

The Redox Proteome*

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The redox proteome consists of reversible and irreversible covalent modifications that link redox metabolism to biologic structure and function. These modifications, especially of Cys, function at the molecular level in protein folding and maturation, catalytic activity, signaling, and macromolecular interactions and at the macroscopic level in control of secretion and cell shape. Interaction of the redox proteome with redox-active chemicals is central to macromolecular structure, regulation, and signaling during the life cycle and has a central role in the tolerance and adaptability to diet and environmental challenges.

Redox biology is the study of oxidation-reduction processes associated with life. Early 20th century research on O₂ as an electron acceptor linked electron transfer reactions to generation of ATP (1, 2). In 1955, research with ¹⁸O₂ and mass spectrometry (MS) showed that atoms from O₂ were introduced into biomolecules (3, 4), stimulating research on mechanisms of O₂ activation (5, 6). This research was focused mostly on enzyme cofactors rather than the redox activity of the translated proteome.

At the same time, protein aggregation and oxidative inactivation introduced artifacts in protein chemistry and enzymology (7). Removal of contaminating trace metals, O₂, and/or inclusion of GSH or dithiothreitol protected against these processes. The susceptibility of protein to oxidation contributed to the study of oxidative damage in biologic systems, termed oxidative stress (8, 9). The discovery of superoxide dismutase (10) fueled this research and the related study of lipid peroxidation, oxidative DNA damage, and oxidative protein chemistry. Advances in cancer biology and molecular biology led to discovery of NADPH oxidases, enzymes that produce reactive oxygen species (superoxide and H₂O₂) for redox signaling (11). At the same time, advances in MS and crystallography supported detailed studies of the proteome.

In this minireview, we provide an overview of redox components of the translated proteome that connect electron transfer to biologic structure and function. We do not include the central enzymology of oxidoreductases, *e.g.* oxidases, hemoproteins, NAD-linked intermediary metabolism, or mitochondrial electron transfer. We focus instead on reversible reactions of

the Cys proteome, which has an inherent tendency to undergo oxidative post-translational modification under aerobic conditions. Irreversible covalent modifications provide a complementary system for prolonged biologic redox signaling and have been addressed elsewhere (12). Both are relevant to oxidative stress and redox signaling and, through interactions with the metabolome, provide an interface with diet and environmental exposures (Fig. 1A). Disruption of these mechanisms contributes to human disease.

Definition of Redox Proteomics

“Redox proteome” is a collective term for components of the proteome that undergo reversible redox reactions and those modified irreversibly by reactive species during oxidative stress. Only three of the amino acids undergo reversible reaction (Cys, Met, and selenocysteine (Sec)²) but many amino acids (*e.g.* Trp, Tyr, and Arg) and the peptide backbone react with products generated during oxidative stress (13). The redox proteome interacts with the redox metabolome, a redox-active subset of the metabolome, with NADPH/NADP⁺, GSH/GSSG, and cysteine/cystine being specifically relevant to the redox proteome. The metabolome includes low molecular mass biochemicals generated by intermediary metabolism and chemicals from the diet, microbiome, pharmaceuticals, commercial products, and environment (14). Many are linked to NADH/NAD and other redox systems and can indirectly impact the redox proteome.

The redox proteome impacts the genome and epigenome, RNA processing and translation, and other post-translational modifications of the translated proteome (Fig. 1A). In analogy to the epigenome and genome, the epiproteome includes the translated proteome with covalent modifications to provide function and regulation beyond that of the translated sequence of amino acids. As a component of the epiproteome, the redox proteome is controlled primarily by endogenous metabolism but responds to chemicals from the diet, air, and other exposures. These redox reactions have a central role in integrating homeostatic, adaptive, and defense responses between the epigenome, epiproteome, and external environment.

Redox Steady State—Thioredoxin (Trx) and GSH provide effective thiol antioxidant systems, yet ongoing oxidation maintains a non-equilibrium steady-state oxidation of protein Cys in cells. This steady-state oxidation is not fully understood because non-enzymatic protein thiol oxidation with physiologic oxidants is relatively slow (15), as illustrated by redox-sensitive green fluorescent protein, which requires minutes to oxidize even with added H₂O₂ (16). However, measurement of multiple proteins in a redox pathway in cells (Fig. 2) shows that reduction is kinetically limited (17). Because the rates of oxidation and reduction of individual thiols in cells are difficult to measure, redox effects are often quantified in terms of the steady-state redox potential (E_h). E_h is calculated using the Nernst equation: $E_h = E_o + RT/nF \ln[\text{oxidized molecule}]/[\text{re-$

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² The abbreviations used are: Sec, selenocysteine; Trx, thioredoxin; MetO, Met sulfoxide; Prx, peroxiredoxin.

