptosis in Jurkat T-cells was observed at intracellular concentration of the oxidant from $7 \cdot 10^{-7}$ to $30 \cdot 10^{-7}$ M; above this level necrosis was observed. It is noteworthy that these concentrations are slightly higher than the physiological level determined for intracellular H_2O_2 (10^{-8}-10^{-7} M) [114]. Cell treatment resulting in change in glutathione status (decrease in its concentration, oxidation or alkylation) made the cells more sensitive to H_2O_2-induced apoptosis but did not change the threshold and saturation H_2O_2 concentrations that induced apoptosis. According to the authors' opinion, these data indicate that the induction of apoptosis by hydrogen peroxide indeed takes place in living cells, and the processes inducing apoptosis under these conditions are associated with Fenton-type reactions, and do not depend on cellular thiol status.

Along with the data indicating the positive role of glutathione in cell defense against apoptosis, results have been published providing evidence that GSH depletion can inhibit apoptosis, switch cells from the apoptotic pathway to necrotic, and prevent caspase activation [115-118]. It appears that the role of GSH in apoptosis is complex and depends on the cell type and inducer [52].

Extracellular thiols and ROS. There is significant data accumulated on thiol-containing proteins localized on the cell surface and in extracellular fluid, which can undergo redox modifications, responding to changes in the redox state of the cellular environment. These proteins include receptors, enzymes and transport system elements. Changes in their activity can influence such cell functions as neurotransmission, secretion, sensitivity to toxicants, immunity, and (indirectly) such processes as apoptosis, proliferation, and differentiation [119]. The signal can be transduced from the outer to inner environment by way of thiol-disulfide exchange between thiol residues passing through the plasma membrane [69]. For instance, it has been demonstrated that change in extracellular thiol-disulfide state can modulate the rate of cell proliferation through the interaction with growth factor signaling pathways independently of intracellular glutathione.

One of the putative mechanisms for redox regulation includes the formation of extracellular ROS as byprod-

ucts of the reaction occurring with the participation of GSH and membrane γ-glutamyl transpeptidase. This enzyme plays a key role in glutathione metabolism, catalyzing its cleavage to cysteinylglycine and glutamate. Formed H_2O_2 can freely permeate across the membrane and have an effect on redox-sensitive molecules. Since the release and degradation of glutathione occur constantly, glutathione catabolism can be a source of discontinuous H_2O_2 current, being a proliferating and antiapoptotic signal ("life signal") [38, 39]. At the same time, cysteinylglycine, being a stronger reductant than GSH, can participate in extracellular detoxication of electrophilic compounds [120].

OXIDATIVE STRESS

The mechanisms by which cells sense the level of antioxidants and activate anti-oxidant defense systems are the most studied in yeast and bacteria [15, 121-124].

In *S. cerevisiae*, a H_2O_2 sensor switches on the oxidant receptor Orp1 and yeast protein AP-1 (Yap1). Yap1-binding protein (Ybp1) is also necessary for signaling transduction [125, 126]. Orp1 is a protein with a molecular weight of 20 kD and amino acid sequence homologous to that of GPx peroxidase family, bearing three cysteine residues. Apart from the major function, participation in redox regulation, Orp1 can catalyze the reduction of peroxides; however, due to low intracellular protein concentration this function is not significant for cellular activity.

Yap1 is a transcription factor whose oxidation by H_2O_2 activates the genes encoding the elements of antioxidant defense [125]. The activation begins in one minute and lasts for 30-45 min. About 100 μM H_2O_2 is sufficient for oxidation of half of Yap1 molecules. Yap1 activation involves two cysteine-rich domains located at the N- and C-termini of Yap1 (n-CRD and c-CRD, respectively). Under normal conditions Yap1 is localized predominantly in the cytoplasm, whereas during oxidative stress it is in the nucleus. Yap1 accumulation in the nucleus is a consequence of its oxidation, which is accompanied by the formation of a disulfide bond between the n-CRD and c-CRD domains and switching off the Crm1 protein, mediating Yap1 export from the nucleus into the cytoplasm [127]. Yap1 is inactivated by its reduction by thioredoxin. In general, the Yap1 sensor functions as a highly autoregulated system with feedback, enabling the maintenance of intracellular H_2O_2 concentration at a level safe for cells [15].

