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Forum Review

The Transfer of Reductive Energy and Pace of Proteome Turnover: A Theory of Integrated Catabolic Control

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ABSTRACT

Hundreds of cell proteins undergo reversible transitions among redox states. Coordinate control and common functions served by redox-modified proteins are unknown. The suspect "redox code" integrating metabolome, proteome, and genome remains undefined. Protein redox control involves coupling of the population redox partition to transfer of reductive energy from source to sink. Lessons in metabolic programs under redox coordination might be found in nutritional desperation where reductive transfer from fuel fails to feed pathways to protein reduction. Upon nutritional interruption, proteolysis initially increases. However, catabolism secondarily declines in later starvation so as to postpone loss of the minimal proteome under synthetic failure and delay death. Integrated proteome turnover is paced by reductive transfer coupled to redox states of proteins serving diverse functions. Some continuing proteolysis is redox-independent. Cathepsin B is a model, redox-responsive, catabolic machine among proteins involved in turnover. The CysHis pair is simultaneously a redox-responsive site, an inhibitory metal-binding site, and a peptidolytic reaction mechanism. Pro-region cleavage generates permissive reaction conditions, but not necessarily the maximal peptidolytic rate. Mature cathepsin B can be inactivated by partition into multiple oxidation states. Cathepsin B can be reductively activated by glutathione or disulfhydryl reductases, and redox-buffered by glutathione homodisulfide/glutathione. Topics in protease regulation include: (a) the rate of total cell transfer of nutrient reductive energy from NADPH source potential to reductive pathways, (b) the distribution of reductive energy routed through parallel interactive pathways to protease, (c) the rate of transfer from protease through pathways to oxygen (reactive oxygen species) acceptor at sink potential, and (d) the linkage of protease state partition to relative rates of reductions and oxidations. Cell iron, sulfur, and oxygen redox are inseparable. The interaction of the CysHis site with iron provides a sensor, integrator, and effector switch coupling cathepsin B to metal-sulfur-oxygen redox. Artificial metal-redox-proton switching is a new concept in protein engineering; however, nature has already applied "nanotechnology" to protein redox control. *Antioxid. Redox Signal.* 7, 982–998.

INTRODUCTION: QUESTIONS IN REDOX CONTROL OF PROTEIN FUNCTIONS, INCLUDING CYSTEINE-HISTIDINE (CYSHIS) PROTEASES

REDOX PROTEOMICS reveals that hundreds of protein sulfur sites can undergo reversible transitions among different redox states (8, 9, 11, 13, 14, 22, 27, 38, 41–43, 48, 72, 73, 81, 94, 114, 115). In complex biologic mixtures, the redox state of a protein site is the net result of its interaction with many sulfur, metal, oxygen, and other redox factors. The redox states of proteins can influence conformation, associative properties, enzyme activities, and other physical properties. Many types of protein functions can be redox-modified, including transcriptional regulations, enzyme reactions, and membrane/cytoskeletal functions. However, it is not known whether groups of redox-modified proteins might share common redox controls and serve coordinate functions. Some protein functions that are redox-modified are also controlled

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by redox-independent factors. Protein functions under dual redox-dependent and redox-independent influences include kinase/phosphatase activities, the ubiquitin conjugation pathway, proteasome activity, and the ATP regulatory subunit of a protease targeted by thioredoxin (8). This background questions the identities of all proteins controlled by redox modifications, the coordinate functions served, the mechanisms of coordinate control, and the metabolic conditions or signals that impose coordinate changes. In addition to these uncertainties, there is no unified definition of the cell redox status, and no cipher to the suspected redox code that integrates metabolome, proteome, and genome.

In seeking to define metabolic programs under redox coordination, it seems useful to consider conditions under which the cell undergoes large changes in the redox status. The transfer of reductive energy from fuel through the redox system can vary in relation to nutritional supply from electron source, as well as oxidative demand from electron sink. In late starvation, the deficit of energy from fuel fails to feed the reductive chains. Among programs under redox coordination might be the proteome–metabolome conversion under progress from fed conditions to late starvation. Under nutritional deficit, the machinery of the proteome can be converted to energy for survival via proteolysis and amino acid metabolism. In microbes and mammals, the proteome–metabolome conversion is accompanied by negative nitrogen balance until loss of the minimal proteome causes death (Fig. 1) (76–79, 106). Pacing of the proteome–metabolome conversion is a form of "death delay" under nutritional desperation. Under prolonged starvation, the interconvertibility of cell structure and energy involves a complex compromise among the need for precursors to replace inactivated proteomic machinery, the opposing need for sufficient energy to operate the machinery, and the need to clear denatured machinery. Upon nutritional interruption, protein degradation initially increases so as to provide precursors for adaptive synthesis. Under later starvation, the advantages of turnover are secondarily compromised so as to postpone catastrophic loss of the minimal proteome under synthetic failure.

Most of the steady-state release of [^{3}H]-leucine postcursor from the proteome of a primary tissue bioassay depends on reductive energy transfer as observed under nonrecirculating perfusion. However, a subcomponent of proteolysis is simultaneously redox-independent (76, 77, 106). Among many proteins involved in integrated proteome turnover, the CysHis peptidolytic reaction mechanism mediates the functions of key proteases. The CysHis pair of cathepsin B (cat B) is simultaneously a redox-reactive site, a metal-binding site, a proton-responsive site, and a peptidolytic site. It is routine practice to assay maximal cysteine protease reaction rate following strong reductant, removal of copurified inhibitory metals, and optimal ionization under appropriate pH. CysHis proteases are known to be controlled by pro-region cleavage, compartmentalization, and tissue protease inhibitors. Intracellular CysHis proteases have been assumed to be fully "on-or-off" in relation to pro-region cleavage. Metal-redox-proton modification of mature protease reaction rate has not been proposed as an intracellular control. Fe is a primordial central redox coordinator of metabolome, proteome, and genome in all cell types (48). Speciated metals, sulfur, and oxygen redox are virtually inseparable, and interactive with many metabolites in complex biologic mixtures. It has been recently proposed that the CysHis site is a *sensor* of multiple redox factors, an *integrator* of simultaneous signals, and a *responder–effector switch* wiring the peptidolytic rate to cell control of metal-redox-proton factors (79).

The cell redox circuitry involves nonequilibrium electron transfer from fuel source to oxygen sink, which differs from electrochemical equilibrium. The variable, dynamic, redox partition of a molecular population can be reciprocally cou-

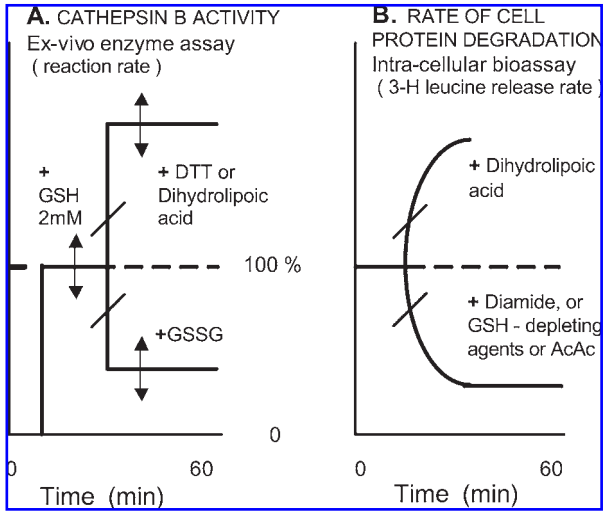

FIG. 1. Experimental redox fluctuations in 100% cathepsin B reaction rate under 2 m*M* GSH, and 100% steady-state release of [^{3}H]leucine postcursor from proteins of a primary perfused tissue bioassay. This composite of data is described quantitatively in several separate reports (76–78, 106). Vertical arrows and slash marks indicate graded, concentration-dependent relationships. (**A**) Cathepsin B was assayed by standard methods in *ex vivo* assay. Air oxidation reversibly inactivates cathepsin B by presumptive oxygenation to sulfenic acid. Addition of endogenous 2 m*M* GSH can promptly reactivate the oxidized protease to a submaximal reaction rate (arbitrary 100% value). In the absence of GSSG, the reaction rate is graded in proportion to the reduced GSH concentration over the endogenous range (data not shown; see 77). The GSH-activated protease is promptly inhibited in proportion to added GSSG. The stronger disulfhydryl reductants dithiothreitol (DTT) (several millimolar) or dihydrolipoic acid (DHLA) (5–50 μ*M*) can cause a higher reaction rate than endogenous GSH monosulfhydryl alone. (**B**) The proteome of an isolated perfused rat heart was biosynthetically labeled with [^{3}H]leucine. The 100% value of integrated proteolysis is the steady-state rate of postcursor release from the proteome via all catabolic pathways under basal reductive energy transfer. Under nonrecirculating perfusion, the basal release of [^{3}H]leucine from the myocardial proteome can be reversibly inhibited by noninjurious infusion of the thiolating agent, diamide (10–100 μ*M*), or intervention in GSH status, or acetoacetate (AcAc) (5 m*M*). Conversely, sustained infusion of 80 μ*M* DHLA causes an elevation above the basal rate of proteome catabolism. The reductive stress of DHLA is followed by injury and death. Constantly sustained tissue exposure to reactive agents occurs within 10 s of infusion.

pled to reductive energy transfer from NADPH source potential to oxygen sink potential. The physics underlying coupling of population redox state partitions and reciprocal energy transfer processes is daunting, and leads beyond the current frontiers of biology. However, present understanding of the chemistry of CysHis proteases, and the biology of proteolytic pathways, provides insights into metal-redox control of other proteins. It appears that evolution has applied "nanotechnology" engineering to the metal-redox-proton control of cell proteins.

THE PACE OF INTEGRATED CELL PROTEIN DEGRADATION IS BIPHASIC DURING PROGRESS FROM FED CONDITIONS TO NUTRITIONAL DESPERATION

Starvation includes many different deficiencies and resulting diseases. Various sensing mechanisms can respond to total energy deficit or selective nutrient deficits, including micronutrients, individual metals, and amino acids. Insufficiencies of energy and/or nutrients elicit complex, multifactorial programs (for reviews, see 7, 36, 39, 46, 56, 57, 64, 69, 75, 85, 105). The molecular biology and pathology of balanced and unbalanced nutritional desperation are currently at the beginning stages, including the bioenergetics of death delay. Among the many metabolic parameters of progressive starvation, changes in glutathione (GSH) and Fe redox, and ketone bodies are particularly relevant to CysHis protease control. Other interesting parameters of progressive starvation are not reviewed here, *e.g.,* ascorbic acid.

Conversion of the proteome to energy by amino acid catabolism is a primordial feature of nutritional economy evolved across phyla. There is some minimal proteome necessary for integrated cell function and survival. Higher organisms have evolved a nutritional buffering system including expenditure of glycogen and fat stores, followed by sacrifice of the proteomes of various tissues at different rates under ketotic conditions. Some human tissues can lose ~90% of their proteome by the time of death, although critical tissues are better defended. Various forms of hibernation are metabolic states evolved to survive starvation under oxidative metabolic shift while preserving the minimal proteome for recovery under better times (18, 19, 63).

Upon nutritional interruption, lysosomal engulfment of cytoplasm initially increases in order to maintain continuing amino acid pools for adaptive synthesis. However, during synthetic failure of later starvation, the advantages of "self eating" change to obvious disadvantage (78). The benefits of constitutive proteome turnover can be compromised under nutritional desperation in order to delay death. In mammalian cells, a distinct subcomponent of ~25% of basal protein degradation continues unabated under supramaximal diamide exposure (76). Redox-independent proteolysis presumably provides continuing clearance of denatured proteins and other functions of proteolysis under late starvation.