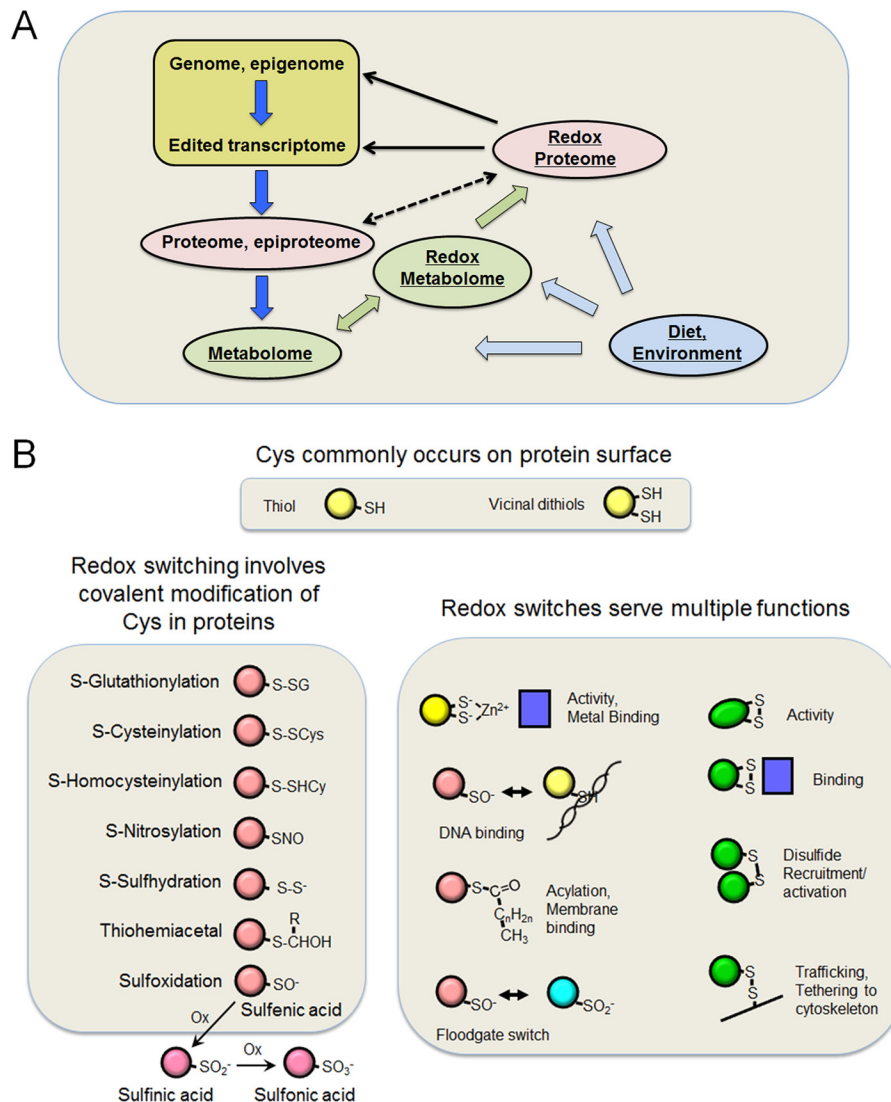


FIGURE 1. Redox proteome as the interface of the epiproteome, diet, and environment. *A*, the redox proteome is a subset of post-translational modifications of protein considered here as the epiproteome (indicated by the *dashed arrow*). This is considered in analogy to the epigenome and genome as a system to provide function and regulation beyond that of the translated sequence of amino acids. The redox proteome includes amino acids that undergo reversible redox reactions (Cys, Met, and Sec) and others that are irreversibly modified by reactive species in oxidative stress (Lys, Trp, and others). The redox proteome impacts the genome and epigenome through altered DNA and RNA binding and altered trafficking, activities, and structures of associated proteins. Metabolic activities, especially in cell signaling, impact the metabolome. The redox metabolome is a subset of the metabolome that alters structure and function of the redox proteome. NADPH/NADP, GSH/GSSG and Cys/cystine are central components of the redox metabolome. The metabolome and redox proteome respond to diet and environmental influences (*blue arrows*) to protect against oxidant stress and other environmental challenges. *B*, covalent modifications of Cys provide a versatile structure-function switching system. Cys commonly exists on the surface of proteins either alone (monothiol) or in close proximity to another Cys residues (vicinal dithiols) (*top*). The reactivities are impacted by vicinal cationic amino acids, which enhance ionization and make Cys more reactive. Vicinal dithiols tend to form disulfides upon oxidation, whereas monothiols undergo reversible oxidation to sulfenic acid and mixed disulfides with low molecular mass thiol chemicals, GSH, Cys, and homocysteine (HCy; *left, inside box*). Under highly oxidizing conditions, sulfenic acid is further oxidized to sulfinic and sulfonic acids (*left, below box*). Other modifications also occur, such as S-nitrosylation, S-sulfhydration, and thiohemiacetal formation. These changes, as well as Zn^{2+} binding and acylation, can result in altered protein-protein interactions, DNA or RNA binding, or membrane interaction (*right*).

duced molecule], where R is the gas constant, T is the absolute temperature, n is the number of electrons transferred, and F is Faraday's constant. The NADPH/NADP couple is maintained at about -400 mV in cells and serves as the principal reductant to reverse oxidation by O_2 ($\text{O}_2/\text{H}_2\text{O}$ at approximately $+600$ mV). The displacement of thiol/disulfide systems from equilibrium is illustrated by GSH/GSSG (Fig. 2), with intermediate E_h in plasma (-138 mV) (18) and in liver (-255 mV) (19). Similarly, Trx1 has an intermediate value (-270 mV) (20).