Treatment of *E. coli* and *S. typhimurium* with low doses of H_2O_2 activates the expression of a number of genes involved in cell defense from peroxide stress [128]. Some of these genes are controlled by the transcription factor OxyR. Like other proteins of the LysR transcription factor family, OxyR contains a DNA-binding region in the N-terminal domain. According to a model suggested by the group of researchers under the direction of G. Storz, the formation of an intramolecular disulfide bond is a signal for a transformation of OxyR into its active state, and the reduction of this bond converts the protein into an inactive form. As a tetramer, oxidized OxyR can bind to promoters of regulated genes and stimulating their transcription by interaction of its N-terminal domain with the α-subunit of RNA polymerase. It can bind to its own promoter in both oxidized and reduced state, thus repressing its own synthesis. Upon activation of OxyR by H_2O_2, a disulfide bond forms between Cys199 and Cys208 [129]. Very high sensitivity of OxyR to H_2O_2 (activating concentrations: *in vivo* 5 μM, *in vitro* 0.05-0.2 μM) is associated with a special positively charged Cys199 environment, which dramatically increases the activity of the sulfur atom. The formation of a disulfide bond results in significant conformational changes in the OxyR regulatory domain, thus enabling the protein to activate transcription [130]. The reduction of this disulfide bond by glutaredoxin 1 in the presence of GSH inactivates OxyR *in vitro* and *in vivo* [129, 131]. It is worth noting that the activation of OxyR in *E. coli* even by relatively high concentrations of H_2O_2 is not accompanied by detectable changes in total intracellular glutathione and the ratio of GSH/GSSG [132]. At the same time, there is proof that OxyR exhibits sensitivity both to H_2O_2 and to the change in intracellular thiol-disulfide state [131].

Later the results of a study by the group of Stamler were published indicating a more complex mechanism of OxyR functioning [133]. These researchers did not observe the formation of intramolecular disulfide bond between Cys199 and Cys208 in native activated OxyR, and thus they conclude that this bond is not involved in regulation. Instead, the authors presented evidence that OxyR activity is controlled by different Cys199 modifications in response to different stress conditions: upon oxidative stress a stable derivative of sulfenic acid (C_{199}-SOH) is formed; the reaction of nitrosothiols results in C_{199}-SNO, whereas disulfide stress leads to a formation of mixed disulfide with glutathione (C_{199}-S-S-G) [133, 134]. These authors also suggested that the Cys199 modifications activate OxyR to different degrees, which allows identifying different transcriptional responses resulting from different stresses. Therefore, instead of a model of thiol-disulfide switch, a model of multiple active states has been put forward. The latter model dramatically changes existing ideas about transduction pathways for redox signals in cells; however, it is evident that further experimental studies are needed for evaluation of its correctness.

Comparing the properties of the sensors discussed above, it should be noted that OxyR is at the same time a sensor, responding to H_2O_2, and a transcription regulator; whereas in yeast these functions are divided between Orp1, playing a role of sensor, and the transcription factor Yap1. It is noteworthy that inactivation of both Yap1

and OxyR occurs with the participation of thiol-containing molecules, thioredoxin and glutaredoxin, respectively. At the same time, in response to the effect of H_2O_2 these regulators activate the pathways involved in thioredoxin and glutaredoxin metabolism, thus resulting in the formation of a homeostatic feedback loop. In yeast thioredoxin simultaneously participated in the reduction of Yap1 and, as a reductant, in the reactions leading to H_2O_2 reduction. In *E. coli*, glutathione is not directly linked to either H_2O_2 reduction or regulation of OxyR activity. GSH is indirectly involved in the latter process as cofactor for glutathione reduction. In turn, OxyR controls the *gor*, *grxA*, and *trxC* genes, encoding the synthesis of glutathione reductase, glutaredoxin, and thioredoxin 2. Therefore, both sensors are involved not only in maintaining the homeostasis of intracellular H_2O_2, but also in the regulation of thiol-disulfide cell status. Since the reduction of oxidized thioredoxin form required NADPH, and glutathione reductase also uses this reductant for reducing GSSG, the functioning of both systems is indirectly linked to constructive metabolism, where NADPH plays an important role.

During the oxidative stress in *E. coli* caused by NO and substances generating superoxide, the *soxRS* regulon (conferring the resistance to these oxidants) becomes activated. Regulation of the regulon is mediated by a constitutively expressed protein SoxR consisting of two subunits, each of which contains a [2Fe-2S] cluster [135]. The [2Fe-2S] clusters in SoxR can be reversibly oxidized and reduced, but it is only the oxidized SoxR form that is a powerful transcription activator specific for the *soxS* promoter [136]. The increase in SoxS synthesis, in turn, activates the expression of the *soxRS* regulon. Therefore, the redox state of SoxR [2Fe-2S] clusters regulates its activity as a transcription factor [122, 137]. During aerobic growth, 95% of these clusters are in the reduced state. Upon treatment with superoxide-producing substances, rapid reversible accumulation of SoxR takes place. It has been shown that the redox signals are transduced to DNA through subunit dimerization. Mutations, disturbing the bond between two SoxR subunits make it incapable of transducing stress signals [138].