ATP requirements for protein degradation range from cytokinetic function to proteolytic enzyme systems. However, biphasic control of proteolysis during progressive starvation cannot be attributed to ATP fluctuations in mammals. In association with requirements for its critical functions, ATP levels are defended into late prelethal starvation. In contrast, transfer through cell redox chains can fluctuate markedly as part of normal cell response to nutritional and endocrine conditions (18, 24, 25, 54, 60, 80, 102, 112). Experimental inhibition of proteolytic pathways with diamide thiolating agent (Fig.1) need not be associated with ATP depletion, contractile dysfunction, or injury (76). Conversely, near-lethal experimental ATP depletion does not simultaneously decrease diamide-responsive proteolytic pathways. Therefore, the ATP requirements for those detectable proteolytic processes requiring ATP are satisfied near the minimal amount of ATP necessary to keep the myocardium alive. In contrast, myocardial protein degradation is quite responsive to noninjurious experimental intervention in GSH pools (77, 79). A small amount of undetected proteolysis might violate these findings on integrated function of major pathways, and some microbial pathways might differ from those in mammals.

Autophagy is a complex process involving multiple controls of vacuolar barrier properties and substrate acquisition (for reviews, see 7, 36, 39, 46, 56, 57, 64, 69, 75, 85, 105). Control of autophagic substrate acquisition is distinct from control of intravacuolar protease function. In diverse organisms, a kinase signaling pathway, TOR (target of rapamycin), senses amino acid levels and adjusts protein synthesis (36, 64). This kinase signal system is suspected of triggering an elevation of vacuolar autophagy in yeast under amino acid deficiency, although the mammalian counterpart is still uncertain. Total energy insufficiency and amino acid deficiencies can vary independently, particularly in organisms relying on dietary amino acids. Whether the TOR autophagic signal system is redox-modified is presently unknown. As starvation progresses, secondary decline of initially elevated proteolysis cannot be explained by any mechanism sensing only deficient amino acid pools, because amino acids continue to decline. Several days of mammalian nutritional interruption cannot be considered to be starvation while metabolism remains nonketotic. Some preemptive inhibitory mechanism must secondarily override control of the initially elevated proteolysis as starvation progresses under continuing amino acid deficiency and ketotic metabolic shift. Implicit in a mechanism delaying death by pacing catabolism is "central authority" dominating diverse other controls influencing proteolysis. Under continuing reductive deficit, oxidative inactivation of diverse catabolic machines might secondarily decrease lysosomal and some extralysosomal proteolysis regardless of all other mechanisms initially promoting autophagic substrate acquisition (78).

A thermodynamic definition of nutritional desperation might include a deficiency of reductive current from source while transfer to sink continues. NADPH is the primary source of reducing energy driving protein reduction and other processes. Oxygen [including reactive oxygen species (ROS)] is the ultimate electron sink permitting flow of metabolic current from source. In most cell types, the major

source of NADPH production and control is believed to be glucose-6-phosphate dehydrogenase (G-6-PD) (2, 3, 33, 98), although other sources and interconversion pathways should be revisited. Under fed conditions, glucose fuel availability does not limit the amount of metabolic current transferred to and through the $NADP^+$/NADPH pool and pathways below. Severe glucoprivation eventually fails to feed the redox chains. Under late starvation, the transfer of reductive energy can be limited by fuel availability regardless of other controls.

Although Fe is a central signal in redox metabolism and transcriptional regulation, the exact relationships between Fe redox state and other parameters of starvation are not well characterized. Fe redox biology began with quinine toxicity observed three centuries ago. "Blackwater fever" is oxidized Fe^{3+} in urine from lysed erythrocytes depleted of reductive energy. Several percent of the human population are genetically deficient in G-6-PD, which was selected for its phenotypic association with resistance to malaria (97). The antimalarial drugs quinine and primaquine disrupt normal reductive pathways. In normal persons, prophylactic dosage with primaquine can sustain 12% methemoglobinemia in stressed erythrocytes without toxicity under long-term dosage. Higher doses of prooxidants can cause erythrocyte GSH depletion and hemolysis. Heritable G-6-PD deficiency can be phenotypically silent until antimalarial drugs or occupational oxidants exceed the ability of mutated G-6-PD to transfer reductive energy into the pathways of erythrocytes (33). The present relevance is that reductive transfer can become limiting in the maintenance of erythrocyte GSH, Fe^{2+}, and protein reduction under moderate, nonlethal source–sink imbalance. Much about metal-sulfur redox actions on cysteine proteases might be learned from the interbiotic parasite–host relationships in erythrocytes and antiparasitic redox pharmacology (for review, see 100).

Among metabolite changes in later starvation, the ketone bodies are central. Transition from glucose utilization to greater reliance upon ketone utilization is a fundamental part of survival metabolism. After several days of total deprivation, mammalian metabolism shifts to increasingly "oxidative" negative energy balance where large 5–10 m*M* amounts of ketone bodies are produced from acetyl CoA (1, 15, 32, 61, 70, 86, 88, 94). Despite depletion of fat stores, ketonemia from acetyl CoA is sustained until death. Lethal diabetic ketoacidosis involves up to 18 m*M* ketonemia. Acetoacetate appears to serve as a redox signal and protein oxidant, as well as currency of exchange between fatty acids and energy. Repeated acetoacetate injections can cause GSH depletion in rats (86, 88), and diabetic ketonemia can cause protein glutathionylation (1, 15, 94).

Progressive starvation is accompanied by GSH pool depletion and oxidation (24, 25, 80, 98, 112). Under artificial prooxidant exposure, protein *S*-glutathionylation appears correlated with the extent of glutathione homodisulfide (GSSG) formation (14). Severe dietary deficiency of cysteine can limit GSH synthesis by glutathione synthetase (112). In late starvation, cell GSH might be synergistically limited by dietary amino acid precursor deficiency, as well as insufficient reductive energy to maintain its reduction. During the severe starvation of hibernation, GSH-GSSG/GSH undergoes prooxidative shift, and ketone bodies from stored fat serve as fuel source. Whether the stupor of hibernation is associated with nitric oxide is controversial and complex (19). Changes in GSH, speciated metals, nitric oxide, ketones, and some other metabolic parameters of starvation are all associated with inhibition of CysHis proteases and cell proteolytic pathways. The combined effects of all individual prooxidative factors on proteases and cell proteolysis are presumably additive or multiplicative.

THE PACE OF MAJOR PROTEOLYTIC PATHWAYS CAN BE COORDINATELY RESPONSIVE TO THE TRANSFER OF REDUCTIVE ENERGY

Basal, steady-state release of [^{3}H]leucine postcursor from the proteome of a primary perfused tissue proceeds at a rate that is intermediate between decreases under prooxidative interventions and increases under proreductive interventions. Diamide causes protein *S*-glutathionylation and GSSG formation. As assayed by nonrecirculating perfusion of rat myocardium, 75% of basal cell protein degradation can be reversibly and noninjuriously inhibited by infusion of diamide; however, an unidentified subcomponent of 25% continues under these prooxidative conditions (Fig. 1) (76). In primary tissue, diamide-inhibitable proteolysis includes all of lysosomal and much of extralysosomal proteolysis. Following diamide washout, reversal of the inhibition of proteolytic pathways can be enhanced by artificial GSH pool repletion under a supraphysiologic exposure to extracellular GSH (78). The diamide-sensitive catabolic subcomponents can also be inhibited by infusion of acetoacetate (5 m*M*) in association with its prooxidative action (78, 86, 88). Metallo exo- and endoproteases are presumably involved in diamide-resistant proteolysis; however, other processes are conceivable. 1-Chloro-2,4-dinitrobenzene (CDNB) is a good substrate for glutathione transferase. CDNB is widely used to deplete tissue GSH with little attack on protein sulfur sites. CDNB mimics the proteolytic inhibition caused by diamide (77); however, CDNB action is irreversible in association with loss of the GSH pool and export of the conjugate from the tissue (58). Proteolysis can be artificially elevated above the basal rate by dihydrolipoic acid (DHLA), serving as permeant surrogate for excess function of the cycliz-ing disulfhydryls of reductases (20) (Fig. 1), *i.e.,* experimental "reductive stress." Although brief diamide exposure is noninjurious, the catabolic elevation caused by supraphysiologic DHLA (Fig. 1B) leads to contractile dysfunction and death. Despite technical obstacles in cultured cells, redox-dependent and -independent subcomponents of protein degradation have been identified in the familiar 3T3 cell (78).

The diversity of redox-responsive machines involved in integrated proteome turnover reveals the pervasiveness of redox catabolic control. Integrated proteome turnover depends on the structures and functions of many cell proteins that can

be reversibly modified by their redox states (13). Profound structural changes following gentle, sublethal, prooxidant exposure can be readily observed in cultured cells with light microscopy, although reductants of the medium can prevent or reverse initial actions of low diamide concentrations.

The lysosomal import of protons and export of Fe both involve redox-dependent transport processes (for review, see 79). Therefore, lysosomal function can be redox-dependent via metal and proton transport controls, as well as protease active-site redox control. Enzymes of the ubiquitin-conjugating system exhibit redox-responsive sulfhydryls, as well as ATP requirement, *i.e.,* dual requirements (92). The chymotryptic 20S subunit of the *S. cerevesiae* proteasome is somehow redox-responsive (31). Redox proteomics revealed the ATP-binding regulatory subunit of the chloroplast clp protease (8) and the regulatory subunit of the 26S proteasome of *Arabidopsis* cytoplasm (114) as targets of thioredoxin; however, roles and controls await characterization. Autophagins are cysteine proteases with unknown present implications (82). Some noncysteine proteases can be biologically redox-responsive via sites remote from the catalytic site. The dimeric retroviral aspartate proteases sense their redox environment via surface sulfhydryls remote from the catalytic site, and activate proteolysis by subunit changes (29). Thus far, the only protease suggested to be inhibited by biologic reduction is extracellular elastase, although the regulatory functions are not clear (30). Multiple redox-modified mechanisms might influence protein degradation indirectly (13). For example, cytoskeletal protein function, membrane structure and flow, compartmental ion transport, interprotein S-S bonding state, and overall cytokinesis can be redox-modified (13, 17, 71, 79, 109). In contrast to cysteine proteases, the reaction mechanisms of metallo, carboxyl, and serine proteases do not require reduction, and need not be inhibited by moderate oxidizing conditions that do not denature proteins.

A PUZZLING DIVERSITY OF DISEASES CAN RESULT FROM HYPERCATABOLISM OR HYPOCATABOLISM INVOLVING NONSELECTIVE CYSHIS PROTEASES

Using knockout mice, cat B and cat L have been objectively discovered in a wide variety of roles unexpected for nonselective proteases (e.g., 55, 56). Excess cell protein degradation can cause hypercatabolic injury, and apoptotic or nonapoptotic death separate from caspases (7, 46, 75). Cathepsin and calpain proteolysis can be either contributory or causative in apoptotic or nonapoptotic death (75). Cat B is fundamentally involved in receptor-mediated apoptosis from tumor necrosis factor-α (TNF) and other causes (23, 48, 74, 86). Cat B has been objectively identified in signal pathways associated with various hypercatabolic pathogenicities (48, 74, 86). Loss of function of the tissue cat B inhibitor, cystatin B, results in hypercatabolic injury and monoclonus epilepsy from cat B hypercatabolism (52, 53). Conversely, insufficient protein degradation can cause failure of protein processing, accumulation of denatured proteins, cell dysfunction, and death. Apoptosis has multiple, confounding triggering mechanisms, including extremes of oxidative and reductive injuries. Apoptosis can be associated with either a hypercatabolic state or prolonged hypocatabolic accumulation of denatured proteins.