Reversible Redox Elements of the Proteome—The thiol of Cys is the most extensively characterized component of the redox

proteome and is the main focus of this minireview. This does not imply that Cys is more important than Sec or Met. Sec is present in only 25 human proteins, but these include central redox enzymes, Trx reductases, and GSH peroxidases (21). The Met proteome is similar to the Cys proteome but is less studied. If one excludes Met for initiation of translation, the human genome encodes 174,000 Met residues (17). A small percentage exists in enzyme active sites, but others contribute to the three-dimensional structure and molecular interactions (22). About 5–10% of the total Met proteome is present *in vivo* as Met sulfoxide (MetO) (23). A family of MetO reductases (Msr)

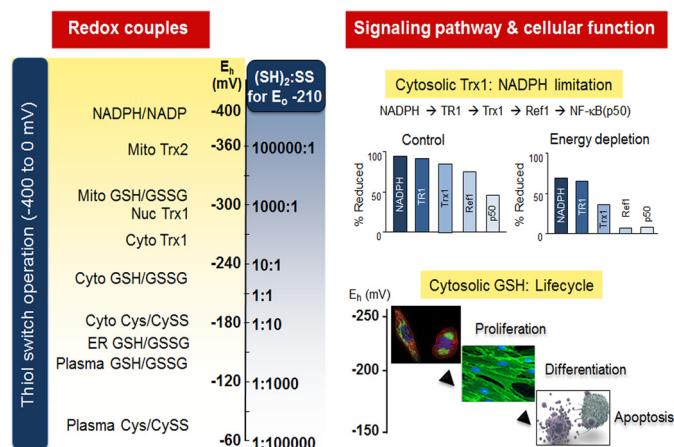


FIGURE 2. **Non-equilibrium steady states of redox couples direct metabolism, structure, and macromolecular trafficking.** Central thiol/disulfide couples (left) serve as redox hubs and include small molecules of the redox metabolome and redox-active proteins, such as Trx1 and Trx2. These exist with a range of steady-state redox potentials (E_h , calculated with the Nernst equation (see text)) spanning nearly 400 mV from mitochondrial NADPH/NADP to plasma Cys/cystine (CySS). The difference between redox couples (ΔE_h) is proportional to $-\Delta G$, describing the energetics of electron transfer. The ratio of (SH)₂ to SS for a dithiol/disulfide couple with $E_o = -210$ mV illustrates the relative abundance of the forms if the protein is equilibrated at the respective E_h value. *Upper right*, kinetic limitation is shown for proteins in the pathway from NADPH to NF- κ B (p50) under both control and energy-limiting conditions in cultured cells. *Lower right*, the E_h of cytosolic GSH/GSSG becomes progressively oxidized in the life cycle of cells from proliferation to differentiation to apoptosis. Such change could, in principle, impact proteins via glutaredoxin-dependent *S*-glutathionylation (30) or reflect changes in H₂O₂ generation and GSH peroxidase activity. *Mito*, mitochondria; *nuc*, nuclear; *cyto*, cytosolic; *ER*, endoplasmic reticulum; *TR1*, Trx reductase 1. Cell images are from Invitrogen and the Genetics Home Reference (<http://ghr.nlm.nih.gov>).

reduces peptidyl-MetO back to peptidyl-Met (24). Genetic manipulation of MsrA impacts longevity (25), suggesting the importance of peptidyl-MetO in sensitivity to oxidative stress but not excluding other redox functions of peptidyl-Met oxidation.

Special Character of the Cys Proteome

Early research showed that about half of all enzymes are sensitive to thiol reagents, and studies of protein structure revealed contributions of disulfides to three-dimensional configuration (26) and to protein processing and trafficking (27). Additionally, x-ray crystallography and molecular biology revealed widespread Zn²⁺ binding to Cys in proteins (28, 29). The fundamental character of Cys as a “sulfur switch” (30), discussed below, was recognized only more recently.

Oxidation of the Cys Proteome—The human Cys proteome includes 214,000 Cys residues encoded in the genome. Only the thiol form is translated due to the specificity of the tRNA^{Cys} synthetase, so all modified forms represent post-translational modifications. In cells and tissues, 5–12% of total protein Cys is oxidized, and this can be increased to >40% by adding oxidants. Oxidation occurs through 1e⁻ or 2e⁻ reactions, producing thiyl radicals or sulfenic acids and disulfides, respectively (Fig. 1B). Introduction of sulfenic acids is termed sulfenylation (31); this reaction and disulfide formation represent central components of the reversible redox proteome. The most commonly studied disulfide formation is *S*-glutathionylation (32), but *S*-cysteinylation and protein-protein disulfide formation also

occurs. Successive oxidation produces sulfinic and sulfonic acids (Fig. 1B).