SoxR belongs to the MerR family of transcription activators, which regulate the genes involved in protection of bacteria from a number of stress effects (influence of heavy metals, organic compounds, etc.). A molecular mechanism by which these proteins activate gene expression is of interest. Promoters, regulated by factors belonging to MerR-family contain an unusual spacer of 19-20 bp, localized between −35 and −10 bases, instead of the 17 bp as in a majority of promoters interacting with σ^{70}-RNA polymerase. In the absence of the activator, the presence of suboptimal spacer prevents the formation of open complex by RNA polymerase. Upon activation of transcription in response to the corresponding signals, the regulatory protein binds to a palindrome promoter sequence between −35 and −10 bp, and changes the conformation of the promoter region by bending DNA in such manner that the distance between the two sites becomes optimal for the formation of the open complex [139-141]. In general, the available data indicate that SoxR functions as a molecular switch, transforming slight changes in the redox state into dramatic alterations of DNA structure [142].

The molecular mechanisms of SoxR oxidation as well as its subsequent reduction and maintenance in a reduced state remain insufficiently studied. It has been assumed that the oxidation of [2Fe-2S] clusters by compounds generating superoxide anion can be mediated by the decrease in activity of a putative reductase, reducing the SoxR protein because of a decrease in NADPH pool, as a possible reductase donor [142]. Such NADPH-dependent SoxR-reductase activity was found in *E. coli*, but the protein responsible for this activity has not been characterized [143]. Recently it was reported that the products of the six genes of the *rsx* operon and *rseC* gene constitute a reducing system for SoxR [144].

SoxR can be activated by $NO^{\bullet}$, whereas the mechanism of its activation is dramatically different from the activation of [2Fe-2S] clusters [145]. $NO^{\bullet}$ reacts with [2Fe-2S] sites, replacing the sulfur atom with the formation of mixed dinitrosyl-Fe-cysteinyl complex [146]. Nitrosylated SoxR form has the same activity as oxidized SoxR. Although nitrosylated SoxR is relatively stable *in vitro*, a rapid removal of nitrosylated complex and its replacement with intact [2Fe-2S] sites occurs *in vivo*. Such type of nitrosylation represents a fundamentally new way of signal transduction with participation of $NO^{\bullet}$ [147]. Apparently, the possibility of existence of at least two different active SoxR forms is in a certain degree of agreement with the model of multiple active states suggested for OxyR.

One of the types of oxidative stress is a disulfide stress, occurring upon increasing level of disulfide bonds in cytoplasm. In this connection, the discovery of a new control system for thiol-disulfide status in the bacterium *Streptomyces coelicolor*, containing of the σ^R factor and anti-σ factor RsrA, is of interest. The latter is a σ^R-specific, Zn-containing redox-sensitive protein. Under normal conditions, RsrA is bound to σ^R and inhibits the σ^R-dependent transcription. During disulfide stress caused by cell treatment with the specific thiol oxidant diamide, an intramolecular disulfide bond in RsrA is formed, resulting in dissociation of the σ^R–RsrA complex and activation of σ^R-dependent transcription of more than 30 genes and operons including *trxBA*. The increase in *trxBA* expression, in turn, leads to thioredoxin-sensitive reduction of the oxidized RsrA, its binding to σ^R, and inhibition of transcription [124].

In recent years, a number of other redox-sensitive proteins have been found in prokaryotic cells; oxidative modifications of these proteins provide a signal for induc-

ing controllable genes or other regulatory events. Transcription regulators OhrR, PpsR/CrtJ, PerR, and chaperone Hsp33 belong to this group. These proteins are activated by different mechanisms: the only cysteine residue on OhrR forms a stable derivative of sulfenic acid (C_{15}-SOH), PpsR/CrtJ repressors form a reversible disulfide bond, and oxidation of Hsp33 is accompanied by the formation of two disulfide bonds and release of zinc followed by dimerization of the protein [124].

Regulation by glutathionylation of significant SH-groups is also possible in prokaryotic cells. One prokaryotic protein for which the possibility of regulation by forming mixed disulfide with glutathione was shown is *E. coli* PAPS reductase [148]. Recently, it has been demonstrated that in *E. coli* cells thioredoxin is capable of binding to 80 proteins involved in different cellular processes [149]. The presence of transcriptional regulators among these proteins indicates a possibility of involvement of thioredoxin in redox-regulation of activity in bacterial cells.

Many bacterial species excrete micromolar amounts of glutathione into the medium [150]. It is noteworthy that the level and redox state of extracellular glutathione in *E. coli* dramatically changes upon the change in cultivation conditions and during stress; however, even upon the action of exogenous oxidants the level of reduced extracellular glutathione markedly exceeds the level of oxidized glutathione [132, 151-153]. As in eukaryotic cells, a significant contribution in the regulation of the level of extracellular glutathione in bacterium *E. coli* is made by GGT [151]. It is assumed that one of the functions of extracellular glutathione is cell defense from toxic compounds "far away". Further study should elucidate whether extracellular glutathione plays a role in redox regulation in bacteria.

