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Current Opinion in Physiology

Redox signaling in inherited diseases of metabolism

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Advances in basic and clinical research present compelling evidence that redox signaling constitutes a specific mechanism that operates at the molecular, cellular, and organ levels. The efficiency of energy producing pathways and coupling of electron transfer establish the thresholds of reactive species concentrations that may activate redox signaling events for function adjustment. A great deal of specificity in redox signaling is achieved by the reactivity of the target and the biophysical properties of its vicinity. Redox modification of proteins in circulation such as the case of Cys oxidation in albumin demonstrate that systemic conditions favorable for redox signaling could elicit organ-specific effects. Results from research on inherited metabolic diseases help to shed light on the intricate mechanisms that connect metabolism and redox signaling in more common chronic disorders such as cardiovascular disease, neurodegeneration and cancer.

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Current Opinion in Physiology 2019, **9**:48–55

This review comes from a themed issue on **Redox regulation**

Edited by **Sruti Shiva** and **Miriam Cortese-Krott**

For a complete overview see the Issue and the Editorial

Available online 2nd May 2019

https://doi.org/10.1016/j.cophys.2019.04.024

The redox code of metabolism: concentration, speciation and compartmentalization

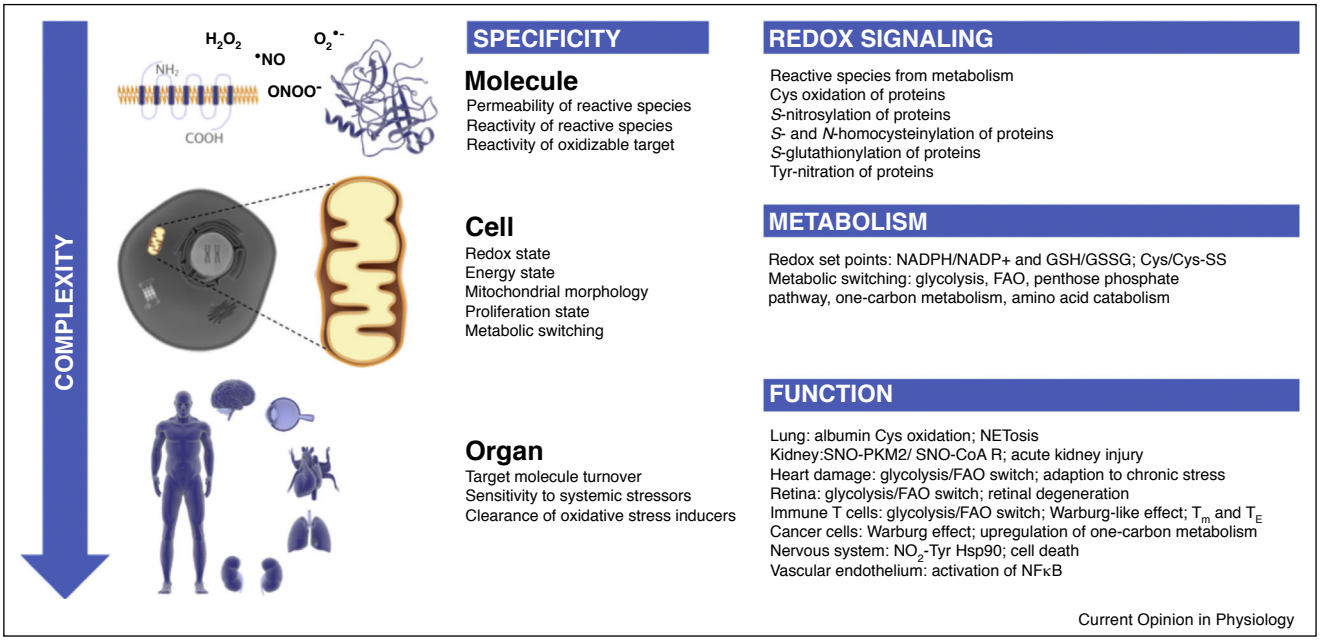
Cellular metabolism in aerobic organisms is driven by multiple reactions of oxidation and reduction. At the molecular level, nicotinamide adenine nucleotide (NAD, NADP) and thiol/disulfide pairs are the major redox systems supporting catabolic and anabolic reactions. Likewise, it is widely appreciated that reactive species including reactive oxygen species and reactive nitrogen species in addition to being byproducts of normal metabolism, act as signaling molecules in a specific manner [1•]. As defined by redox pioneers Dean Jones and Helmut Sies, cell metabolism is organized according to a redox code: 'The redox code is a set of principles that defines the positioning of the nicotinamide adenine dinucleotide (NAD, NADP) and thiol/disulfide and other redox systems as well as the thiol redox proteome in space and time in biological systems' [2••].