Other Modifications of the Cys Proteome—Peptidyl-Cys reacts with other biologic molecules to support complementary mechanisms, such as *S*-nitrosylation and *S*-sulfhydration (Fig. 1B). Inclusion of such modifications into systems biology models of the redox proteome requires a multidimensional description because some specific peptidyl-Cys residues can undergo different modifications, *i.e.* a specific Cys may be glutathionylated, cysteinylated, nitrosylated, or sulfhydrated (33–37). *S*-Nitrosylation of proteins also occurs (38), and *S*-nitroso groups can be transferred between protein thiols (39). Sulfhydration of protein thiols by H₂S also occurs (40, 41) and could support a system of sulfane transfer (42) analogous to nitrosyl transfer. How multiple modifications are integrated within the proteome remains uncertain because global proteomic analysis showed overlap between protein Cys undergoing *S*-nitrosylation and sulfenylation, but the extent of this overlap is limited (43). Reversible reaction with aldehydes, including monosaccharides, to form thiohemiacetals is also possible, but the extent and biologic functions of such modifications are not known. Thiol modifications that result from post-translational modifications of the proteome by electrophiles are termed the adductome (44). These include a biochemical signature of environmental exposure to reactive chemicals (44) and also of endogenously generated reactive lipids and lipid products from radical reactions of oxidative stress that can function in signaling (12).

Sulfur Switches

Sulfur within the redox proteome has a fundamentally important use as a structure-function switch. At the molecular level, there is often no meaningful separation of structure and activity related to redox effects on sulfur. At the macroscopic level, however, structure and activity can be measured independently. Because of this, we list types of switches (Fig. 3) as a framework to consider redox links between metabolism, biologic structure, and activity.

Types of Sulfur Switches—An “on-off” switch provides a qualitative activity change, such as inactivation of protein-tyrosine phosphatases by oxidation in kinase signaling (45). An allosteric switch adjusts activity, as occurs in regulation of enzymes, such as Trx1 (46). An interaction switch provides a change in binding character. Examples include oxidation of Cys in the transcription factor NF- κ B, which prevents its binding to DNA (47), and palmitoylation/myristoylation of endothelial nitric-oxide synthase to enhance binding to membrane (48). Such changes activate complex formation in cell signaling (49) and sequential processing in protein maturation and secretion (50). Finally, different thiolation at a specific Cys residue can change the function of a protein. For example, a switch in cysteinylation of Cys⁶⁰ to glutathionylation results in a change from glycosylation-inhibiting factor activity (51, 52) to macrophage migration inhibitory factor activity (53). Together, these sulfur switches provide diverse capabilities for integration of biologic structures and functions. These sulfur switching mechanisms of the redox proteome evolved with the genome to provide a common system to coordinate and optimize complex biologic systems.