In general, the data accumulated at present indicate that bacterial cytoplasm and periplasm possess redox pathways enabling the maintenance of thiol-disulfide equilibrium. Their importance for cell viability is confirmed by the fact that many of them can, preserving certain specificity, have overlapping functions [154]. Thiol-containing redox proteins are capable of functioning as reductases or oxidases; this ability is determined by redox conditions in the cell compartment where they are located, and by the presence or absence of redox partners capable of maintaining them in the reduced or oxidized state. Expression of several components of the redox systems is regulated in response to changes in the environmental conditions and redox state of the cell [154].

Taken together, the available data provide evidence that, although the study of redox regulation of cellular processes both in prokaryotic and eukaryotic cells is just beginning, it is evident that the intimate balance between oxidative and reductive molecules is an important tool in regulating cellular activity and transducing cellular signals [155].

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REFERENCES

1. Schafer, F. Q., and Buettner, G. (2001) *Free Rad. Biol. Med.*, **30**, 1191-1212.
2. Cross, A. R., and Jones, O. T. G. (1991) *Biochim. Biophys. Acta*, **1057**, 281-298.
3. Skulachev, V. P. (1998) *Biochim. Biophys. Acta*, **1363**, 100-124.
4. Cadenas, E. (2004) *Mol. Aspects Med.*, **25**, 17-26.
5. Andreyev, A. Yu., Kushnareva, Yu. E., and Starkov, A. A. (2005) *Biochemistry (Moscow)*, **70**, 200-214.
6. Jezek, P., and Hlavata, L. (2005) *Int. J. Biochem. Cell. Biol.*, **37**, 2478-2503.
7. Gamaley, I. A., and Klyubin, I. V. (1999) *Int. Rev. Cytol.*, **188**, 203-255.
8. Boldyrev, A. A. (1995) *Biochemistry (Moscow)*, **60**, 1173-1178.
9. Nakamura, H., Nakamura, K., and Yodoi, J. (1997) *Annu. Rev. Immunol.*, **15**, 351-369.
10. Thannickal, V. J., and Fanburg, B. L. (2000) *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **279**, L1005-L1028.
11. Turpaev, K. T. (2002) *Biochemistry (Moscow)*, **67**, 281-292.
12. Pastori, G. M., and Foyer, C. H. (2002) *Plant Physiol.*, **129**, 460-468.
13. Stone, J. R. (2004) *Arch. Biochem. Biophys.*, **422**, 119-124.
14. Antunes, F., and Cadenas, E. (2001) *Free Rad. Biol. Med.*, **30**, 1008-1018.
15. Toledano, M. B., Delaunay, A., Monceau, L., and Tacnet, F. (2004) *Trends Biochem. Sci.*, **29**, 351-357.
16. Poole, L. B., Karplus, P. A., and Claiborne, A. (2004) *Annu. Rev. Pharmacol. Toxicol.*, **44**, 325-347.
17. Giaccia, A. J., Simon, M. C., and Johnson, R. (2004) *Genes Dev.*, **18**, 2183-2194.
18. Bailey-Serres, J., and Chang, R. (2005) *Ann. Botany*, **96**, 507-518.
19. Semenza, G. L. (2004) *Physiology*, **19**, 176-182.
20. Chandel, N. S., McClintock, D. S., Feliciano, C. E., Wood, T. M., Melendez, J. A., Rodriguez, A. M., and Schumacker, P. T. (2000) *J. Biol. Chem.*, **275**, 25130-25138.
21. Bell, E. L., Emerling, B. M., and Chandel, N. S. (2005) *Mitochondrion*, **5**, 322-332.
22. Lopez-Barneo, J., Toro, R., Levitsky, K. L., Chiara, M. D., and Ortega Saenz, G. P. (2003) *J. Appl. Physiol.*, **96**, 1187-1195.
23. Zitomer, R. S., and Lowry, C. V. (1992) *Microbiol. Rev.*, **56**, 1-11.
24. Kwast, K. E., Burke, P., and Poyton, R. O. (1998) *J. Exp. Biol.*, **201**, 1177-1195.
25. Hon, T., Dodd, A., Dirmeier, Gorman, N., Sinclair, P. R., Zhang, L., and Poyton, R. O. (2003) *J. Biol. Chem.*, **278**, 50771-50780.
26. Satroutdinov, A. D., Kuriyama, H., and Kobayashi, H. (1992) *FEMS Microbiol. Lett.*, **77**, 261-267.
27. Sohn, H. Y., Murray, D. B., and Kuriyama, H. (2000) *Yeast*, **16**, 1185-1190.
28. Murray, D. B., Engelen, F., Lloyd, D., and Kuriyama, H. (1999) *Microbiology*, **145**, 2739-2745.