Alternative pathways might compensate for some, but not all, defective redox-dependent pathways (34, 90). Knockout of cat B alone is reportedly dispensable, although incompletely characterized. Knockout of cat L alone causes a hyperplasia syndrome (90). In combined knockout of cat B and cat L, mice can survive until death at 2 months from accumulation of denatured brain proteins, and perhaps other mechanisms of injury (34). Thus, lethal hypocatabolic cell damage and apoptosis appear to require severe cathepsin inhibition and a prolonged time for accumulation of a lethal burden of denatured proteins. The list of mutations contributory to oncogenesis now includes failure of autophagic proteolysis via the Beclin-1 gene (for review, see 46). Lysosomal inhibitors alone are not known to be oncogenic, although they could conceivably contribute to failure of normal apoptosis or perhaps tumor growth rate. Chronic, sublethal hypocatabolism might provide a subtle contribution to a wide variety of other diseases ranging from atherogenesis to several neuropathic "failure of catabolism" syndromes (26, 84, 104). Multiple simultaneous controls can interact with redox-responsive proteins (4–6, 16, 26, 28, 35, 37, 44, 45, 47, 50, 51, 59, 65, 67, 93, 95, 96, 99, 103, 113).

The exact noninjurious range of fluctuations above and below the basal rate of proteome degradation are not known. Injury or apoptosis caused by either increase or prolonged decrease in nonselective proteolysis cautions against generalizations as to triggering mechanisms. The ±75% span of experimental degradative fluctuations observable under redox interventions (Fig. 1B) is presumably greater than the *in vivo* range of noninjurious catabolism. The repeated reversibility of diamide action eliminates the possibility that pro-region cleavage alone regulates all redox-dependent proteolysis on a minute-to-minute basis. However, the cell contains large amounts of inactive pro-proteases. Generalized hypercatabolism is widely believed to cause inappropriate intra- or intermolecular activation of pro-proteases to mature proteases (7, 46, 75). Increase in the ongoing reaction rates of mature proteases could increase the intra- or intermolecular activation of caspases, cathepsins, and calpains. A threshold of increased pro-region cleavages could cause an accelerating activation with positive feedback and pan-proteolytic activation. The escape mechanisms permitting injurious hypercatabolism with positive feedback could include elevation of nonselective ongoing protease reaction rates by reductive stress. Hypoxic deficiency of electron sink could be contributory to reductive stress and pathogenic hypercatabolism, although control of the reductive source–sink balance is largely uncharacterized (56).

THE STATE PARTITION OF A POPULATION OF REDOX-RESPONSIVE PROTEINS CAN BE RECIPROCALLY COUPLED TO THE TRANSFER OF REDUCTIVE ENERGY FROM SOURCE TO SINK, AND REDOX COUPLING CAN BE VARIABLE

Protein sites can be partitioned among alternative post-translational energy (redox) states that are related to alternative functional states. Dynamic partition of the chemical states of intracellular protein populations is not a theory, but rather a well established fact supported by diverse model proteins (21, 40, 68, 83, 89, 107). For example, a protein sulfur site can exist in four different oxygenation states: protein monosulfhydryl (Pr-SH), protein sulfenic acid (Pr-SO), protein sulfinic acid (Pr-SO_2), and protein sulfonic acid (Pr-SO_3) (21, 111). Some sulfenic acid derivatives of protein sulfur sites appear to be nonenzymatically reversed by sufficient GSH. Sulfinic acid is suggested to be reversed enzymatically. Sulfonic acid derivatives might not be reversed under most biologic conditions. The peroxiredoxin model illustrates oxidation, "overoxidation," and deep oxidation (oxygenation) states of a protein sulfhydryl site in biologic regulation. The OxyR transcriptional regulator has been suggested to spend its time partitioned among a disulfide-bonded state, oxygenation product, and nitrosation product (68). In the endoplasmic reticulum (ER), a nascent protein population in progress to the native state can exist as Pr(-$SH)_2$, Pr(-SH, -SG), Pr(-SG,-SG), Pr-S-S-Pr, or internally bonded Pr(S-S) (10, 13, 40, 89, 107). The intermediate states can be reshuffled by the ER redox system (reviewed below). In proteins with more pairs of disulfide bonds, the number of possible states is greater. Hemoglobin can be glutathionylated under prooxidants such as acetoacetate (1, 15, 94). Distinct glutathionylation states of hemoglobin have been identified with unknown significance in hemoglobin conformation and packing. Intermolecular protein disulfide formation (Pr-S-S-Pr) has been recently appreciated as another protein redox state partition (for review, see 13). Under *ex vivo* conditions, reversible transitions of papain and/or cat B have been observed among the reduced permissive states, and air-inactivated, presumptive sulfoxide states, GSH-activated reduced states, GSSG-inhibited states, various metal-bound states (Figs. 2 and 3) (76–79, 106), and nitrosylated states of unknown significance (6); others are likely.

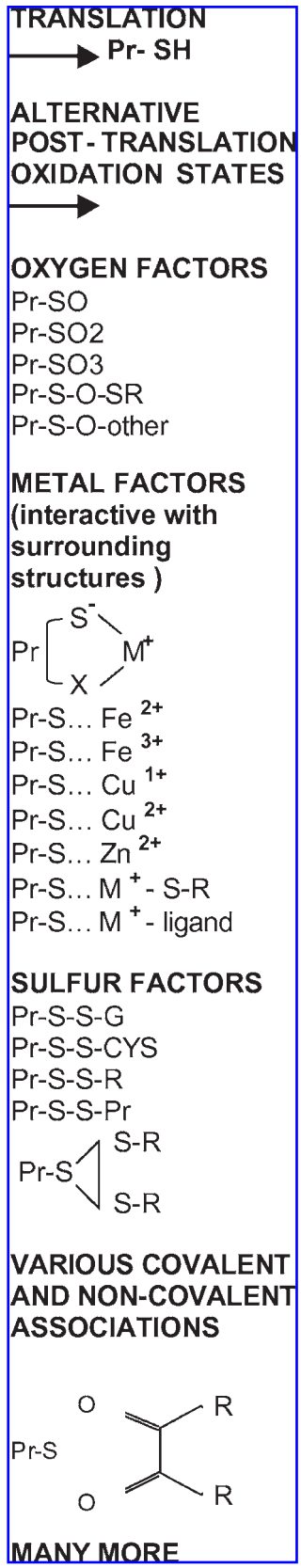

FIG. 2. Some of the many known and predictable posttranslational oxidation states of a protein monosulfur site formed in a complex biologic mixture. In a biologic mixture, protein surface sulfur can undergo many reactions to many products (see text). The possibility of protein-mixed hetero-trisulfide, as well as hetero-disulfide, has been speculatively discussed (43). Various interactions of oxygen with protein and nonprotein sulfur to form mixed oxygenation products are also speculative (54). The exact metal interactions with protein monosulfur or multisulfur sites can depend on surrounding constellations of amino acids, which can increase or decrease the metal–sulfur interactions. A protease CysHis site exhibits all reactions of a protease monosulfur site; however, the additional reactivities of His and the bound metal confer far greater complexity. If a protein function is differentially responsive to the redox state of a bound speciated metal, then the metal can serve as a sensor, redox signal integrator, and switch.

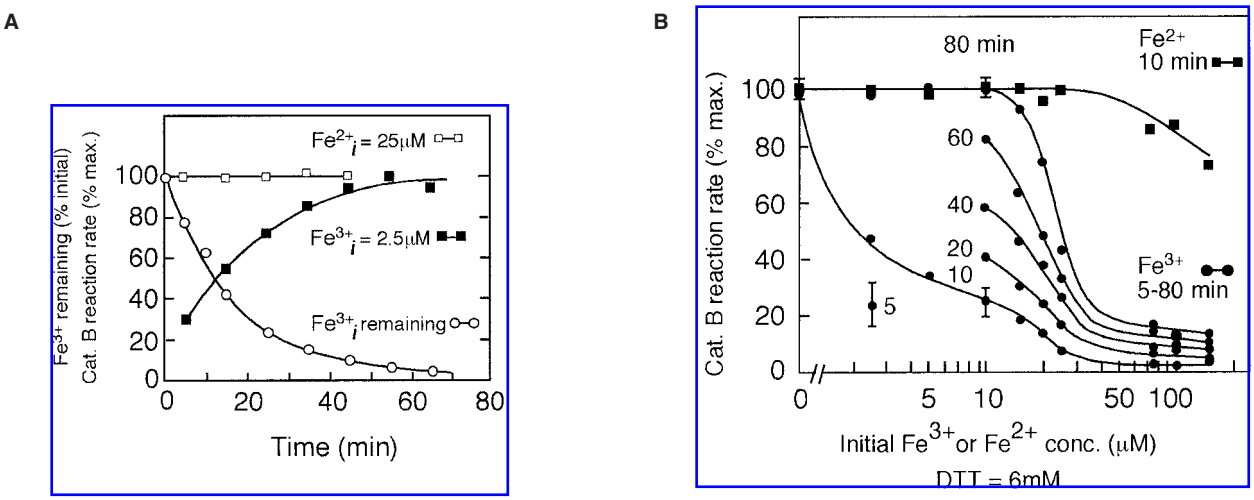

FIG. 3. (A) Reversal of the initial inhibitory action of Fe^{3+} on cat B reaction rate correlated with the time course of reduction of Fe^{3+} to Fe^{2+} caused by DTT. *Ex vivo* enzyme assays of Figs. 1A, 3A, and 3B represent approach to equilibrium conditions *ex vivo*, and not steady-state cell transfer conditions (see 20). Cat B was initially exposed to Fe^{3+} (2.5 μ*M*) or 10-fold higher Fe^{2+} (25 μ*M*) prior to addition of a large excess of DTT (6 m*M*) (79). The oxidized Fe^{3+} state initially inhibited the protease; however, the reduced Fe^{2+} state was only marginally inhibitory at endogenously relevant concentrations. DTT is known to reduce Fe^{3+} to Fe^{2+} with a half-time of ~12–14 min at pH 5–6. As measured separately, the time course of Fe^{3+} reduction to noninhibitory Fe^{2+} by DTT was correlated with the time course of reversal of initial cat B inhibition by Fe^{3+}. Results were similar with GSH reduction of Fe^{3+} although the maximal protease reaction rate sustained under GSH in the absence of Fe^{3+} is less than that with DTT (77). (**B**) Concentration dependence of the initial inhibition of cat B by Fe^{3+} in the presence of excess DTT and progressive reversal over 80 min. The endogenous exchangeable pool of Fe is ~100 μ*M*, and is maintained largely reduced under constitutive conditions of most cell types. Endogenous 100 μ*M* Fe^{2+} caused only a slight apparent inhibition of cat B, and much of this apparent inhibition can be attributed to the artifact of unavoidable initial 1% oxidation of the 100 μ*M* Fe^{2+} under exposure to oxygen in water. The threshold for Fe^{3+} inhibitory action is poised at only several percent oxidation of the endogenous 100 μ*M* pool of Fe^{2+}.

Metabolic reductive energy flows from source potential (largely NADPH) through parallel interactive transfer pathways to sink potential (largely oxygen). Rapid progress is describing the cell reductive transfer circuitry from electron source to electron sink in various cell compartments. Reversible transitions among protein redox states can be coupled to the transfer of electrons from NADPH source potential to oxygen sink potential. Parallel-reciprocal pathways transfer energy from source to sink and also exchange energy between them (Fig. 4). The definition of "irreversible" transfer refers to irreversible acceptance of the source current by the sink; however, reciprocal exchange among pathways can occur in both directions.