Experimental research over decades establishes four principles of the redox code. The first principle is metabolic organization by electron transfer reactions involving the NAD/NADP systems operating near equilibrium to furnish ATP production, catabolism, and anabolism. The second principle establishes that metabolism is linked to the structural flexibility of proteins through kinetically controlled redox switches that determine tertiary structure, macromolecular interactions, trafficking, and function. The specificity of biological processes is controlled by the abundance of proteins and the reactivity of their built-in redox switches with oxidants, which can vary over several orders of magnitude. Indeed, redox proteomics of cells and tissues show that Cys occurs with different steady-state oxidation in proteins [3,4]. The third principle concerns redox signaling by H_2O_2, the major redox metabolite operative in redox sensing, signaling and redox regulation [5]. The cyclic activation and deactivation of H_2O_2 production support redox signaling and spatiotemporal organization of complex cellular processes. While localized elevation of H_2O_2 production constitutes a small fraction of total cellular O_2 utilization, the diffusion rates and rapid metabolism of the oxidant confines the redox signal to the vicinity of its production site. The fourth principle of the redox code states that 'redox networks form an adaptive system to respond to the environment from microcompartments through subcellular systems to the levels of cell and tissue organization' [2••]. These principles are summarized in Figure 1, invoking specific examples of metabolic plasticity and redox signaling in genetic and non-genetic diseases of metabolism.

The mitochondrion is a hub for metabolic and redox signaling

The spatial and temporal organization of redox signaling relies primarily on the site of formation of reactive oxygen species and the capacity of those species to undergo diffusion and partition into cellular compartments. The mitochondrion is the primary source of ROS production, followed by the endoplasmic reticulum during oxidative protein folding [6] and membrane-bound NADPH oxidases [7]. Mitochondrial production of ROS derives from the reaction of oxidoreductases that support metabolism. Substrates and products of these reactions are metabolites with recently ascribed signaling roles, mediating cell–cell

Figure 1



Specificity of redox signaling at the molecular, cellular, and organ levels.
Reactive species produced during metabolism permeate membranes and/or react at their site of production with proteins. Reversible post-translational modification of proteins via Cys oxidation, *S*-nitrosylation, *S*-homocysteinylation, and *S*-glutathionylation constitutes central elements of redox signaling. Redox modification events that lead to irreversible modification of proteins, such as *N*-homocysteinylation and Tyr-nitration trigger profound loss of function such as cell death, and are only cleared upon protein degradation. Cells adapt to redox stress to replenish homeostatic energy and redox balance in the form of NADPH/NADP+, and GSH/GSSG, Cys/Cys-SS ratios, respectively by switching substrate utilization for energy production (glycolysis, fatty acid oxidation, pentose phosphate oxidation, amino acid catabolism). Redox signaling events and metabolic adaptions determine organ-specific changes in function. The study of inherited diseases of metabolism characterized by selective organ-injury permits the detailed examination of organ-specific phenotypes.

communication and function shift via post-translational modification of proteins. This includes, for example, lactate [8] and the Krebs cycle intermediate acetyl-CoA [9]. The dual role of Krebs cycle metabolites in energy production and signaling has been reviewed elsewhere [10].

Molecular specificity of redox signaling

Given the widespread utilization of the NAD/NADP systems, the ubiquity of Cys residues that could serve as redox switches, the broad reactivity of reactive oxygen species and the existence of multiple sites of ROS production in the cell, it is fair to question how redox signaling could harbor specificity. Specificity of redox signaling is achieved by a combination of factors that include the permeability of reactive species across cellular compartments, the intrinsic chemical reactivity of reactive species and the reactivity of the oxidizable target.