29. Klevecz, R. R., Bolen, J., Forrest, G., and Murray, D. B. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 1200-1205.
30. Tu, B., Kudlicki, A., Rowicka, M., and McKnight, S. L. (2005) *Science*, **310**, 1152-1158.
31. Kiley, P. J., and Beinert, H. (1999) *FEMS Microbiol. Rev.*, **22**, 341-352.
32. Unden, G., Achebach, S., Holighaus, G., Tran, H. G., Wackwitz, B., and Zeuner, Y. (2002) *J. Mol. Microbiol. Biotechnol.*, **4**, 263-268.
33. Alexeeva, S., Hellingwerf, K. J., and Mattos, M. J. T. (2003) *J. Bacteriol.*, **185**, 204-209.
34. Alexeeva, S., Kort, B., Sawers, G., Hellingwerf, K. J., and Mattos, M. J. T. (2000) *J. Bacteriol.*, **182**, 4934-4940.
35. Malpica, R., Franco, B., Rodriguez, C., Kwon, O., and Georgellis, D. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 13318-13323.
36. Taylor, B. L., and Zhulin, I. B. (1999) *Microbiol. Mol. Biol. Rev.*, **63**, 479-506.
37. Meister, A., and Anderson, M. E. (1983) *Ann. Rev. Biochem.*, **52**, 711-760.
38. Del Bello, B., Paolicchi, A., Comporti, M., Pompella, A., and Maellaro, E. (1999) *FASEB J.*, **13**, 69-79.
39. Paolicchi, A., Dominici, S., Pieri, L., Maellaro, E., and Pompella, A. (2002) *Biochem. Pharmacol.*, **64**, 1027-1035.
40. Klatt, P., and Lamas, S. (2000) *Eur. J. Biochem.*, **267**, 4928-4944.
41. Fratelli, M., Demol, H., Puype, M., Casagrande, S., Villa, P., Eberini, I., Vandekerckhove, J., Gianazza E., and Ghezzi, P. (2003) *Proteomics*, **7**, 1154-1161.
42. Thomas, J. A., and Mallis, R. J. (2001) *Exp. Gerontol.*, **36**, 1519-1526.
43. Shelton, M. D., Chock, P. B., and Mieyal, J. J. (2005) *Antiox. Redox. Signal.*, **7**, 348-366.
44. Shenton, D., Perrone, G., Quinn, K. A., Dawes, I. W., and Grant, C. M. (2002) *J. Biol. Chem.*, **277**, 16853-16859.
45. Lind, C., Gerdes, R., Schuppe-Koistinen, I., and Cotgreave, I. A. (1998) *Biochem. Biophys. Res. Commun.*, **247**, 481-486.
46. Casagrande, S., Bonetto, V., Fratelli, M., Gianazza, E., Eberini, I., Massignan, T., Salmona, M., Chang, G., Holmgren, A., and Ghezzi, P. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 9745-9749.
47. Klatt, P., Molina, E. P., De Lacoba, M. G., Padilla, C. A., Martinez-Galesteo, E., Barcena, J. A., and Lamas, S. (1999) *FASEB J.*, **13**, 1481-1490.
48. Molina, E. P., Klatt, P., Vazquez, J., Marina, A., De Lacoba, M. G., Perez-Sala, D., and Lamas, S. (2001) *Biochemistry*, **40**, 14134-14142.
49. Obin, M., Shang, F., Gong, X., Handelman, G., Blumberg, J., and Taylor, A. (1998) *FASEB J.*, **12**, 561-569.
50. Humphries, K. M., Juliano, C., and Taylor, S. S. (2002) *J. Biol. Chem.*, **277**, 43505-43511.
51. Holmgren, A. (1989) *J. Biol. Chem.*, **264**, 13963-13966.
52. Kern, J., and Kehrer, J. P. (2005) *Frontiers Biosci.*, **10**, 1727-1738.
53. Ritz, D., and Beckwith, J. (2001) *Annu. Rev. Microbiol.*, **55**, 21-48.
54. Chae, H. Z., Kang, S. W., and Rhee, S. G. (1999) *Meth. Enzymol.*, **300**, 219-226.
55. Hofmann, B. (2002) *Biol. Chem.*, **383**, 347-364.
56. Wood, Z. A., Schroder, E., Harris, J. R., and Poole, L. B. (2003) *Trends Biochem. Sci.*, **28**, 32-40.
57. Seaver, L. C., and Imlay, J. A. (2001) *J. Bacteriol.*, **183**, 7173-7181.