The dynamic state partition of protein redox sites can be linked to the flow of metabolic reductive current from fuel source through parallel pathways to oxygen sink. Under dynamic, steady-state partition, the flow of reductive energy into a protein population is balanced by the oxidative flow of energy out of the population. The steady-state partition of a protein population involves the balance between rates of protein reduction and oxidation, which are coupled to the flow of reductive current between source and sink. A population of protein sulfur sites in a complex biological mixture can be distributed in "more energized" or "less energized" states in relation to the relative rates of reductions versus oxidations from all interactive participants (Figs. 4–7). Some protein populations might be distributed in a dynamic partition among more than two states coupled to reductive transfer, *e.g.,* Pr-SH, Pr-S-S-G, metal complexes, nitrosations, Pr-SO, Pr-SO_2, etc. (Fig. 2). The redox coding and decoding of a protein among its chemical states is presumably determined by the intrinsic reactivity of each state with colliding intermediaries of transfer pathways, and many conceivable factors that might change such coupling, *e.g.,* metal homeostasis, metabolite interactions (ketone bodies), nitric oxide signaling, etc.

In reciprocal exchange of energy between intermediaries of parallel pathways, the intermediary with more stored energy can effectively buffer the redox energy state of the reciprocal intermediary with less stored energy. Intermediaries in source to sink pathways can store different amounts of reductive energy. An intermediary in one transferring pathway can exchange its stored energy with an intermediary in a parallel pathway. In the absence of a redox buffer, a slight perturbation in the steady-state source/sink balance could cause wild fluctuations in the redox state of an unbuffered protein or the entire redox proteome. GSSG/GSH, the ratio of oxidized to reduced protein sulfur sites (Pr-S-ox/Pr-S-red), or proteome-S-ox/proteome-S-red are among many interactive intermediaries in the "parallel" pathways of reductive energy transfer (Figs. 4 and 5). Redox buffering over time depends on the relative concentrations/contents and relative transfer rates among all participants interacting (Fig. 5B, and see below).

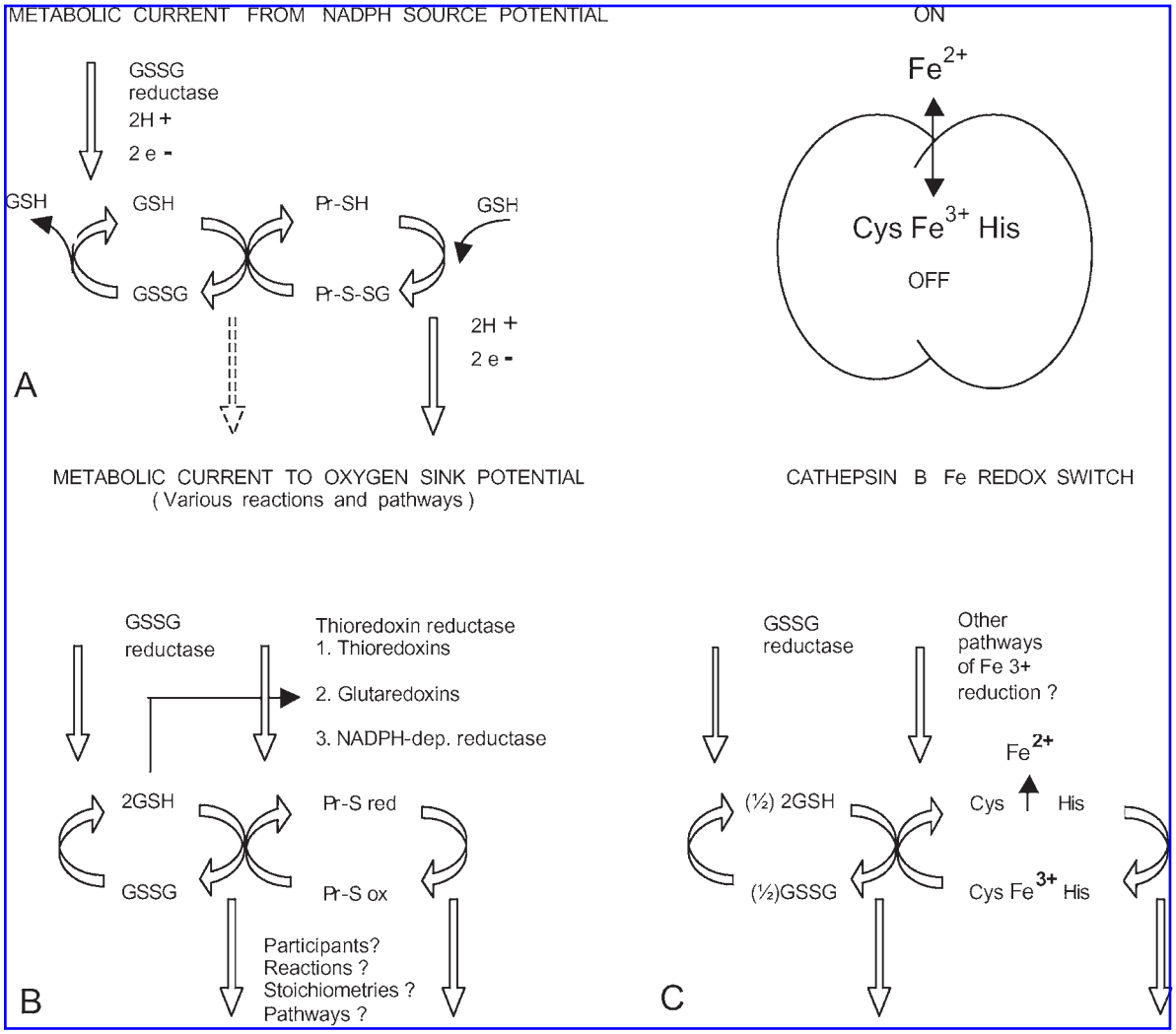

FIG. 4. Topics in CysHis protease redox control under steady-state, nonequilibrium transfer of reductive energy from source potential, through parallel interactive pathways, to sink potential. In A–C, simultaneous redox reactions of protein sulfur sites are represented as dynamic transfer processes as they might occur within the cell redox chains. Peptidolytic activity requires reduction of both Cys and His, removal of inhibitory metals, and pH-dependent ionizations to thiolate anion and imidazolium cation. His can also undergo oxygenation at the 2 position, and covalent adduction with oxidants, although only sulfur oxidation is diagrammed here. Multiple simultaneous reactions can influence the peptidolytic rate of a mature CysHis protease population, and the exact cellular/compartmental picture is not yet defined. Although these three types of transfer processes can occur simultaneously in the identical protease population, they must be represented as three different processes for diagrammatic practicality. (**A**) Dynamic redox buffering of CysHis proteases by GSH, GSSG/GSH.(**B**) Protease reductions by thioredoxins, glutaredoxins, and NADPH-dependent reductases, interactive with redox buffering. These three distinct enzymatic pathways can transfer from NADPH to various oxidized states of a protein site. Some thioredoxin reductases can reportedly transfer to glutaredoxins also (see 112). These pathways are distinct and parallel, but cross-reactive (see text). (**C**) Fe redox switching of CysHis proteases. The CysHis site is "wired" to the cell transfer of reductive energy from source to sink (19). In the presence of excess GSH or DTT, Fe^{3+} inhibits cat B; however, endogenous Fe^{2+} is noninhibitory (Fig. 3). Differential sensitivity of cat B to the redox state of a bound speciated metal reveals a sensor, integrator, and responder-switch linking peptidolysis to all factors that determine Fe concentration and redox ratio. Among several known candidates, the predominant reactions and pathways of Fe redox are uncertain.

Topics in protein redox control include variable regulations of (a) the rate of transfer of nutrient reductive energy from NADPH source potential to reductive pathways, (b) the distribution and rates of reductive energy transfer routed through parallel interactive pathways to proteins, (c) the rate of reductive energy transfer from proteins through pathways to oxygen (ROS) acceptor at sink potential, and (d) the variable coupling of protein state partition to relative rates of all involved transfer processes by reductions relative to oxidations. Organization of these topics suggests the biophysical basis with which to search for the redox code linking individual proteins to cell controls. The redox state partition of a protein population can be determined either by change in the flow of reductive current or by change in the coupling of the protein to constant flow of reductive current (Figs. 4 and 5). Deficient nutrients could cause deficiency of current from

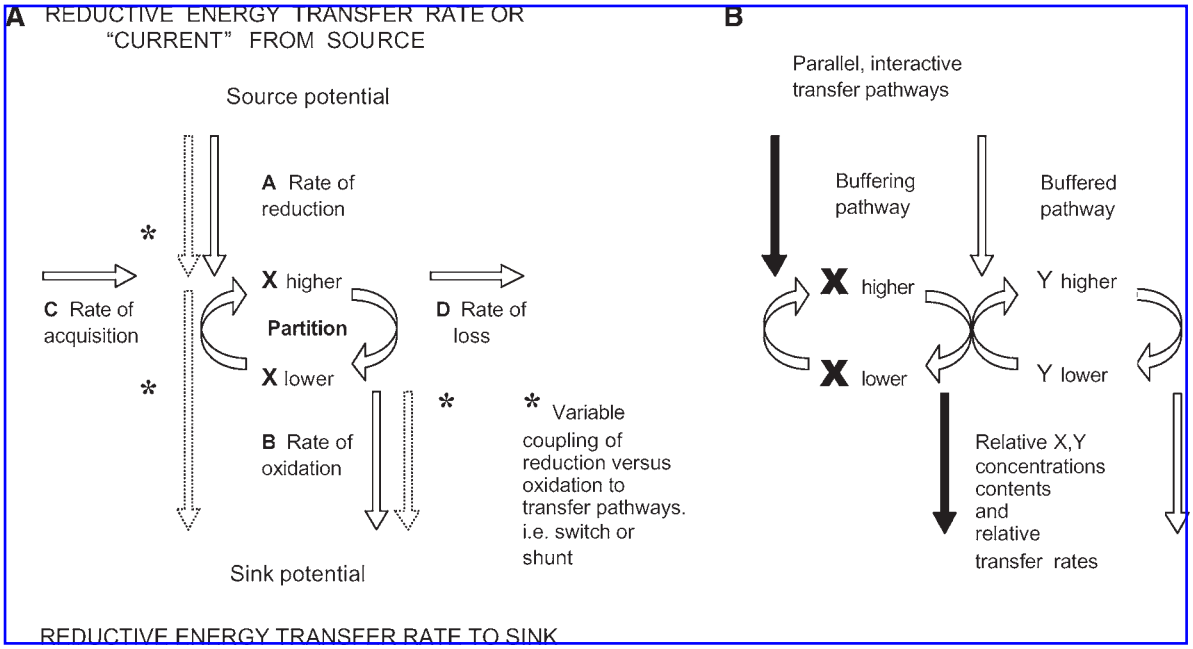

FIG. 5. Hypothetical redox buffering and control of intermediaries in parallel, interactive pathways of reductive energy transfer from source to sink. (**A**) The rate of reductive energy transfer can be considered to be an irreversible current from source potential, distributed through parallel pathway intermediaries, to sink potential. The lower energy state of the hypothetical intermediary, X, can accept reductive energy from current and be promoted (reduced) to a higher energy state (A). The higher state of X can donate electron energy to an intermediary transferring eventually to sink and be demoted (oxidized) to a lower energy state (B). The partition of X between the two states is determined by the relative rates of its promotion versus its demotion. Independent of promotion versus demotion, X can be acquired by the compartment (C) (uptake or synthesis) and can be lost from the compartment (D) (degradation or export). X is interactive with some transfer pathways, but can be "insulated" from some other pathways by nonreactivity with intermediaries. Independent of changes in current flow, the interactions of X with transfer pathways can be increased or decreased by additional participants that change reactivity (asterisks), *e.g.,* Fe^{3+} or redox-active metabolites. (**B**) Reciprocal intermediaries of the transfer pathways can exchange energy between them. An intermediary with greater stored energy can buffer and control a reciprocal intermediary with lesser energy as described in the text. Buffering over time also depends on all relative rates of all processes involved in energy transfer.

source, and deficient oxygen delivery could result in deficiency of current to sink. The protein coupling to constant current might change due to metabolic modifying factors (*e.g.,* acetoacetate, nitric oxide, and others) or "on-off" metal switches (*e.g.,* Fe redox) (79) (Fig. 4). Two different proteins, side by side, could exhibit different coupling and responses to the identical reductive transfer due to differences in intrinsic reactivities of the proteins, *e.g.,* binding of metal intermediaries or switches. Conversely, the identical proteins in different species or compartments of the same cell could be exposed to very different metal-redox-proton environments and energy transfer rates.