Permeability of reactive species

A review of the diffusion properties of reactive species shows that their ability to permeate through membranes is similar to their diffusion in water [11]. This property of reactive species enables far-reaching redox signaling events, such as the activation of guanylate cyclase residing in smooth muscles cell by nitric oxide produced in endothelial cells to elicit vasodilation [12]. Likewise, reactive species produced in the mitochondrion can exert effects outside the organelle. However, differences exist between reactive species. Nitric oxide ($^{\bullet}NO$) and oxygen (O_2) exhibit high permeability across cells and intracellular compartments due to their favorable partition into membranes and high diffusion coefficient in lipids. By means of comparison, the diffusion of the gasotransmitter H_2S is 10-fold slower than that of $^{\bullet}NO$ and O_2, and those of the oxidants peroxynitrous acid (ONOOH) and hydrogen peroxide (H_2O_2) is 10^5-fold slower [11].

Chemical reactivity of reactive species

Reactive species that derive from reduction of O_2 harbor intrinsic chemistry that determines their preferred targets. For example, hydroxyl radical ($HO^{\bullet}$) has indiscriminate reactivity with biological molecules, modifying lipids, DNA and amino acids, and thus, it is not considered a redox signal per se. In stark contrast, parent O_2 itself oxidizes iron–sulfur clusters but it is essentially

harmless to all other biological molecules. Superoxide anion ($O_2^{\bullet -}$) oxidizes iron–sulfur clusters and reacts with other radicals such as $^{\bullet}NO$ and semiquinones, whereas H_2O_2 oxidizes iron–sulfur clusters, metalloenzymes as well as Cys and Met residues in proteins. These aspects have been covered comprehensively elsewhere [1•].

The thiol balance influences the ability of the cell to rid itself from reactive species. Glutathione is the most abundant low molecular weight antioxidant in the cell, yet it reacts very slowly with reactive species *in vivo* [13]. The main antioxidant capacity of glutathione is exerted through its serving as a substrate for glutathione peroxidases and by forming *S*-glutathionylated adducts with oxidized Cys in proteins (protein-sulfenic acids) [1•]. Thus, abnormal ratios of oxidized and reduced glutathione could alter the ability of glutathione-dependent enzymes to cope with excessive concentration of reactive species.

Target reactivity

Within target groups, there is an additional layer of specificity and selectivity brought about by the properties of the target molecule and its surroundings. Cysteine residues in proteins are uniquely suited for redox signaling as they can undergo reversible oxidation, and at the same time, their reactivity toward reactive species is finely influenced by surrounding amino acids and solvent accessibility in the protein structure [14,15]. Only the oxidized forms of Cys that can be reduced participate in redox signaling. This includes sulphenic, disulphide bonds, sulphenamide and less often $–SO_2H$ [1•]. The pKa of Cys residues in a protein dictates its reactivity with reactive species. The pKa of Cys residues is influenced by the presence of basic and acidic amino acids in its surroundings. Positively charged amino acids (such as Arg) stabilize the thiolate form of Cys by electrostatic interactions thereby decreasing the pKa of the residue compared to free Cys (pKa of free Cys is 8.3) [1•].

One of the best studied examples of the specificity of redox signaling is that of human serum albumin (HSA) [16–18]. Human serum albumin possesses a unique free Cys residue that was shown to undergo oxidation to sulphenic acid, and subsequent reaction with other oxidants [16–18]. In virtue of the abundance of HSA in human plasma in the range 0.6−0.8 mM [19••], the reactivity of its thiol with oxidants influences systemic redox status, and as will be discussed later, it can also exert organ-specific effects [20••]. An important aspect is the recent suggestion that assessing redox targets may hold more physiological relevance than measuring reactive species themselves, due to the substantial variability of reactive species concentration in biological systems during short-term assessments and the intrinsic technical limitations of detecting transient reactive species [21].