58. Immenschuh, S., and Baumgart-Vogt, E. (2005) *Antiox. Redox. Signal.*, **7**, 768-777.
59. Seo, M. S., Kang, S. W., Kim, K., Baines, I. C., Lee, T. H., and Rhee, S. G. (2000) *J. Biol. Chem.*, **275**, 20346-20354.
60. Poole, L. B., Karplus, P. A., and Claiborne, A. (2004) *Annu. Rev. Pharmacol. Toxicol.*, **44**, 325-347.
61. Saurin, A. T., Neubert, H., Brennan, J. P., and Eaton, P. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 17982-17987.
62. Biteau, B., Labarre, J., and Toledano, M. B. (2003) *Nature*, **425**, 980-984.
63. Budanov, A. V., Sablina, A. A., Feinstein, E., Koonin, E. V., and Chumakov, P. M. (2004) *Science*, **304**, 596-600.
64. Chang, T. S., Jeong, W., Woo, H. A., Lee, S. M., Park, S., and Rhee, S. G. (2004) *J. Biol. Chem.*, **279**, 50994-51001.
65. Woo, H. A., Jeong, W., Chang, T. S., Park, K. J., Park, S. J., Yang, J. S., and Rhee, S. G. (2005) *J. Biol. Chem.*, **280**, 3125-3128.
66. Rhee, S. G., Chae, H. Z., and Kim, K. (2005) *Free Rad. Biol. Med.*, **38**, 1543-1552.
67. Matsuzawa, A., and Ichijo, H. (2005) *Antiox. Redox Signal.*, **7**, 472-481.
68. Irani, K., Xia, Y., Zweier, J. L., Sollott, S. J., Der, C. J., Fearon, E. R., Sundaresan, M., Finkel, T., and Goldschmidt-Clermont, P. J. (1997) *Science*, **275**, 1649-1652.
69. Filomeni, G., Rotilio, G., and Ciriolo, M. R. (2002) *Biochem. Pharmacol.*, **64**, 1057-1064.
70. Liu, H., Nishitoh, H., Ichijo, H., and Kyriakis, J. M. (2000) *Mol. Cell Biol.*, **20**, 2198-2208.
71. Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K., and Ichijo, H. (1998) *EMBO J.*, **17**, 2596-2606.
72. Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., Pincus, M. R., Sardana, M., Henderson, C. J., Wolf, C. R., Davis, R. J., and Ronai, Z. (1999) *EMBO J.*, **18**, 1321-1334.
73. Gabbita, S. P., Robinson, K. A., Stewart, C. A., Foyd, R. A., and Hensley, K. (2000) *Arch. Biochem. Biophys.*, **376**, 1-13.
74. Baeuerle, P., and Baltimore, D. (1996) *Cell*, **87**, 13-20.
75. Hirota, K., Murata, M., Sachi, Y., Nakamura, H., Takeuchi, J., Mori, K., and Yodoi, J. (1999) *J. Biol. Chem.*, **274**, 27891-27897.
76. Mitomo, K., Nakayama, K., Fujimoto, K., Sun, X., Seki, S., and Yamamoto, K. (1994) *Gene*, **145**, 197-203.
77. Rao, G. N., Katki, K. A., Madamanchi, N. R., Wu, Y., and Birrer, N. J. (1999) *J. Biol. Chem.*, **274**, 6003-6010.
78. Xanthoudakis, S., Miao, G., Wang, F., Pan, Y. C., and Curran, T. (1992) *EMBO J.*, **11**, 3323-3335.
79. Arrigo, A. P. (1999) *Free Rad. Biol. Med.*, **27**, 936-944.
80. Buzek, J., Latonen, L., Kurki, S., Pelponen, K., and Laiho, M. (2002) *Nucleic Acids Res.*, **30**, 2340-2348.
81. Allen, R. G., and Balin, A. K. (1989) *Free Rad. Biol. Med.*, **6**, 631-661.
82. Smith, J., Ladi, E., Mayer-Proschel, M., and Noble, M. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 10032-10037.
83. Aw, T. Y. (2003) *News Physiol. Sci.*, **18**, 201-204.
84. Noble, M., Smith, J., Power, J., and Mayer-Proschel, M. (2003) *Ann. N. Y. Acad. Sci.*, **991**, 251-271.
85. Shackelford, R. E., Kaufmann, W. K., and Paules, R. S. (2000) *Free Rad. Biol. Med.*, **28**, 1387-1404.