Pending better understanding of protein coupling to the cell reductive circuitry, the interactions between protein redox buffering and enzymatic control pathways can best be considered with hypothetical transfer pathways and redox intermediaries. Helpful general principles underlying the cell reductive circuitry can be found in steady-state, nonequilibrium thermodynamics of irreversible processes. Coupling of the energy states of molecular populations to the flow of reductive energy from source to sink also involves concepts of statistical mechanics. Figure 5A is a hypothetical illustration of the regulation of the dynamic state partition of a redox intermediary by its coupling to the flow of reductive current from source to sink potentials. In this pretense, X is a generalized redox intermediate that can exist in only a single higher energy state (*i.e.,* reduced) or a single lower energy state (*i.e.,* oxidized), ignoring various other forms of internal molecular energy. The higher energy state of X could correspond to a reduced protein site, GSH, speciated metal, ascorbic acid, other metabolites, etc., and the lower state of X could be their oxidized counterparts.

In theory, parallel transfer pathways can be intrinsically interactive or noninteractive; however, coupling of pathway intermediaries can be increased or decreased by additional participants. In the hypothetical illustration of Fig. 5A, the reductive source current can be routed through multiple pathways, one of which is coupled to transfer of reductive energy into the internal energy of X. The transferring intermediary of the coupled pathway has a high probability of reductive interaction with X upon collision. The other transfer pathway is parallel, but effectively "insulated" from X. The intermediary of the second pathway has a low probability of reaction with X upon collision. Interactions of X with transferring intermediaries might be increased or decreased by hypothetical coupling/decoupling factors or blocking switches (Fig. 5, asterisks). If X can exist in more than two states (Fig. 2), then the

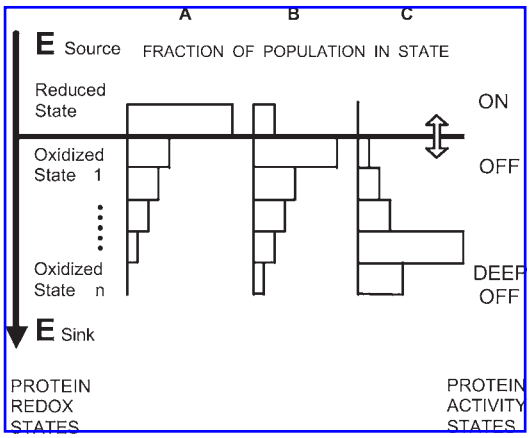

FIG. 6. Questions for future understanding of protein redox coding–decoding. This illustration involves only one reduced "ON" state and five discrete oxidized "OFF" states; however, some proteins can exist in a far greater number of states (Fig. 2). Questions in protein redox coding–decoding include: What protein structures and reaction mechanisms couple the dynamic redox partition of a protein population to the transfer of reductive energy from source to sink? What is the relationship between the redox partition of a protein population and its functional partition? What is the basal redox partition of a protein population under constitutive cell/compartmental conditions, and how does the distribution change under various other metabolic conditions? Condition B might describe the basal status of some cysteine proteases as shown in Figs. 1 and 7. The relationships between some protein functional states and redox states might be opposite to that shown, including graded or biphasic linkages.

interactivity of each different state with intermediaries of transfer pathways could differ markedly, and such could be part of the redox coding of the X population distribution. In real proteins, a functional dependence of CysCys, CysHis, or HisHis metal-binding sites upon the redox state of a speciated metal might permit metals to serve as sensors, integrators, and switches responding to all factors that determine the metal redox state (Figs. 3 and 4C) (discussed in 79).

Change in reductive source availability (nutrition) or sink availability (oxygen delivery or other electron sinks), or both, could change the state partition of an X population linked to reductive current. An increase in reductive current coupled to X might increase the energy of the population partition to a distribution closer to the source potential in the absence of any other changes. Conversely, during late starvation, a severe decrease in current flow might cause a shift in the population partition to an energy distribution closer to sink potential. If the rate of X oxidation by the downstream electron acceptor fluctuates under constant reduction from source, then the X population might shift its partition among higher and lower distributions among redox states. The properties of some redox cycling agents can "short circuit" the transfer pathways, *e.g.,* shunt electrons from NADPH or thioredoxin directly to oxygen sink. Various redox cycling agents create new transfer pathways, or "short circuit" from source to sink. Other drugs can serve as terminal electron acceptors.

The reductive energy stored in the higher state of X is a reservoir between source and sink potentials. The energy stored in the higher state of X is proportional to the metabolically recoverable energy difference in the higher and lower states, and the total amount of higher X in the compartment. In this illustration, X can also be acquired by the compartment (C) and lost from the compartment (D), as is GSH (24, 58, 112). Thus, the amount of energy in higher state X is dependent on the relative rates of its acquisition versus loss and also its reduction versus oxidation. The reciprocity between exchanging intermediaries can blunt the effects of sudden source/sink disparity on the redox partition of the buffered intermediary. The predominance of a buffering intermediary in parallel reciprocal pathways depends on the relative total reservoirs of energy in each reciprocal intermediary, as well as the relative rates of all transfer processes influencing each intermediary. A relatively high rate of transfer into an intermediary present at low concentration (*e.g.,* thioredoxin) might oppose the buffering action of another intermediary present at relatively higher concentration (*e.g.,* GSSG/GSH).

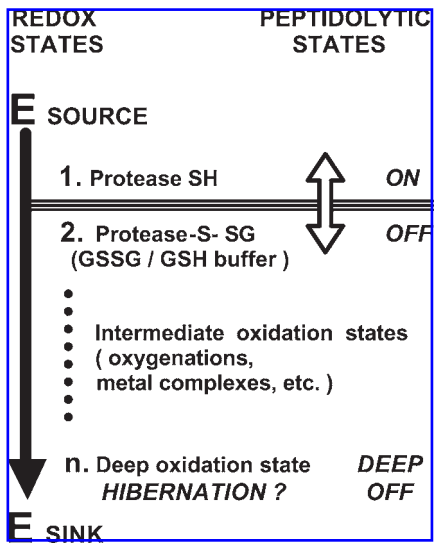

FIG. 7. Proposed basal buffering of intracellular cat B function at a submaximal rate, and preemptive removal of control from the buffer by shift in redox conditions. Following pro-region cleavage, some intracellular cysteine protease populations and proteolytic pathways might function under a basal redox buffer creating a submaximal reaction rate as schematized in Fig. 6B. As described in the text, the submaximal basal reaction rate of the protease population can be increased or decreased by either change in reductive transfer or the reciprocal coupling of protease molecules to transfer intermediaries. Deep oxidation states of the protease or perhaps its metal complexes might correspond to "hibernating" inactive proteases, which require a larger amount of energy to reactivate than a protease hetero-disulfide. Partial hibernation of redox-responsive proteolysis might be pharmacologically achievable in mammals with nutriceutical ketonemia, nitric oxide, or perhaps other agents.

MULTIPLE REACTIONS CAN CREATE RECIPROCITY BETWEEN PARALLEL PATHWAYS OF ENERGY TRANSFER AND THE STATE PARTITION OF CYSHIS PROTEASES

The reduction of various proteins might switch their functions either on or off, or cause graded change in their functions. Current questions in the redox control of proteases are best illustrated when participants and reactions are illustrated in artificial separation as intermediaries in source to sink transfer processes as in Fig. 4 (discussed in 79). Redox pathways are created by the intrinsic properties of redox-active moieties and protein structures of transferring intermediaries, *e.g.,* pyridine nucleotides, flavin and disulfur sites of enzymes, metabolites, metals, and oxygen (ROS), although not reviewed here.

The basis for redox coding–decoding lies in the reciprocal interactions that couple individual protein functions with reductive transfer routed through parallel pathways from source to sink. GSSG/GSH and protease-S-ox/protease-S-red can be thermodynamically interpreted as interactive intermediaries in parallel reciprocal transfer pathways as illustrated with hypothetical X and Y of Fig. 5. Reductive energy transfer from NADPH source, through pathways, to oxygen sink can be reciprocal to the chemical state partition of individual proteins and total redox-responsive proteome. The notion of parallel transfer pathways with reciprocal interactivities can be recognized in the interaction of the redox buffer with the three different reductase chains schematized in artificial isolation in Fig. 4. These multiple inseparable transfer processes are simultaneously under way in a complex mixture of other interactive intermediaries.

Reciprocity in reductive transfer through parallel reciprocal pathways is an essential interpretive tool in protein (protease) redox control; however, evolution has complicated thermodynamic simplicity. Interpretation with idealized thermodynamic formalisms would require that the transfer between pathway intermediaries be linear and equal in both directions. Complex interactions between the parallel reductive pathway intermediaries are implied by many studies (*e.g.,* 2, 4, 9, 12, 20, 28, 45, 50, 54, 56, 62, 76, 99, 101). Interactions between some parallel pathways appear to have evolved such that some transfer between pathway intermediaries is neither linear nor equal in both directions. In the evolution of biologic redox, it appears that nature has complicated the ideal thermodynamic simplicity of reciprocity among parallel transfer pathways as illustrated by the nonbiologic X and Y of Fig. 5.

Multiple mechanisms appear to regulate the distribution of current through interactive transfer pathways and/or reciprocity between pathways below NADPH. Thioredoxin is reportedly glutathionylated under extreme oxidative conditions, which suggests that its transfer might be responsive to conditions determining GSH, GSSG/GSH status (20). Transfer through some thioredoxins is switched by an independent protein (57). Thioredoxin reductase is reported to also transfer to some glutaredoxins, thereby providing additional interactions between these parallel pathways (62). It is suggested that glutaredoxins are regulated enzymes (35, 101). The observations of Fig. 3 reveal that oxidized Fe^{3+} concentration, and Fe^{3+}/Fe^{2+} redox ratio, can preemptively modify the transferring interactions of cat B with S-S/SH redox factors until the Fe^{3+} is reduced to Fe^{2+}. Metals and metabolites might modify thioredoxin and glutaredoxin transfer reactions and protein targeting (28, 59, 113, 114).

GSH-GSSG/GSH redox buffering of protease function is interactive with and inseparable from enzymatic control of protease redox by reductase systems. In pioneering studies, the content of hepatic protein sites forming mixed disulfides was reported to be 20–30 nmol/g of liver (14). GSH is present at 1–10 m*M* concentrations and might store a considerably greater reservoir of reductive energy than does the entire cell content of redox-responsive protein sites. Regardless of exact amounts, the concentrations and total contents of all reversible protein surface thiolation sites of the proteome are less than the contents of GSH and GSSG/GSH buffer sites. At several millimolar, the GSH, GSSG/GSH concentration, and redox ratio can presumably buffer the sulfur redox sites of the proteome, although control also depends on relative rates of active redox processes interacting with the buffer.