Advances in redox proteomics permit the identification of biological targets of reactive species yielding mechanistic information that could hold relevance in pathology and prospective therapeutic developments [21,22•]. However, elucidating the function of post-translational modifications is notoriously challenging because the production of such proteins *in vivo* or *in vitro* results in a heterogeneous mixture of modified and unmodified variants. Genetic code expansion has emerged as a powerful tool to overcome this hurdle by introducing site-specific oxidative post-translational modifications using noncanonical amino acids [23•]. The insertion of non-canonical amino acids is performed by means of engineered aminoacyl-tRNA synthetase-tRNA pairs that encode a non-canonical amino acid in positions of nonsense (e.g. an amber stop codon) or a frameshift codon [23•].

Examples of successful target identification with direct functional associations are illustrated by the nitration of cytochrome c [24], mitochondrial SOD [24] and Hsp90 [25]. Nitration of specific Tyr residues in cytochrome c elicits more facile conversion to the alkaline transition of the protein. This conformational change switches the function of cytochrome c from electron carrier into a peroxidase and apoptotic mediator [26,27]. Nitration of mitochondrial SOD (SOD2, Mn-SOD) leads to loss of its enzymatic activity and increases mitochondrial redox stress [24].

Nitration of specific Tyr residues in Hsp90 elicits signaling cascades of cell death, of particular relevance in nervous system cells [28,29]. Studies with purified Hsp90 and with cultured PC12 cells demonstrated that nitration of Hsp90 at Tyr33 but not in any other four targetable Tyr residues reduced mitochondrial activity. The authors showed that Hsp90 nitration at specific sites can afford cell-specific modulation of metabolism [28]. Nitration of Tyr33 or Tyr56 transforms Hsp90 into a toxic protein, eliciting cell death. Immunohistochemistry using the antibody anti-nitro-Tyr demonstrated the presence of nitrated Hsp90 in motor neurons of patients with amyotrophic lateral sclerosis as well as in an animal model of the disease with and without spinal cord injury [29]. These findings substantiate the importance of identifying targets to interpret results in the context of cellular-specificity and gain or loss of function.

Redox signaling in inherited disorders of metabolism

Homocystinurias

Homocystinurias are a group of inherited disorders of metabolism that lead to elevation of total plasma homocysteine (tHcy, hyperhomocysteinemia) beyond the normal reference range of 5–15 μM in healthy subjects [30] and to elevated levels of homocysteine in the urine. Homocystinurias are caused by mutations in canonical genes that control Hcy homeostasis such as methionine synthase, cystathionine β-synthase, methylenetetrahydrofolate reductase, as well as in genes of the vitamin B12 transport (*TCN2* and *CD320*) and intracellular

trafficking (*cblC*, *epi-cblC*, *cblD*, *cblE*, *cblF*, *cblJ*, *cblG*, *cblX*) pathways that ultimately affect the activity of downstream methionine synthase (cblG) by impeding cobalamin delivery [31–33]. Abnormal elevation of tHcy is associated with increased risk of cardiovascular disease, thrombosis, and stroke, an observation first noted by McCully almost five decades ago [34].

Aside of the canonical Hcy genes, there are genetic polymorphisms that lead to elevated homocysteine in blood without the onset of symptoms (reviewed in Ref. [32]). Elevated tHcy is also associated with neurological and cognitive dysfunction such as Alzheimer's disease, dementia and neural tube defects [35,36]. Hyperhomocysteinemia results in the formation of protein-Cys-Hcy adducts [protein-*S*-homocysteinylation] both *in vitro* and *in vivo* [37,38]. Experimental studies have demonstrated the involvement of Hcy in oxidative stress and redox signaling (reviewed elsewhere in Ref. [39]), with major discoveries suggesting activation of NF-κB [40], modification of endothelial cell transition into apoptosis or proliferation by influencing NO bioavailability [41], and the post-translational modification of proteins by *S*-homocysteinylation [42•] and *N*-homocystinylation [43••].