86. Menon, S. G., Sarsour, E. H., Spitz, D. R., Higashikubo, R., Sturm, M., Zhang, H., and Goswami, P. C. (2003) *Cancer Res.*, **63**, 2109-2117.
87. Havens, C. G., Ho, A., Yoshioka, N., and Dowdy, S. F. (2006) *Mol. Cell Biol.*, **26**, 4701-4711.
88. Sel'kov, E. E. (1970) *Biofizika*, **15**, 1065-1073.
89. Lioyda, Lemara, K. M., Salgadoa, L. E. J., Goulda, T. M., and Murraya, D. B. (2003) *FEMS Yeast Res.*, **3**, 333-339.
90. Hutter, D. E., Till, B. G., and Greene, J. J. (1997) *Exp. Cell Res.*, **232**, 435-438.
91. Hoffman, A., Spetner, L. M., and Burke, M. (2002) *Carcinogenesis*, **23**, 1961.
92. Murakami, A., Takahashi, D., Kinoshita, T., Koshimizu, K., Kim, H. W., Yoshihiro, A., Nakamura, Y., Jiwajinda, S., Terao, J., and Ohigashi, H. (2002) *Carcinogenesis*, **23**, 795-802.
93. Menon, S. G., Coleman, M. C., Walsh, S. A., Spitz, D. R., and Goswami, P. C. (2005) *Antiox. Redox. Signal.*, **7**, 711-718.
94. Gulbins, E., Jekle, A., Ferlinz, K., Grassme, H., and Lang, F. (2000) *Am. J. Physiol. Renal. Physiol.*, **279**, 605-615.
95. Ghibelli, L., Fanelli, C., Rotilio, G., Lafavia, E., Coppola, S., Colussi, C., Civitareale, P., and Ciriolo, M. R. (1998) *FASEB J.*, **12**, 479-486.
96. Kwon, Y. W., Masutani, H., Nakamura, H., Ishii, Y., and Yodoi, J. (2003) *J. Biol. Chem.*, **384**, 991-996.
97. Migliaccio, E., Giorgio, V., and Mele, S. (1999) *Nature*, **402**, 309-313.
98. Trinei, M., Giorgio, M., Cicalese, A., et al. (2002) *Oncogene*, **21**, 3872-3878.
99. Giorgio, M., Migliaccio, E., Orsini, F., et al. (2005) *Cell*, **122**, 221-233.
100. Nemoto, S., and Finkel, T. (2002) *Science*, **295**, 2450-2452.
101. Skulachev, V. P., and Longo, V. D. (2005) *Ann. N. Y. Acad. Sci.*, **1057**, 145-164.
102. He, Y. Y., Huang, J. L., Ramirez, D. C., and Chignell, C. F. (2003) *J. Biol. Chem.*, **278**, 8058-8064.
103. Grierson, A. W., Nicholson, R., Talbot, P., Webster, A., and Kemp, G. (1994) *J. Gen. Virol.*, **75**, 2761-2764.
104. Davis, D. A., Newcomb, F. M., Starke, D. W., Ott, D. E., Mieyal, J. J., and Yarchoan, R. (1997) *J. Biol. Chem.*, **272**, 25935-25940.
105. Hammond, C. L., Madejczyk, M. S., and Ballatori, N. (2004) *Toxicol. Appl. Pharmacol.*, **195**, 12-22.
106. Macho, A., Hirsch, T., Marzo, I., Marchetti, P., Dallaporta, B., Susi, S. A., Zamzami, N., and Kroemer, G. (1997) *J. Immunol.*, **158**, 4612-4619.
107. Sandstrom, P. A., Mannie, M. D., and Buttke, T. M. (1995) *J. Leukoc. Biol.*, **55**, 221-226.
108. Voehringer, D. W. (1999) *Free Rad. Biol. Med.*, **27**, 945-950.
109. Mirkovic, N., Voehringer, D. W., Story, M. D., McConkey, D. J., McDonnel, T. J., and Meyn, R. E. (1997) *Oncogene*, **15**, 1461-1470.
110. Meredith, M. J., Cusick, C. L., Soltaninassab, S., Sekhar, K. S., Lu, S., and Freeman, M. L. (1998) *Biochem. Biophys. Res. Commun.*, **248**, 458-463.
111. Voehringer, D. W., McConkey, D. J., McDonnel, T. J., Brisbay, S., and Meyn, R. E. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 2960.
112. Hirota, K., Matsui, M., Iwata, S., Nishiyama, A., Mori, K., and Yodoi, J. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 3633-3638.
113. Andoh, T., Chock, P. B., and Chiueh, C. C. (2002) *J. Biol. Chem.*, **277**, 9655-9660.
114. Chance, B., Sies, H., and Boveris, A. (1979) *Physiol. Rev.*, **59**, 527-605.
115. Fernandes, R. S., and Cotter, T. G. (1994) *Biochem. Pharmacol.*, **48**, 675-681.
116. Nobel, C. S. I., Burgess, D. H., Zhivotovsky, B., Burkitt, M. J., Orrenius, S., and Slater, A. F. G. (1997) *Chem. Res. Toxicol.*, **10**, 636-643.
117. Hentze, H., Kunstle, G., Volbracht, C., Ertel, W., and Wendel, A. (1999) *Hepatology*, **30**, 177-185.
118. Hentze, H., Schmitz, I., Latta, M., Krueger, A., Krammer, P. H., and Wendel, A. (2002) *J. Biol. Chem.*, **277**, 5588-4595.
119. Moriarty-Craige, S. E., and Jones, D. P. (2004) *Annu. Rev. Nutr.*, **24**, 481-509.
120. Pompella, A., Visvikis, A., Paolicchi, A., de Tata, V., and Casini, A. (2003) *Biochem. Pharmacol.*, **66**, 1499-1503.
121. Zheng, M., and Storz, G. (2000) *Biochem. Pharmacol.*, **59**, 1-6.
122. Pomposiello, P. J., and Demple, B. (2001) *Trends Biotechnol.*, **19**, 109-114.
123. Demple, B., Ding, H., and Jorgensen, M. (2002) *Meth. Enzymol.*, **348**, 355-364.
124. Paget, M. S. B., and Buttner, M. J. (2003) *Annu. Rev. Genet.*, **37**, 91-121.
125. Delaunay, A., Isnard, A. D., and Toledano, M. B. (2000) *EMBO J.*, **19**, 5157-5166.
126. Delaunay, A., Pflieger, D., and Barrault, M. B. (2002) *Cell*, **111**, 471-481.
127. Kuge, S., Toda, T., Iizuka, N., and Nomota, A. (1998) *Genes Cells*, **3**, 521-532.
128. Zheng, M., Wang, X., Templeton, L. J., Smulski, D. R., LaRossa, R. A., and Storz, G. (2001) *J. Bacteriol.*, **183**, 4562-4570.
129. Zheng, M., Aslund, F., and Storz, G. (1998) *Science*, **279**, 1718-1721.
130. Choi, S. J., Kim, H. J., Mukhopadhyay, P., Cho, S., Woo, J. R., Storz, G., and Ryu, S. E. (2001) *Cell*, **105**, 103-113.
131. Aslund, F., Zheng, M., Beckwith, J., and Storz, G. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 6161-6165.
132. Smirnova, G. V., Muzyka, N. G., Glukhovchenko, M. N., and Oktyabrsky, O. N. (2000) *Free Rad. Biol. Med.*, **28**, 1009-1016.
133. Kim, S. O., Merchant, K., Nudelman, R., Beyer, W. F., Jr., and Keng, T. (2002) *Cell*, **109**, 383-396.
134. Hausladen, A., Privalle, C. T., Keng, T., DeAngelo, J., and Stamler, J. S. (1996) *Cell*, **86**, 719-729.
135. Hidalgo, E., and Demple, B. (1996) in *Regulation of Gene Expression in Escherichia coli* (Lin, E. C. C., and Lynch, A. S., eds.) R. G. Landes Company, Austin, pp. 435-452.
136. Hidalgo, E., and Demple, B. (1994) *EMBO J.*, **13**, 138-146.
137. Ding, H., Hidalgo, E., and Demple, B. (1996) *J. Biol. Chem.*, **271**, 33173-33175.
138. Ding, H., and Demple, B. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 8445-8449.
139. Outten, C. E., Outten, F. W., and O'Halloran, T. V. (1999) *J. Biol. Chem.*, **274**, 37517-37524.