Consistent with theory, passive, *ex vivo* redox buffering of cat B involves both concentration of GSH and its redox ratio, as observed in the absence of metals (77, 78). The reaction rate of cat B is proportional to the concentration of reduced GSH without added GSSG. The difference between sustained cat B reaction rate under 0.5 and 5 m*M* reduced GSH is nearly 10-fold as measured *ex vivo* under steady-state reaction conditions without GSSG (22, 78). Thus, fluctuations in cell concentration of reduced GSH, in the absence of GSSG, could influence cat B reaction rate. Even when GSH is completely reduced, the endogenous 1–10 m*M* GSH concentration does not drive the maximally reduced function of the protease population (28, 43). Following cat B activation by several millimolar GSH, a small concentration of 10 μ*M* DHLA can cause further activation to a higher reaction rate (Fig. 1). Cat B also exhibits a steady-state dependence on the GSSG/GSH redox ratio at any particular GSH concentration (77, 78). Decreased GSH concentration or change in GSSG/GSH redox ratio conspire to cooperatively decrease the cat B reaction rate. Less than 10% oxidation of GSH to GSSG can cause an appreciable inhibitory action on the GSH activation of cat B. Stable, artificial disulfide agents, or sufficient concentrations of GSSG, might interact with some protein sulfur sites to form a hetero-trisulfide (43). In the mechanism(s) of protease redox buffering, it is difficult or impossible to distinguish an inhibitory effect of a mixed hetero-trisulfide from protein sulfhydryl–disulfide exchange into a covalent heterodisulfide. Cell GSH content is regulated by relative rates of uptake and synthesis from precursors versus loss and catabolism, as well as relative rates of reduction versus oxidation (24, 70, 112) (Fig. 5A). Although useful, the concept of GSH,

GSSG/GSH protein redox buffering (Fig. 4A) might be an oversimplification of complex reaction mechanisms involving additional participants.

In thermodynamic theory, reductive stress might result from either an excess of enzymatic reductive transfer into the pathways to protein relative to sink or a hypoxic deficiency of acceptance by electron sink or both. The buffering capacity of a passive, *ex vivo* redox buffer is analogous to other passive buffer systems. However, buffering actions involving interactive intermediaries in dynamic, transfer pathways also depend on the relative rates of all transfer processes influencing the buffering and buffered intermediaries. When the GSH buffer is almost entirely reduced, then there is theoretically less protection against spurious changes under enzymatic reductive stress or deficiency of oxygen electron sink. Some redox-active metabolites might also serve as protein redox buffers or modifiers. Under late starvation, several millimolar acetoacetate in conjunction with GSSG might oppose reductive protease activation by the cyclizing disulfhydryl enzyme pathways. The hypothetical possibilities of reductive stresses resulting from sudden deficiency of oxygen sink or activation of reductase chains are not yet verified.

The pathways of transfer from reduced proteins to oxygen are uncertain except in the ER; however, speciated metals, ROS, and a few metabolites are among candidates. The function of the ER protein redox pathway is not to regulate proteins via surface redox sites, but rather to mediate protein sulfur oxido-reductions and final formation of internal disulfide bonds of native proteins. The oxygen electron sink diffuses freely through compartmental barriers; however, the enzymatic and nonenzymatic means of transfer from proteins to oxygen are highly compartmentalized. Recent advances in the ER redox control reveal corresponding gaps in understanding of protein redox outside this compartment (for reviews, see 10, 40, 89, 107). Reductive transfer within the ER compartment corresponds to "far from equilibrium" conditions of energy transfer, whereas other cell compartments might be interpreted as "near-equilibrium" conditions of transfer. The reducing conditions of the cytoplasm are not favorable for the rapid formation of disulfide bonds converting a nascent unfolded protein into a native folded protein. Unassisted diatomic oxygen is not sufficiently reactive to serve as a direct electron acceptor for rapid protein oxidation to internal disulfide bonds. Previous speculations as to the protein-reactive electron acceptor in the ER centered around dehydroascorbic acid. Interestingly, Fe and ascorbate are highly reactive, and ascorbate is a major contributor to Fe^{3+} reduction. However, a compartmentalized FAD-transferring enzyme is now shown to be the electron acceptor interacting with proteins in a rapid relay to oxygen (107). As the result of rapid transfer of electrons to sink, a large fraction of the glutathione in the ER is found in disulfide linkage with unfolded protein sulfur sites (10). Nascent protein sulfhydryls can be oxidatively mismatched to inappropriate internal disulfides, or *S*-glutathionylated. Regulation of the content of flavoprotein transfer protein of this pathway to sink is adjusted to oxygen tension (40). This important finding suggests that variable delivery of the oxygen electron sink might be a rate-limiting factor in other protein redox regulation. Indeed, sustained inhalation of high oxygen is lethal in mammals, and toxic even to lung tissue.

In contrast to the rapid enzymatic electron relay within the ER, the reactions and pathways transferring from protein to oxygen sink are largely unknown outside the ER. *Ex vivo* chemistry suggests that a mutually reactive, speciated metal could provide an important reaction mechanism and pathway from proteins to oxygen sink. After accepting an electron from reduced proteins, speciated metals can increase the reactivity of diatomic oxygen. Other candidate electron acceptors from proteins include various metabolites, such as dehydroascorbic acid, and metal–metabolite interactions. Oxidative signal generators are another unknown, *e.g.,* NADPH oxidases, nitric oxide production, and others.

One electron transition between Fe^{3+} and Fe^{2+} can operate a redox switch on the cat B peptidolytic mechanism, and might also serve as a conduit pathway from protein to oxygen sink, i.e., to "wire" the protein to reductive transfer (Figs. 3 and 4C). Speciated metal switching is a new concept in protein engineering (113), and some of these ideas can be borrowed in understanding protease control (79, 106). Metal, sulfur, and oxygen redox are virtually inseparable, and can also be interactive with protonations. In the presence of excess GSH or (DTT), Fe^{3+} is inhibitory to cat B; however, the endogenous concentration of Fe^{2+} is almost noninhibitory (Fig. 3). Fe^{3+} can inhibit reduced CysHis proteases by binding to the reduced catalytic pair so as to interfere with substrate hydrolysis. In mixtures, speciated metals might also catalyze protease hetero-disulfide or oxygenation products (65). In addition to direct CysHis active-site switching, the electron-accepting action of Fe^{3+} might oxidize the protease and create a transferring pathway from reduced protease to oxygen.

Fe^{3+}/Fe^{2+} is a central intermediary redox couple in pathways from source to sink (16, 37, 48). Tissue fluctuations in Fe redox during starvation have apparently not been studied. If the endogenous 100 μM Fe pool were entirely reduced, it might be almost inactive against cat B, and the S-S/SH controls would then predominate. Multiple enzymatic and nonenzymatic reactions and pathways can reduce or oxidize Fe^{3+} in various cell types and compartments; however, the exact contribution of each is unknown. Fe^{2+} is rapidly oxidized to Fe^{3+} in air-equilibrated water, and oxidizes instantaneously with ROS. In *ex vivo* assay, excess GSH or DTT can reduce inhibitory Fe^{3+} to noninhibitory Fe^{2+}, and also reduce oxidized states of the protease in the absence of Fe^{3+} (Fig. 3) (79). In addition to GSH reduction, pathways to metal reductions involve the disulfur reductases, lipoylated enzymes, and ascorbic acid cycling; however, the predominant pathways of Fe^{3+} reductions are unknown. Oxidation of a small fraction of the endogenous 100 μM Fe pool concentration is the threshold for inhibition of reduced cat B in the presence of excess GSH (Figs. 3 and 4C) (79).

There might be no absolute intracellular chemical state of some redox-responsive proteins, but rather a dependence

of their posttranslational redox partition upon coupling to energy transfer. Relative to some basal reference redox partition, a population of protein sulfur sites might fluctuate between a "more reduced" and "more oxidized" distribution among alternative posttranslational oxidation states in response to the rate of current flow or coupling to it (Figs. 6 and 7). Under basal conditions or moderate oxidative conditions, the predominant partition might consist largely of Pr-SH and Pr-S-SG functioning at submaximal peptidolytic rate. A protease population partitioned among deep oxidation states might be considered to be in hibernation (Fig. 7). A protease population activated above the constitutive buffer might be hypercatabolic, causing degradative injury or secondary pan-protease activation.

THERAPEUTIC INCREASES OR DECREASES IN REDOX-DEPENDENT PROTEIN DEGRADATION MIGHT BE USEFUL IN DIFFERENT DISEASES OF INSUFFICIENT OR EXCESS PROTEOLYSIS

Metal homeostasis and redox metabolism can be pathogenically deranged and pharmacologically manipulated in humans. Targeting of Fe to the active sites of CysHis proteases by cobinding drug ligands has previously been discussed in relation to development of redox-active, antiparasitic protease inhibitors (106). The antilysosomal antiinflammatory action of colloidal gold in phagocytic cells is most likely related to the present findings.

Cytotoxic chemotherapy seeks to cause tolerable apoptosis; however, the responsiveness of apoptotic programs is decreased in resistant neoplastic cells. Cat B is somehow involved in the apoptotic process caused by TNF (23, 48). Thus, TNF is an endogenous proapoptotic agent acting by hypercatabolic mechanisms requiring cat B. Following cytotoxic chemotherapy, the apoptotic decision might be pharmacologically enhanced by reductive activation of cat B, as well as other cysteine proteases. Synthetic disulfur agents and metal chelators can be simultaneously tolerated by humans. It might be possible to increase mature cat B reaction rate, and activation of various pro-proteases in neoplastic cells (100), by simultaneous intervention in metal-redox if combined toxicities prove to be acceptable.

Mammalian hibernation has long been a model with which to study medical or surgical protection of brain or heart under temporary ischemia of hours to days. Severe, prolonged hypocatabolism is eventually pathogenic; however, injurious accumulation of denatured proteins does not occur immediately upon inhibition of proteolysis (see above). Under hibernation, proteome protection in mammals is accompanied by ketonemia and GSH oxidation, and perhaps nitric oxide formation. Ketonemia, high nitric oxide, and deficient transfer of reductive energy can all inhibit CysHis proteases (79). Protection of the hypoxic brain with nitric oxide is already under investigation in association with other proposed mechanisms (not reviewed here). Ketone bodies are already safely administered to humans for hyperinsulinemic hypoglycemia and other applications (for review, see 108). It seems quite possible that the temporarily hypoxic human brain or heart could be safely protected against hypercatabolism by redox inhibition of cysteine proteases with simultaneously administered ketone bodies, nitric oxide, or perhaps other antiproteolytic agents to be developed. A role of protein redox sites in coordinate metabolic regulation was proposed long ago (116); this should be revisited in light of many subsequent advances.

ABBREVIATIONS

cat B, cathepsin B; CDNB, 1-chloro-2,4-dinitrobenzene; CysHis, cysteine-histidine; DHLA, dihydrolipoic acid; DTT, dithiothreitol; ER, endoplasmic reticulum; G-6-PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSSG, glutathione homodisulfide; Pr-SH, protein monosulfhydryl; Pr-SO, protein sulfenic acid; Pr-SO_2, protein sulfinic acid; Pr-SO_3, protein sulfonic acid; Pr-S-ox/Pr-S-red, ratio of oxidized to reduced protein sulfur sites; Pr-S-S-G, protein *S*-glutathione hetero-disulfide; ROS, reactive oxygen species; TNF, tumor necrosis factor-α.