Oxidative stress and redox signaling have been documented consistently in homocystinurias [44], albeit little is known about the cell-specific and organ-specific effects of chronic elevation of Hcy. The trans-sulfuration pathway responsible for the first half of the glutathione biosynthesis pathway, that is, from homocysteine to cysteine, is sensitive to redox status [45]. Pro-oxidant conditions inactivate cysteine biosynthesis, whereas antioxidant enzymes such superoxide dismutase and catalase support glutathione biosynthesis [45]. Thus, redox modulation of the flux of Hcy through the transsulfuration pathway influences glutathione biosynthesis in cells [45]. It would be critical to investigate how elevated Hcy influences glutathione biosynthesis and its oxidized and reduced balance under conditions that intrinsically favor oxidative stress as observed in homocystinuria.

Organic acidurias

Organic acidurias are a group of rare inherited disorders that lead to the accumulation of toxic organic acids (e.g. methylmalonic acid, propionic acid, isovaleric acid and glutaric acid) [46]. The occurrence of oxidative stress has been documented in methylmalonic aciduria [47], propionic aciduria [48] and glutaric aciduria [49,50], yet the existence of specific redox signaling events remains to be identified. A common denominator in organic acidurias is the finding of mitochondrial dysfunction [46] and disturbed thiol metabolism [51]. In the case of methylmalonic aciduria, mitochondrial dysfunction and oxidative stress have been documented in animal models of the disease as well as in patients [52,53]. The studies point to a possible mechanism whereby disruption of methylmalonyl-CoA mutase leads to mitochondrial dysfunction and chronic kidney disease.

The occurrence of oxidative stress in propionic aciduria is well-documented [54]. The disease is characterized by elevated reactive species and increased apoptosis. Administration of antioxidants ameliorates oxidative stress in a mouse model of propionic academia [48]. Mutation of the genes causing propionic aciduria in *Caenorhabditis elegans* revealed disturbed energy metabolism, pointing to abnormal mitochondrial function [55]. In the case of glutaric aciduria, buildup of glutaric acid increases production of reactive oxygen species and induction of pro-inflammatory responses via activation of NF-κB. Treatment with L-carnitine aimed at the removal of the toxic organic acid abolishes oxidative stress [49]. Oxidative stress in glutaric aciduria has been proposed as a major contributor to neurodegeneration [56].

Inherited diseases of glutathione metabolism: GSS and NRf2

Inborn errors caused by mutations in glutathione synthetase (*gss*) lead to deficiency of glutathione in cells with concomitant elevation of 5-oxoproline (pyroglutamic acid) due to compensatory upregulation of gamma-glutamylcysteine [57]. Mutations in the *gss* gene give rise to mild, moderate, and severe clinical phenotypes that correlate with loss of GSS enzyme activity and reduction in intracellular levels of GSH [57]. Patients with mild phenotypes present with hemolytic anemia, those with the moderate presentation additionally have metabolic acidosis and severely affected patients exhibit as well progressive dysfunction of the central nervous system [57]. Indeed, it has been recommended that all neonatal patients presenting with unexplained hemolytic anemia be screened for GSS deficiency [58].

Two independent studies uncovered new aspects of the role of transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) on the redox status of the cell. Nrf2 controls the expression of antioxidant and cytoprotective enzymes, including those involved in *de novo* synthesis of glutathione. One study utilized a knock out mouse model of Nrf2 and showed that Nrf2 controls the activity of endothelial nitric oxide synthase and how NO pools could afford protection against ischemia-reperfusion damage under conditions of compromised antioxidant defense [59].

Another study demonstrated that in humans, mutations that cause chronic activation of Nrf2 lead to early onset disease with multisystem involvement including failure to thrive, immunodeficiency and neurological symptoms [60••]. At the molecular level, chronic activation of Nrf2 unraveled in cytosolic redox imbalance with hypohomocysteinemia and stimulation of the proteins and enzymes of the cellular stress response such as glutathione

reductase, glutathione synthase, thioredoxin, and heme oxygenase 1. As a result, reductive stress was identified as a contributor to pathogenesis [60••]. Imbalances in glutathione concentrations and/or in the proportions of reduced and oxidized species have been documented in other metabolic diseases [61], yet, significantly less is known about the origin of such changes in these diseases. Thus, both deficiency and hyperactivation of the glutathione pathway could elicit oxidative stress and potentially abnormal events of redox signaling.