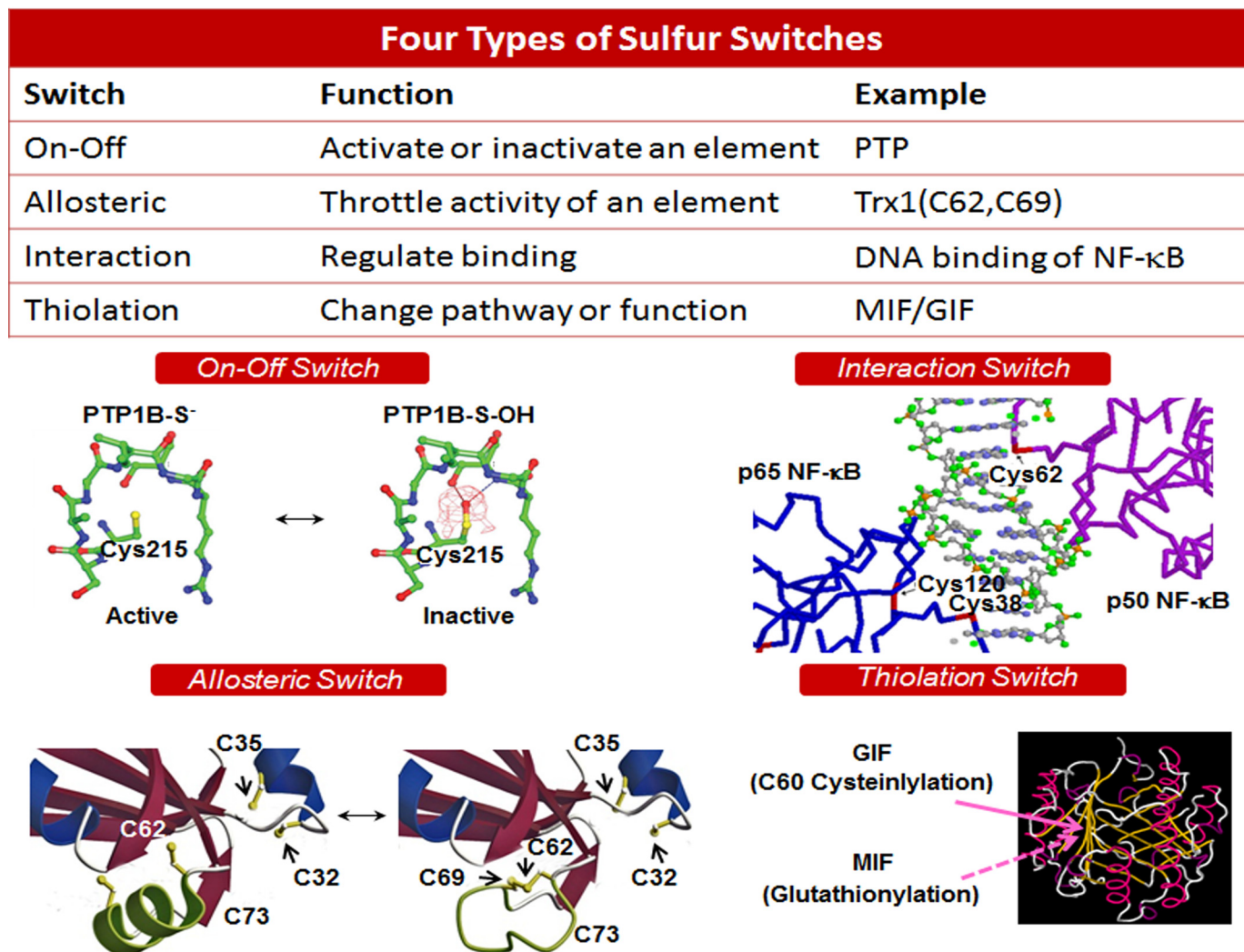


FIGURE 3. **Types of sulfur switches.** Sulfur switches can turn systems on and off, provide allosteric regulation, change the binding interactions, and/or have a chameleon-like effect on the character of a protein (*upper*). Crystal structures are provided to illustrate each. In PTP1B (PTP), oxidation at the active site Cys²¹⁵ residue turns off activity (*middle left*) (45). Oxidation of Cys³⁸ in NF- κ B (p65) or Cys⁶² in NF- κ B (p50) results in loss of DNA binding (*middle right*). Formation of a disulfide (Cys⁶²–Cys⁶⁹) in a surface α -helix in Trx1 (20) results in an allosteric-like decrease in interaction with Trx reductase (*lower left*). A switch in cysteinylolation of Cys⁶⁰ (Protein Data Bank) to glutathionylation results in a change from glycosylation-inhibiting factor (GIF) activity to macrophage migration inhibitory factor (MIF) activity (*lower right*).

Operation of Sulfur Switches—The reversible nature of thiol oxidation to sulfenate and disulfide makes the thiol a versatile element for switching structure and function. Schafer (30) defined sulfur switches in terms of stoichiometry of electron transfer involving $1e^-$ processes, such as glutathionylation, and $2e^-$ processes, such as disulfide bond formation. The activity of the former varies as a function of thiol/disulfide ratio, whereas that of the latter varies as a function of $[GSH]^2/[GSSG]$ (19). The latter is also expressed in terms of the corresponding E_h to allow comparison with other redox-active systems. Several physiologic mechanisms involving protein glutathionylation occur (32), and hundreds of proteins are glutathionylated during oxidative stress (54). Oxidation of a dithiol (Cys⁶²–Cys⁶⁹) in a surface α -helix of human Trx1 provides an example of a $2e^-$ oxidation (20). The Keap1 control system for the transcription factor Nrf2 has 26 Cys residues and appears to have Cys residues undergoing both $1e^-$ and $2e^-$ oxidation (55, 56). Some active sites, e.g. kinase and phosphatases, are also considered to

be sulfur switches because they are reversibly inactivated by oxidation.

Distinction of Redox Signaling and Redox Sensing—The concepts of sulfur switching were derived from studies of redox signaling, but most sulfur switches do not function in discrete signaling pathways as described for analogous kinase signaling. Redox-sensing Cys residues serve as sulfur switches to regulate and integrate biologic functions (57). By operating through distinct Cys residues, redox-sensing Cys residues provide orthogonal control (Fig. 4A) for redox signaling and other activities by changing structure and function without changing molecular mechanisms.

Sulfur Switches and Evolution of the Cys Proteome—Based upon comparison of the percentage of tRNA^{Cys} among all tRNAs (3.28%) and the percentage of Cys in proteins (<2.2%), there is an evolutionary selection pressure against Cys in the proteome (58). Despite this, the percentage of Cys has increased from \sim 0.5% in prokaryotes to 2.2% in mammals,