140. Chander, M., and Demple, B. (2004) *J. Biol. Chem.*, **279**, 41603-41610.
141. Newberry, K. J., and Brennan, R. G. (2004) *J. Biol. Chem.*, **279**, 20356-20362.
142. Liochev, S. I., and Fridovich, I. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 5892-5896.
143. Kobayashi, K., and Tagawa, S. (1999) *FEBS Lett.*, **451**, 227-230.
144. Koo, M. S., Lee, J. H., Rah, S. Y., Yeo, W. S., Lee, J. W., Lee, K. L., Koh, Y. S., Kang, S. O., and Roe, H. R. (2003) *EMBO J.*, **22**, 2614-2622.
145. Nunoshiba, T., DeRojas-Walker, T., Tannenbaum, S. R., and Demple, B. (1995) *Infect. Immun.*, **63**, 794-798.
146. Ding, H., and Demple, B. (2000) *J. Biol. Chem.*, **97**, 33173-33175.
147. Pomposiello, P. J., and Demple, B. (2002) *Adv. Microb. Physiol.*, **46**, 319-341.
148. Vlamis-Gardikas, A., Potamitou, A., Zarivach, R., Hochman, A., and Holmgren, A. (2002) *J. Biol. Chem.*, **277**, 10861-10868.
149. Kumar, J., Tabor, S., and Richardson, C. C. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 3759-3764.
150. Owens, R. A., and Hartman, P. E. (1986) *J. Bacteriol.*, **168**, 109-114.
151. Suzuki, H., Kumagai, H., and Tochikura, T. (1987) *J. Bacteriol.*, **169**, 3926-3931.
152. Smirnova, G. V., Krasnykh, T. A., and Oktyabrsky, O. N. (2001) *Biochemistry (Moscow)*, **66**, 973-978.
153. Smirnova, G. V., and Oktyabrsky, O. N. (2005) *Biochemistry (Moscow)*, **70**, 1199-1211.
154. Ritz, D., and Beckwith, J. (2001) *Annu. Rev. Microbiol.*, **55**, 21-48.
155. Cross, J. V., and Templeton, D. (2004) *J. Cell. Biochem.*, **93**, 104-111.