REFERENCES

1. Al-Abed Y, Van Patten S, Li H, Lawson JA, FitzGerald GA, Manogue KR, and Bucala R. Characterization of a novel hemoglobin–glutathione adduct that is elevated in diabetic patients. *Mol Med* 7: 619–623, 2001.
2. Amir-Ahmady B and Salati LM. Regulation of the processing of glucose-6-phosphate dehydrogenase mRNA by nutritional status. *J Biol Chem* 276: 10514–10523, 2001.
3. Andres A, Satrustegui J, and Machado A. Development of NADPH-producing pathways in rat heart. *Biochem J* 186: 799–803,1980.
4. Arner ES and Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 267: 6102–6109, 2000.
5. Arunachalam B, Phan UT, Geuze HJ, and Cresswell P. Enzymatic reduction of disulfide bonds in lysosomes: characterization of a gamma-interferon-inducible lysosomal thiol reductase (GILT). *Proc Natl Acad Sci U S A* 97: 745–750, 2000.
6. Ascenzi P, Salvati L, Bolognesi M, Colasanti M, Polticelli F, and Venturini G. Inhibition of cysteine protease activity by NO-donors. *Curr Protein Pept Sci* 2: 137–153, 2001.
7. Assuncao Guimaraes C and Linden R. Programmed cell deaths. Apoptosis and alternative death styles. *Eur J Biochem* 271: 1638–1650, 2004.
8. Balmer Y, Koller A, del Val G, Manieri W, Schurmann P, and Buchanan BB. Proteomics gives insight into the regulatory function of chloroplast thioredoxins. *Proc Natl Acad Sci U S A* 100: 370–375, 2003.
9. Balmer Y, Vensel WH, Tanaka CK, Hurkman WJ, Gelhaye E, Rouhier N, Jacquot JP, Manieri W, Schurmann P, Droux M, and Buchanan BB. Thioredoxin links redox to the regulation of fundamental processes of plant mito-

chondria. *Proc Natl Acad Sci U S A* 101: 2642–2647, 2004.
10. Bass R, Ruddock LW, Klappa P, and Freedman RB. A major fraction of endoplasmic reticulum-located glutathione is present as mixed disulfides with protein. *J Biol Chem* 279: 5257–5262, 2004.
11. Baty JW, Hampton MB, and Winterbourn CC. Detection of oxidant sensitive thiol proteins by fluorescence labeling and two-dimensional electrophoresis. *Proteomics* 2: 1261–1266, 2002.
12. Biaglow JE, Donahue J, Tuttle S, Held K, Chrestensen C, and Mieyal J. A method for measuring disulfide reduction by cultured mammalian cells: relative contributions of glutathione-dependent and glutathione-independent mechanisms. *Anal Biochem* 281: 77–86, 2000.
13. Brennan JP, Wait R, Begum S, Bell JR, Dunn MJ, and Eaton P. Detection and mapping of widespread intermolecular protein disulfide formation during cardiac oxidative stress using proteomics with diagonal electrophoresis. *J Biol Chem* 279: 41352–41360, 2004.
14. Brigelius R, Muckel C, Akerboom TP, and Sies H. Identification and quantitation of glutathione in hepatic protein mixed disulfides and its relationship to glutathione disulfide. *Biochem Pharmacol* 32: 2529–2534, 1983.
15. Bursell SE and King GL. The potential use of glutathionyl hemoglobin as a clinical marker of oxidative stress. *Clin Chem* 46: 145–146, 2000.
16. Cabantchik ZI, Kakhlon O, Epsztejn S, Zanninelli G, and Breuer W. Intracellular and extracellular labile iron pools. *Adv Exp Med Biol* 509: 55–75, 2002.
17. Caplan JF, Filipenko NR, Fitzpatrick SL, and Waisman DM. Regulation of annexin A2 by reversible glutathionylation. *J Biol Chem* 279: 7740–7750, 2004.
18. Carey HV, Rhoads CA, and Aw TY. Hibernation induces glutathione redox imbalance in ground squirrel intestine. *J Comp Physiol [B]* 173: 269–276, 2003.
19. Carey HV, Andrews MT, and Martin SL. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev* 83: 1153–1181, 2003.
20. Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massignan T, Salmona M, Chang G, Holmgren A, and Ghezzi P. Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. *Proc Natl Acad Sci U S A* 99: 9745–9749, 2002.
21. Chevallet M, Wagner E, Luche S, van Dorsselaer A, Leize-Wagner E, and Rabilloud T. Regeneration of peroxiredoxins during recovery after oxidative stress: only some overoxidized peroxiredoxins can be reduced during recovery after oxidative stress. *J Biol Chem* 278: 37146–37153, 2003.
22. Collison MW, Beidler D, Grimm LM, and Thomas JA. A comparison of protein *S*-thiolation (protein mixed-disulfide formation) in heart cells treated with *t*-butyl hydroperoxide or diamide. *Biochim Biophys Acta* 23: 58–67, 1986.
23. Costelli P, Aoki P, Zingaro B, Carbo N, Reffo P, Lopez-Soriano FJ, Bonelli G, Argiles JM, and Baccino FM. Mice lacking TNFalpha receptors 1 and 2 are resistant to death and fulminant liver injury induced by agonistic anti-Fas antibody. *Cell Death Differ* 10: 997-1004, 2003.
24. Cotgreave IA. Analytical developments in the assay of intra- and extracellular GSH homeostasis: specific protein *S*-glutathionylation, cellular GSH and mixed disulphide compartmentalisation and interstitial GSH redox balance. *Biofactors* 17: 269–277, 2003.
25. Cotgreave IA, Goldschmidt L, Tonkonogi M, and Svensson M. Differentiation-specific alterations to glutathione synthesis in and hormonally stimulated release from human skeletal muscle cells. *FASEB J* 16: 435–437, 2002.
26. Crabb JW, O'Neil J, Miyagi M, West K, and Hoff HF. Hydroxynonenal inactivates cathepsin B by forming Michael adducts with active site residues. *Protein Sci* 11: 831–840, 2002.
27. Cumming RC, Andon NL, Haynes PA, Park M, Fischer WH, and Schubert D. Protein disulfide bond formation in the cytoplasm during oxidative stress. *J Biol Chem* 279: 21749–21758, 2004.
28. Danon A. Redox reactions of regulatory proteins: do kinetics promote specificity? *Trends Biochem Sci* 27: 197–203, 2002.
29. Davis DA, Brown CA, Newcomb FM, Boja ES, Fales HM, Kaufman J, Stahl SJ, Wingfield P, and Yarchoan R. Reversible oxidative modification as a mechanism for regulating retroviral protease dimerization and activation. *J Virol* 77: 3319–3325, 2003.
30. del Val G, Hagie FE, and Buchanan BB. Thioredoxin-(dithiol-)linked inactivation of elastase. *Mol Immunol* 38: 759–763, 2002.
31. Demasi M, Silva GM, and Netto LE. 20S proteasome from *Saccharomyces cerevisiae* is responsive to redox modifications and is *S*-glutathionylated. *J Biol Chem* 278: 679–685, 2003.
32. Dincer Y, Akcay T, Alademir Z, and Ilkova H. Effect of oxidative stress on glutathione pathway in red blood cells from patients with insulin-dependent diabetes mellitus. *Metabolism* 51: 1360–1362, 2002.
33. Djerassi L. Hemolytic crisis in G6PD-deficient individuals in the occupational setting. *Int Arch Occup Environ Health* 71 Suppl: S26–S28, 1998.
34. Felbor U, Kessler B, Mothes W, Goebel HH, Ploegh HL, Bronson RT, and Olsen BR. Neuronal loss and brain atrophy in mice lacking cathepsins B and L. *Proc Natl Acad Sci U S A* 99: 7883–7888, 2002.
35. Fernandes AP and Holmgren A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antioxid Redox Signal* 6: 63–74, 2004.
36. Fingar DC and Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 23: 3151–3171, 2004.
37. Finney LA and O'Halloran TV. Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. *Science* 300: 931–936, 2003.
38. Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M, Bonetto V, Mengozzi M, Duffieux F, Miclet E, Bachi A, Vandekerckhove J, Gianazza E, and Ghezzi P.

Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc Natl Acad Sci U S A* 99: 3505–3510, 2002.
39. Furuta S, Hidaka E, Ogata A, Yokota S, and Kamata T. Ras is involved in the negative control of autophagy through the class I PI3-kinase. *Oncogene* 23: 3898–3904, 2004.
40. Gess B, Hofbauer KH, Wenger RH, Lohaus C, Meyer HE, and Kurtz A. The cellular oxygen tension regulates expression of the endoplasmic oxidoreductase ERO1-Lalpha. *EurJ Biochem* 270: 2228–2235, 2003.
41. Ghezzi P and Bonetto V. Redox proteomics: identification of oxidatively modified proteins. *Proteomics* 3: 1145–1153, 2003.
42. Ghezzi P, Romines B, Fratelli M, Eberini I, Gianazza E, Casagrande S, Laragione T, Mengozzi M, Herzenberg LA, and Herzenberg LA. Protein glutathionylation: coupling and uncoupling of glutathione to protein thiol groups in lymphocytes under oxidative stress and HIV infection. *Mol Immunol* 38: 773–780, 2002.
43. Gilbert HF. Thiol/disulfide exchange equilibria and disulfide bond stability. *Methods Enzymol* 251: 8–28, 1995.
44. Giles NM, Watts AB, Giles GI, Fry FH, Littlechild JA, and Jacob C. Metal and redox modulation of cysteine protein function. *Chem Biol* 10: 677–693, 2003.
45. Gladyshev VN. Methionine sulfoxide reduction in mammals: characterization of methionine-*R*-sulfoxide reductases. *Mol Biol Cell* 15: 1055–1064, 2004.
46. Gozuacik D and Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. *Oncogene* 23: 2891–2906, 2004.
47. Gravina SA and Mieyal JJ. Thioltransferase is a specific glutathionyl mixed disulfide oxidoreductase. *Biochemistry* 32: 3368–3376, 1993.
48. Guicciardi ME, Miyoshi H, Bronk SF, and Gores GJ. Cathepsin B knockout mice are resistant to tumor necrosis factor-alpha-mediated hepatocyte apoptosis and liver injury: implications for therapeutic applications. *Am J Pathol* 159: 2045–2054, 2001.
49. Hentze MW, Muckenthaler MU, and Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. *Cell* 117: 285–297, 2004.
50. Hirt RP, Muller S, Embley TM, and Coombs GH. The diversity and evolution of thioredoxin reductase: new perspectives. *Trends Parasitol* 18: 302–308, 2002.
51. Honda K, Casadesus G, Petersen RB, Perry G, and Smith MA. Oxidative stress and redox-active iron in Alzheimer's disease. *Ann N Y Acad Sci* 1012: 179–182, 2004.
52. Houseweart MK, Vilaythong A, Yin XM, Turk B, Noebels JL, and Myers RM. Apoptosis caused by cathepsins does not require Bid signaling in an in vivo model of progressive myoclonus epilepsy (EPM1). *Cell Death Differ* 10: 1329–1335, 2003.
53. Houseweart MK, Pennacchio LA, Vilaythong A, Peters C, Noebels JL, and Myers RM. Cathepsin B but not cathepsins L or S contributes to the pathogenesis of Unverricht–Lundborg progressive myoclonus epilepsy (EPM1). *J Neurobiol* 56: 315–327, 2003.
54. Huang KP and Huang FL. Glutathionylation of proteins by glutathione disulfide *S*-oxide. *Biochem Pharmacol* 64: 1049–1056, 2002.
55. Huang Q, Raya A, DeJesus P, Chao SH, Quon KC, Caldwell JS, Chanda SK, Izpisua-Belmonte JC, and Schultz PG. Identification of p53 regulators by genome-wide functional analysis. *Proc Natl Acad Sci U S A* 101: 3456–3461, 2004.
56. Hui TY, Sheth SS, Diffley JM, Potter DW, Lusis AJ, Attie AD, and Davis RA. Mice lacking thioredoxin-interacting protein provide evidence linking cellular redox state to appropriate response to nutritional signals. *J Biol Chem* 279: 24387–24393, 2004.
57. Inoki K, Zhu T, and Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115: 577–590, 2003.
58. Ishikawa T and Sies H. Cardiac transport of glutathione disulfide and S-conjugate. Studies with isolated perfused rat heart during hydroperoxide metabolism. *J Biol Chem* 259: 3838–3843, 1984.
59. Jacob C, Giles GI, Giles NM, and Sies H. Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew Chem Int Ed Engl* 42: 4742–4758, 2003.
60. Jaeschke H and Wendel A. Diurnal fluctuation and pharmacological alteration of mouse organ glutathione content. *Biochem Pharmacol* 34: 1029–1033, 1985.
61. Jain SK and McVie R. Hyperketonemia can increase lipid peroxidation and lower glutathione levels in human erythrocytes in vitro and in type 1 diabetic patients. *Diabetes* 48: 1850–1855, 1999.
62. Johansson C, Lillig CH, and Holmgren A. Human mitochondrial glutaredoxin reduces *S*-glutathionylated proteins with high affinity accepting electrons from either glutathione or thioredoxin reductase. *J Biol Chem* 279: 7537–7543, 2004.
63. Kabine M, El Kebbaj MS, Hafiani A, Latruffe N, and Cherkaoui-Malki M. Hibernation impact on the catalytic activities of the mitochondrial D-3-hydroxybutyrate dehydrogenase in liver and brain tissues of jerboa (*Jaculus orientalis*). *BMC Biochem* 4: 11, 2003.
64. Kamada Y, Sekito T, and Ohsumi Y. Autophagy in yeast: a TOR-mediated response to nutrient starvation. *Curr Top Microbiol Immunol* 279: 73–84, 2004.
65. Kanazawa H, Fujimoto S, and Ohara A. On the mechanism of inactivation of active papain by ascorbic acid in the presence of cupric ions. *Biol Pharm Bull* 17: 789–793, 1994.
66. Kaznelson DW, Bruun S, Monrad A, Gjerlov S, Birk J, Ropke C, and Norrild B. Simultaneous human papilloma virus type 16 E7 and cdk inhibitor p21 expression induces apoptosis and cathepsin B activation. *Virology* 320: 301–312, 2004.
67. Kerblat I, Drouet C, Chesne S, and Marche PN. Importance of thioredoxin in the proteolysis of an immunoglobulin G as antigen by lysosomal Cys-proteases. *Immunology* 97: 62–68, 1999.
68. Kim SO, Merchant K, Nudelman R, Beyer WF Jr, Keng T, DeAngelo J, Hausladen A, and Stamler JS. OxyR: a molecular code for redox-related signaling. *Cell* 109: 383–396, 2002.
69. Kirkegaard K, Taylor MP, and Jackson WT. Cellular autophagy: surrender, avoidance and subversion by microorganisms. *Nat Rev Microbiol* 2: 301–314, 2004.

70. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev* 15: 412–426, 1999.
71. Landino LM, Moynihan KL, Todd JV, and Kennett KL. Modulation of the redox state of tubulin by the glutathione/glutaredoxin reductase system. *Biochem Biophys Res Commun* 314: 555–560, 2004.
72. Lind C, Gerdes R, Hamnell Y, Schuppe-Koistinen I, von Lowenhielm HB, Holmgren A, and Cotgreave IA. Identification of *S*-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. *Arch Biochem Biophys* 406: 229–240, 2002.
73. Linke K and Jakob U. Not every disulfide lasts forever: disulfide bond formation as a redox switch. *Antioxid Redox Signal* 5: 425–434, 2003.
74. Liu N, Raja SM, Zazzeroni F, Metkar SS, Shah R, Zhang M, Wang Y, Bromme D, Russin WA, Lee JC, Peter ME, Froelich CJ, Franzoso G, and Ashton-Rickardt PG. NF-kappaB protects from the lysosomal pathway of cell death. *EMBO J* 22: 5313–5322, 2003.
75. Lockshin RA and Zakeri Z. Caspase-independent cell death? *Oncogene* 23: 2766–2773, 2004.
76. Lockwood TD. Redox-dependent and redox-independent subcomponents of protein degradation in perfused myocardium. *Am J Physiol* 276: E945–E954, 1999.
77. Lockwood TD. Cathepsin B responsiveness to glutathione and lipoic acid redox. *Antioxid Redox Signal* 4: 681–691, 2002.
78. Lockwood TD. Redox pacing of proteome turnover: influences of glutathione and ketonemia. *Arch Biochem Biophys* 417: 183–193, 2003.
79. Lockwood TD. Cys-His proteases are among the wired proteins of the cell. *Arch Biochem Biophys* 432: 12–24, 2004.
80. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J* 13: 1169–1183, 1999.
81. Mannervik B, Axelsson K, Sundewall AC, and Holmgren A. Relative contributions of thioltransferase- and thioredoxin-dependent systems in reduction of low-molecular-mass and protein disulphides. *Biochem J* 213: 519–523, 1983.
82. Marino G, Uria JA, Puente XS, Quesada V, Bordallo J, and Lopez-Otin C. Human autophagins, a family of cysteine proteinases potentially implicated in cell degradation by autophagy. *J Biol Chem* 278: 3671–3678, 2003.
83. Mawatari S and Murakami K. Different types of glutathionylation of hemoglobin can exist in intact erythrocytes. *Arch Biochem Biophys* 421: 108–114, 2004.
84. McGrath ME. The lysosomal cysteine proteases. *Annu Rev Biophys Biomol Struct* 28: 181–204, 1999.
85. Meiling-Wesse K, Barth H, Voss C, Eskelinen EL, Epple UD, and Thumm M. Atg21 is required for effective recruitment of Atg8 to the preautophagosomal structure during the Cvt pathway. *J Biol Chem* 279: 37741–37750, 2004.
86. Michallet MC, Saltel F, Preville X, Flacher M, Revillard JP, and Genestier L. Cathepsin-B-dependent apoptosis triggered by antithymocyte globulins: a mechanism of T-cell depletion. *Blood* 102: 3719–3726, 2003.
87. Nath MC and Sivakumar B. Studies on acetoacetate inhibition of sulfhydryl enzymes. *Enzymologia* 36: 86–92, 1969.
88. Nath MC, Hatwalne VG, and Hadgil IS. Progressive depletion in the reduced glutathione content of the blood following acetoacetate injections. *Biochem J* 53: 479–481, 1953.
89. Nerini Molteni S, Fassio A, Ciriolo MR, Filomeni G, Pasqualetto E, Fagioli C, and Sitia R. Glutathione limits Ero1-dependent oxidation in the endoplasmic reticulum. *J Biol Chem* 279: 32667–32673, 2004.
90. Nishimura F, Naruishi H, Naruishi K, Yamada T, Sasaki J, Peters C, Uchiyama Y, and Murayama Y. Cathepsin-L, a key molecule in the pathogenesis of drug-induced and I-cell disease-mediated gingival overgrowth: a study with cathepsin-L-deficient mice. *Am J Pathol* 161: 2047–2052, 2002.
91. Niwa T, Naito C, Mawjood AH, and Imai K. Increased glutathionyl hemoglobin in diabetes mellitus and hyperlipidemia demonstrated by liquid chromatography/electrospray ionization–mass spectrometry. *Clin Chem* 46: 82–88, 2000.
92. Obin M, Shang F, Gong X, Handelman G, Blumberg J, and Taylor A. Redox regulation of ubiquitin-conjugating enzymes: mechanistic insights using the thiol-specific oxidant diamide. *FASEB J* 12: 561–569, 1998.
93. Paget MS and Buttner MJ. Thiol-based regulatory switches. *Annu Rev Genet* 37: 91–121, 2003.
94. Park EM and Thomas JA. The mechanisms of reduction of protein mixed disulfides (dethiolation) in cardiac tissue. *Arch Biochem Biophys* 274: 47–54, 1989.
95. Poole LB, Karplus PA, and Claiborne A. Protein sulfenic acids in redox signaling. *Annu Rev Pharmacol Toxicol* 44: 325–347, 2004.
96. Rouhier N, Gelhaye E, and Jacquot JP. Redox control by dithiol–disulfide exchange in plants: II. The cytosolic and mitochondrial systems. *Ann N Y Acad Sci* 973: 520–528, 2002.
97. Ruwende C and Hill A. Glucose-6-phosphate dehydrogenase deficiency and malaria. *J Mol Med* 76: 581–588, 1998.
98. Salati LM and Amir-Ahmady B. Dietary regulation of expression of glucose-6-phosphate dehydrogenase. *Annu Rev Nutr* 21: 121–140, 2001.
99. Sarma GN, Savvides SN, Becker K, Schirmer M, Schirmer RH, and Karplus PA. Glutathione reductase of the malarial parasite *Plasmodium falciparum*: crystal structure and inhibitor development. *J Mol Biol* 328: 893–907, 2003.
100. Sen CK, Sashwati R, and Packer L. Fas mediated apoptosis of human Jurkat T-cells: intracellular events and potentiation by redox-active alpha-lipoic acid. *Cell Death Differ* 6: 481–491, 1999.
101. Shenton D, Perrone G, Quinn KA, Dawes IW, and Grant CM. Regulation of protein *S*-thiolation by glutaredoxin 5 in the yeast *Saccharomyces cerevisiae. J Biol Chem* 277: 16853–16859, 2002.
102. Soderdahl T, Enoksson M, Lundberg M, Holmgren A, Ottersen OP, Orrenius S, Bolcsfoldi G, and Cotgreave IA. Visualization of the compartmentalization of glutathione

and protein-glutathione mixed disulfides in cultured cells. *FASEB J* 17: 124–126, 2003.
103. Stephen AG, Powls R, and Beynon RJ. Activation of oxidized cysteine proteinases by thioredoxin-mediated reduction in vitro. *Biochem J* 291: 345–347, 1993.
104. Storer AC and Menard R. Catalytic mechanism in papain family of cysteine peptidases. *Methods Enzymol* 244: 486–500, 1994.
105. Stromhaug PE, Reggiori F, Guan J, Wang CW, and Klionsky DJ. Atg21 is a phosphoinositide binding protein required for efficient lipidation and localization of Atg8 during uptake of aminopeptidase I by selective autophagy. *Mol Biol Cell* 15: 3553–3566,2004.
106. Sweeney D, Raymer ML, and Lockwood TD. Antidiabetic and antimalarial biguanide drugs are metal-interactive antiproteolytic agents. *Biochem Pharmacol* 66: 663–677, 2003.
107. Tu BP and Weissman JS. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol* 164: 341–346, 2004.
108. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids* 70: 309–319, 2004.
109. Wang J, Boja ES, Tan W, Tekle E, Fales HM, English S, Meiyal JJ, and Chock PB. Reversible glutathionylation regulates actin polymerization in A431 cells. *J Biol Chem* 276: 47763–47766, 2001.
110. Williams AC and Ford WC. Functional significance of the pentose phosphate pathway and glutathione reductase in the anti-oxidant defenses of human sperm. *Biol Reprod* 71: 1309–1316, 2004.
111. Woo HA, Chae HZ, Hwang SC, Yang KS, Kang SW, Kim K, and Rhee SG. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. *Science* 300: 653–656, 2003.
112. Wu G, Fang YZ, Yang S, Lupton JR, and Turner ND. Glutathione metabolism and its implications for health. *J Nutr* 134: 489–492, 2004.
113. Xiao Y, Patolsky F, Katz E, Hainfeld JF, and Willner I. "Plugging into Enzymes": nanowiring of redox enzymes by a gold nanoparticle. *Science* 299: 1877–1881, 2003.
114. Yamazaki D, Motohashi K, Kasama T, Hara Y, and Hisabori T. Target proteins of the cytosolic thioredoxins in *Arabidopsis thaliana. Plant Cell Physiol* 45: 18–27, 2004.
115. Yano H, Kuroda S, and Buchanan BB. Disulfide proteome in the analysis of protein function and structure. *Proteomics* 2: 1090–1096, 2002.
116. Ziegler DM. Role of reversible oxidation–reduction of enzyme thiols–disulfides in metabolic regulation. *Annu Rev Biochem* 54: 305–329, 1985.

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