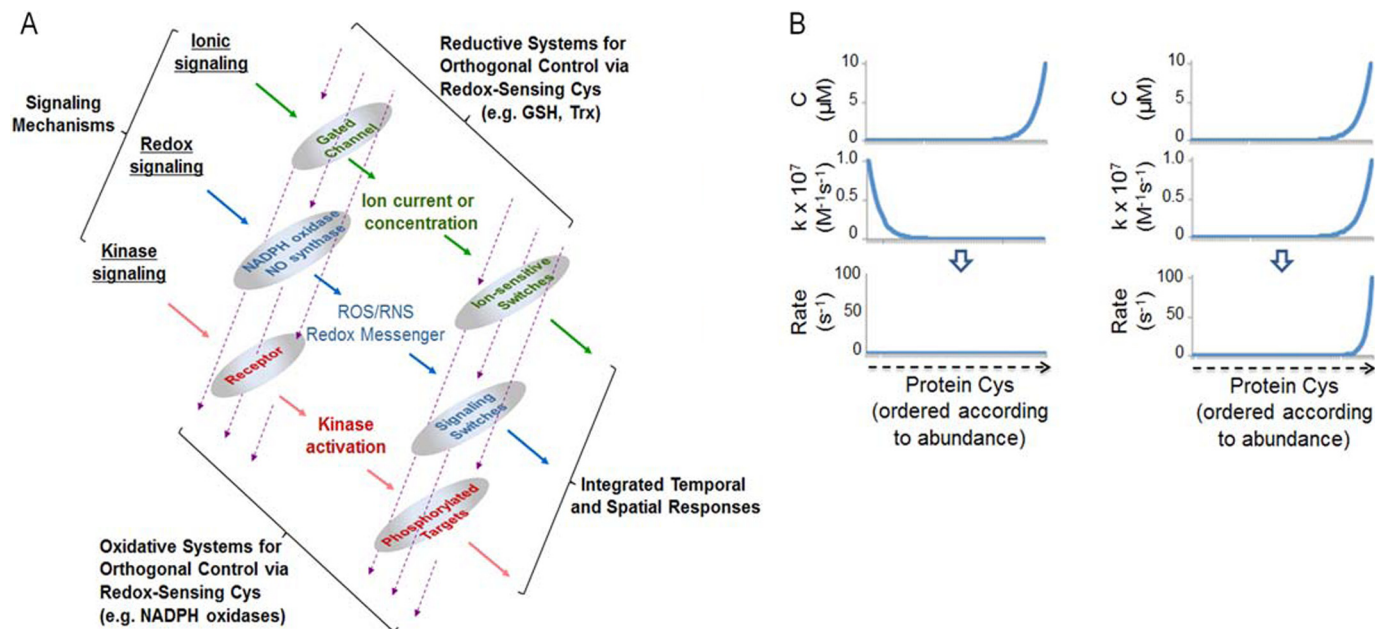


FIGURE 4. Integrated function of the redox proteome. *A*, redox-sensing Cys residues provide an orthogonal control system to regulate and integrate biologic systems without impacting mechanisms. Redox-signaling mechanisms are presented as pathways (diagonal right arrows) that serve to signal cell stress and other responses. These include controlled H_2O_2 production by regulated NADPH oxidases and redox-signaling Cys. They function in parallel with other signaling pathways, such as kinase signaling and ion-gated signaling (diagonal right arrows). These are viewed as providing relatively rapid and transient signals. Redox-sensing Cys residues integrate these signaling pathways without impacting their mechanisms (diagonal left arrows). Although poorly defined experimentally, the latter can be conceived as a background poise of the cellular H_2O_2 generation by mitochondria, Nox4, and other oxidases (lower left) and opposed by reductant systems dependent upon Trx and GSH (upper right). These have slow kinetics relative to signaling mechanisms and thereby provide a more long-term phenotypic control. This allows a single signaling mechanism to be used effectively in different cell types and states of cell division, growth, differentiation, or apoptosis. These signaling and sensing Cys switches operate within the non-equilibrium states maintained by the central redox hubs (Fig. 2) and together provide a versatile system for integrated spatial and temporal control of cell structure and function. ROS/RNS, reactive oxygen and nitrogen species. *B*, broad ranges of protein thiol reactivity and abundance are known to occur in the redox proteome. These provide a redox proteomic structure to support localized redox signaling within an inherently stable redox system. Upper left panel, protein abundance (C) is given on the y axis from femtomolar to $10 \mu\text{M}$, expressed as a function of each respective protein, listed in order of abundance on the x axis. Middle left panel, a hypothetical condition is shown in which the second-order rate constant (k) for protein thiol reaction with H_2O_2 , from 1 to $10^7 \text{ M}^{-1} \text{ s}^{-1}$ (73), is varied in opposition to abundance for the same proteins. Lower left panel, the product of the rate constant and concentration ($k \cdot C$) for each protein under this condition shows that all proteins contribute equivalently to the rate of H_2O_2 metabolism. Right, the rate constant (middle panel) is varied in proportion to abundance (upper panel). The product ($k \cdot C$) for this condition shows that the most abundant protein thiols contribute 14 orders of magnitude more to the rate of H_2O_2 metabolism than the least abundant protein thiols (lower panel). This comparison shows that opposing co-evolution of abundance and reactivity of specific Cys residues within the proteome can account for an inherent stability of the redox proteome while also having specialized subsets of peptidyl-Cys for redox sensing and redox signaling.

showing an increase in Cys with evolution of complexity (58). This includes increased content of conserved/evolved Cys content (57). With an evolutionary selection against Cys, this implies a function for the evolved Cys, such as more extensive redox-signaling and redox-sensing activities. Analyses of conserved Cys residues in mitochondrial proteins detected by MS showed that >90% of the Cys residues were conserved among vertebrates, whereas only ~70% of other amino acids in the same proteins were conserved (59).