The case of cblC: a combined phenotype of homocystinuria and methylmalonic aciduria with disturbed glutathione metabolism

A striking case is that of the cblC disease, also known as MMACHC, characterized by combined homocystinuria and methylmalonic aciduria, caused by mutations in the *mmachc* gene [54–56]. At the cellular level, the cblC disorder is accompanied by elevated production of reactive oxygen species and increased apoptosis [63]. Examination of cblC patient lymphocytes uncovered abnormal ratios of reduced and oxidized glutathione, with increased oxidized glutathione and protein *S*-glutathionylation of proteins compared to controls [62]. Two independent proteomic studies performed in fibroblasts [64] and lymphocytes [65] showed abnormal expression of glutathione reductases, glutathione transferases and peroxiredoxins in cblC patients compared to healthy controls, consistent with oxidative stress. Therefore, it is possible that combined elevation of homocysteine and methylmalonic acid disturb glutathione pools, thus impairing cellular coping mechanisms against oxidative stress. The identification of *S*-glutathionylated proteins as well as the functional consequences of this redox signaling event in the cblC disease remains to be investigated.

Cellular specificity of redox signaling

While in many cases, oxidative stress and the eliciting of redox signaling events can be considered widespread or systemic, some studies suggest cell-specific and organ-specific features that determine redox signaling phenotypes. For example, a study where glutathione biosynthesis was specifically blocked in murine T-cells showed that cells underwent activation, but they were unable to sustain demands for energy and biosynthesis routes [66••]. Glutathione deficiency compromised mTOR activation and the expression of transcription factors NFAT and Myc responsible for metabolic switching to glycolysis and glutaminolysis [66••]. Glutathione deficiency led to an *in vivo* phenotype of protection against autoimmune illness but impaired antiviral defense [66••]. It was concluded that glutathione is essential for metabolic reprogramming associated with T-cell function.

In retinal cells, defense against oxidative stress is tightly linked to energy production pathways, determining functional coupling between cones and rods. The rod-derived cone viability factor long (RdCVFL) protects rod photoreceptors against light-induced oxidative damage. Studies performed in a mouse model of retinal degeneration (rd1) showed that RdCVFL reduces the oxidation of polyunsaturated fatty acids brought about by photoreceptor degeneration due to electron leakage from the respiratory chain [67]. Cone survival relies on the ability of rod-derived cone viability factor, RdCVF, a thioredoxin-like protein encoded by the *NXNL1* gene, to stimulate aerobic glycolysis [68,69]. During the early stage of retinal disease, rod death is elicited by the loss of expression of RdCVF [68,69]. Deficiency of RdCVF is characterized by lipid peroxidation in the form of HNE (4-hydroxynonenal) adducts. The functional coupling of RdCVFL and RdCVL links energy metabolism and oxidative stress, such that recycling of oxidized RdCVFL requires the activity of upstream RdCVF to produce reducing power in the form of NAPDH. It has been shown that RdCVFL in its reduced form reestablishes aerobic glycolysis in cones after oxidative stress. Further, it has been proposed that patients with retinitis pigmentosa present a molecular phenotype whereby loss of RdCVF expression by rods render cones dysfunctional and prone to death over the years [67].

Kalucka *et al.* showed that quiescent endothelial cells employ fatty acid oxidation to maintain redox balance and prevent endothelial cell dysfunction [70]. Unlike proliferating endothelial cells, quiescent endothelial cells reprogram metabolism to support NADPH regeneration which is in turn expended by NADPH-dependent enzymes for redox homeostasis and vascular protection [70].

A striking case is the deregulation of glutathione metabolism in cancer cells [71]. Examination of tumor cells indicate elevated intracellular glutathione and upregulation of biosynthetic enzymes and regulatory factors that support glutathione biosynthesis. Elevated intracellular glutathione confers tumor cells anti-apoptotic capacity and is thought to mediate resistance to chemotherapy [71]. Cancer cells are unique in their fine tuning of energy metabolism to reprogram one-carbon metabolism enabling fast proliferation and survival. Aside from modulating cellular methylation status and, therefore, gene expression, one carbon-metabolism pathways generate the precursors for NADPH and glutathione biosynthesis, two central players in redox homeostasis [72]. As discussed earlier, the largest contributor to cytosolic NADPH concentrations is the oxidative pentose phosphate pathway. Results from flux analysis by Fan *et al.* revealed that a comparable contribution to NADPH regeneration comes from serine-driven one-carbon metabolism [73••]. These reactions are catalyzed by methylenetetrahydrofolate dehydrogenases (MTHFD) that convert methylenetetrahydrofolate into 10-formyl-tetrahydrofolate coupled to the reduction of $NADP^+$ to form NADPH [73••]. Cells deficient in either cytosolic or mitochondrial MTHFD exhibited decreased ratios of both