Methods for Study of the Redox Proteome

Detailed methods for the study of the redox proteome are available in the protein chemistry literature. Biologic studies have often included global measurements, such as total protein thiol measured by colorimetric methods; immunoassays, such as redox Western blotting of reduced and oxidized forms of specific proteins; and MS-based assays, such as the redox isotope-coded affinity tag methods for thiols (59–61) or DAz-2 reagents for sulfenic acids (62). Global measurements of protein thiols during experimental challenge provide a basis to evaluate the abundance of switchable redox elements in the Cys proteome. For instance, analysis of the rate of autoxidation of

thiols in isolated nuclei shows that ~10% of protein thiols are oxidized in 1 h and another 10% autoxidize more slowly, over a period of several hours. Extrapolation from such data suggests that 20,000–40,000 peptidyl-Cys residues are redox-sensitive. These could function as redox sensors (57) as discussed above or as decoy thiols to protect critical active sites from oxidants and electrophiles (63).

Redox Western Blot Analysis—Immunoassays are useful to measure oxidation of individual proteins in cell and tissue extracts (20, 64, 65). Common methods include alkylation of thiols for separation from other forms by electrophoresis, followed by Western blotting (66). Labeling with thiol reagents containing biotin allows selection and/or detection with streptavidin to enhance flexibility in assay. Applications show that oxidation of individual proteins differs among compartments and varies with physiologic challenge and life cycle (64, 65, 67). The methods are useful to detect changes but can be difficult to calibrate because of multiple thiols in proteins, nonlinear responses, and epitope changes with thiol oxidation or alkylation. This is often a method of choice because of the ability to specifically examine redox changes

in most proteins with standard biochemical laboratory equipment.

Mass Spectrometry-based Redox Proteomics—Adaptation of differential labeling approaches to the evaluation of the redox proteome has become increasingly popular (60–62, 68, 69). As with immunoassays, labeling with thiol reagents containing biotin allows detection or selection of peptides according to thiol content. These are useful with both two-dimensional gel electrophoresis coupled with MS/MS and two-dimensional chromatography-MS/MS methods. Oxidation of up to 4000 specific peptidyl-Cys residues, or ~2% of the entire mammalian proteome, can be measured in a single experiment. Such data are useful in identifying specific Cys residues in cells. Many proteins are partially oxidized even under normal physiologic conditions (59), and possible functions of these Cys residues can be inferred by examination of the positions in x-ray crystal structures. Extensive application of these methods to Alzheimer disease, Parkinson disease, and other neurodegenerative diseases, as well as other human diseases and animal and cell models, has established the value of these approaches in improving the mechanistic understanding of redox biology and human disease (70).

Integrated Redox Function of the Cys Proteome

Integration of detailed information on large numbers of redox-sensitive Cys residues requires systems biology approaches (46) and systematic data on the reactivity of specific thiols, protein abundance, spatial distribution, and organization into redox networks. A current view is that the Cys proteome exists in a dynamic steady state with organization into a branching network structure in which oxidation of specific thiols by O_2 , H_2O_2 , and other oxidants is balanced by reduction by NADPH through Trx- and/or GSH-dependent systems (71). The E_h values of Trx1 and GSH/GSSG couples are different, and neither is equilibrated with NADPH/NADP. In analogy to the use of regulons in genetics to describe groups of operons controlled by common elements, intermediate hubs are termed redox regulons (71). For instance, a Trx1 redox regulon and a GSH redox regulon regulate different subsets of the Cys proteome. An opposing set of oxidative redox regulons, operating in multiple subcellular compartments with secondary hubs, provides a bilateral redox network structure to accommodate specific control of each of the 214,000 Cys residues in the proteome (71). In this bilateral design, NADPH is used both to support reductive redox regulons and to generate H_2O_2 to support oxidative redox regulons. This provides an inherent stability by linking rates of oxidation and reduction to a common precursor (72).

Reactivity—The reactivity of peptidyl-Cys residues differs considerably according to the specific location within the three-dimensional structure of a protein. Measures of thiol reactivity with H_2O_2 show that rate constants differ by >6 orders of magnitude (73). This range of reactivity forces critical examination of the definition of network structure, as discussed above, in which multiple redox elements have variable reactivity with other elements (71). Many possible variations in circuitry can occur, and more complete definition of reactivities,

as well as more complete measures of *in vivo* responses, will be needed to define the networks.

Abundance—Measures of absolute protein abundance are often not available, and lack of such data limits redox model development. The importance of abundance is apparent from the use of rate constants and abundance to calculate rate (Fig. 4B). Protein abundance varies by at least 7 orders of magnitude (74), and there are two systematic ways that this can be combined with rate constants to determine rates, either scaled proportionally or scaled inversely. Using the experimentally determined range for second-order rate constants (k) for protein thiol reaction with H_2O_2 ($1-10^7 M^{-1} s^{-1}$) (73), one can see that variation of the rate constant (Fig. 4B, *middle left panel*) in opposition to abundance (C ; *upper left panel*) results in the product ($k \cdot C$), such that all proteins have the same rate of H_2O_2 metabolism (*lower left panel*). If one considers variation of the rate constant (Fig. 4B, *middle right panel*) in proportion to abundance (*upper right panel*), the product shows that the most abundant protein thiols contribute 14 orders of magnitude more to H_2O_2 metabolism than the least abundant protein thiols (*lower right panel*). Thus, co-evolution of mechanisms to control abundance and reactivity can account for H_2O_2 signaling by a subset of proteins while at the same time having an overall system that is inherently stable to changes in H_2O_2 production.

Spatial Distribution—D'Autréaux (75) addressed spatial distribution of redox elements, which provides specificity in redox signaling. As they discussed, compartment- and microcompartment-specific control through local concentrations of oxidants and reductants may be a common biologic principle. Directional movement through the secretory pathway involves O_2 -driven oxidation of peptidyl-Cys by the endoplasmic reticulum oxidizing system, thiol/disulfide exchange by protein-disulfide isomerases, and other sequential modifications during protein processing (50). A similar oxidative system for protein import into mitochondria occurs (76). Changes in extracellular redox potential regulate intracellular metabolism, but also changes in intracellular metabolism affect redox poise in the extracellular compartment (77).

Partial Oxidation of Proteins under Normal Aerobic Conditions—Early research on glutathionylation of actin at Cys³⁷⁴ (78) and redox Western analysis of protein-disulfide isomerase (79) and Trx1 (20) showed partial oxidation under physiologic conditions. MS studies on yeast extended this to show that many peptidyl-Cys residues are oxidized under control conditions (69), and studies with *Escherichia coli* (60), cultured cells (43), developing *Caenorhabditis elegans* (80), mouse aortic endothelial cells (81), and human colon carcinoma HT29 cells (59) confirmed and extended this finding. Functional pathway analysis showed that peptidyl-Cys mapped to functional networks according to percent oxidation (59). Proteins in cell regulation and actin cytoskeletal proteins appeared as different functional modules (81). Tubulins and other cytoskeletal protein, as well as docking proteins, such as 14-3-3, heat shock proteins, and many heteronuclear ribonucleoproteins, associate into functional networks (59). Many of these Cys residues are highly conserved in vertebrate evolution, indicating that this chemomorphic redox switching system contributes to the

increased percentage of Cys in the proteome with evolution of complexity.

Actin-associated Redox Proteome—Actin provides an example of the complexity of redox systems and also illustrates the importance of dynamic regulation in cell functions. Disulfide bond formation (Cys²⁸⁵–Cys³⁷⁴) inhibits depolymerization of filamentous actin in red blood cells of sickle cell disease (82). Oxidation of cytoskeletal Cys causes protein aggregates, membrane blebbing, and cell death in other cells (83). Actin and other membrane-associated proteins (integrin α 4 and myosin) are targets of oxidation in human peripheral blood mononuclear cells (84), and oxidation of extracellular E_n increases F-actin formation (59), regulating actin (Cys²⁵⁷ and Cys²⁸⁵), several actin-associated proteins, and integrins (81). Glutathionylation of Cys³⁷⁴ by integrin-stimulated reactive oxygen species is essential for F-actin formation in cell spreading (85). Trx1 (Cys⁶²) interaction with actin controls actin dynamics and is essential for anti-apoptotic function (86). Trx reductase is responsible for actin denitrosylation in neutrophils for cytoskeletal control and integrin β 2 function (87). The association of Ref-1 (redox factor-1) with actin in thyroid nuclei modulates proliferation and differentiation (88). Sulfiredoxin acts on hyperoxidized peroxiredoxins (Prxs) (89, 90) to regulate deglutathionylation of actin (91). Cyclophilin B interaction with actin improves Prx activity in synaptosomes (92), and cyclophilin A (Cys¹⁶¹) controls glutathionylation/deglutathionylation of GAPDH, cofilin, and Prx. The cell survival mediator serine/threonine kinase Akt also functions in redox control of actin (93, 94). The number and complexity of these interactions illustrate that sulfur switches evolved as a network of control elements to integrate structure and function.

Interactions with the Metabolome

The study of redox metabolism predates redox proteomics especially in understanding the central hubs of the redox metabolome. NADH/NAD is central to catabolism and ATP production, whereas NADPH/NADP is central to anabolism and control of the redox proteome (95). These hubs are not in redox equilibrium with each other and are not in redox equilibrium with low molecular mass redox hubs (GSH/GSSG and cysteine/cystine) or with protein redox hubs (Trx1 and Trx2). Nonetheless, as shown in Fig. 2, these hubs provide a context to maintain organization of redox metabolism and function of the redox proteome in which directionality is maintained by a stable non-equilibrium state. Temporal and spatial changes in the hubs provide a system for polarity in growth, differentiation, and other functions.

The recent development of high resolution metabolomics using high resolution MS and advanced data extraction (96, 97) has provided the capability to measure >20,000 chemicals in biologic extracts. Application to metabolomics of mitochondria shows the effect of Trx2 overexpression on hundreds of metabolites (98). Integration of metabolomics with redox proteomics provides an approach to elucidate adverse and beneficial interactions of diet and environment through the redox proteome.

Summary

The redox proteome serves as an interface between genome-directed biologic structure and functions and the environmental determinants of those structures and functions. As such, the redox proteome is a central focus in biomedical research. The Cys proteome is particularly important in that the thiol of Cys serves as a versatile sulfur switch to link redox chemistry with structure and function. Advances in understanding the network structure of the redox proteome and interactions with the metabolome can be expected to improve the mechanistic understanding of redox signaling and oxidative stress impacting many aspects of human health and disease.

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