NADPH/NADP$^+$ and GSH/GSSG and elevated sensitivity to oxidative stress [73••].

Organ specificity of redox signaling

Diseases where specific events of oxidative stress and redox signaling have been identified as key contributors to their onset and progression do exhibit organ-specificity at times. For example, a study by Stamler *et al.* showed that the *S*-nitrosylation of the glycolytic enzyme pyruvate kinase M2 (PKM2) contributes to kidney disease [74••]. Metabolic profiling coupled with unbiased mass spectrometry-based SNO-protein identification revealed that the *S*-nitroso-CoA reductase (SNO-CoA-SCoR) enzyme system, whose function in humans was previously unknown, affords protection against acute kidney injury [74••].

Protection against renal damage by the SNO-CoA-SCoR system is mediated by inhibitory *S*-nitrosylation of PKM2, a process that balances fuel utilization through glycolysis with redox protection through stimulation of the pentose phosphate shunt, that preserves NADPH/NADP$^+$ ratios and supports glutathione biosynthesis [74••]. The study showed that deletion of PKM2 from renal proximal tubules recapitulated the protective effect of SNO-CoA-SCoR, whereas introduction of a Cys-mutant variant of PKM2 unable to undergo *S*-nitrosylation abrogated its protective role. It was concluded that redox signaling via *S*-nitrosylation reprograms metabolism.

A study performed on an animal model of NOX4 deficiency, a NADPH oxidase enzyme highly expressed in the kidney, showed that NOX4 redirects glucose metabolism to fatty acid oxidation to support cardiac energetics during acute or chronic stress [75]. The study described a NOX4-ATF4 axis that mediates O-linked *N*-acetylglucosamine addition to the fatty acid transporter CD36 thereby stimulating fatty acid utilization, favoring energetic adaptation of the heart to chronic stress [75].

Bratman *et al.* demonstrated that oxidation of albumin causes plasma redox imbalance and this promotes lung-predominant NETosis, the formation of neutrophil extracellular traps that promote cancer metastasis under inflammatory stimuli, and pulmonary cancer metastasis [20••]. Results from an animal model deficient for albumin demonstrated that absence of albumin reduces the total plasma free thiol pool by 90%, and that this redox imbalance is sufficient to trigger NETosis even in the absence of a massive systemic inflammatory response [20••]. Thus, local oxidation of the unique Cys thiol in human albumin by H_2O_2 becomes a systemic event due to the location of the protein, yet, it triggers effects that are vastly specific for the lung [20••,76]. However, as mentioned above, diseases that induce systemic responses to cellular stress or abrupt redox imbalance could also lead to multi-organ dysfunction, such as observed in human mutations leading to chronic Nrf2 activation [60••]. Understanding organ specificity emphasizes the importance of spatial and temporal organization of redox signaling.

Outlook

Analysis of the literature shows that establishing the involvement of oxidative stress in inherited diseases of metabolism as well as in non-genetic abnormalities has occurred faster and more extensively than the elucidation of specific and relevant redox signaling targets. The identification of targets and the reversibility of target modification are essential pieces to discriminate between homeostatic versus pathogenic formation of reactive species. An additional important aspect is the need for integration of genetic, proteomic and metabolomic information to decode the mechanisms that underlie pathology and the exact contribution of redox signaling to such processes. The discrete genotypes and the multiplicity of phenotypes of monogenic inborn errors of metabolism provide an excellent opportunity to investigate specificity and reversibility of redox signaling at the molecular, cellular, and organ levels.

Conflict of interest statement

Nothing declared.

Acknowledgement

The authors apologize to authors whose work could not be included due to space restrictions.

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