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إشارات الأكسدة-الاختزال: تحكم متعامد في دورة الخلية واشارات موت الخلايا المبرمج

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الملخص
تستخدم الأنظمة الحيوية ثلاث آليات رئيسية في الاتصال الخلوي: المسارات التي تعتمد على وجود الجزيئات عالية الطاقة، والمسارات التي تعتمد على حالة الأكسدة والاختزال الخلوية، وتدفّق الأيونات إلى داخل الخلية وخارجها عبر الأغشية الخلوية من خلال القنوات ذات البوابات. وينبغي النظر إلى الإشارات الخلوية المتكاملة من منظور مفاهيمي يشمل هذه الأنواع الثلاثة جميعها. وقد أظهرت التطورات الحديثة في بيولوجيا الأكسدة والاختزال أن شبكات الثيول/ثنائي الكبريتيد تُعد حالات ديناميكية غير متوازنة، وتصبح تدريجيًا أكثر أكسدة طوال عمر الخلية، كما تمتلك جهود أكسدة واختزال مميزة عبر العضيات المختلفة داخل الخلية. وقد استُخدمت هذه الملاحظات لتكوين تمييز واضح بين استشعار الأكسدة والاختزال، وهو أنظمة حيوية واسعة تنظّم توازن الأكسدة والاختزال وتحافظ عليه، وبين إشارات الأكسدة والاختزال، التي تتضمن نقل إشارات تنشيطية أو تثبيطية محددة. ويستخدم كلاهما مفاتيح جزيئية قائمة على الكبريت، ولا سيما بقايا الحمض الأميني السيستئين في البروتينات، التي يمكن أن تتفاعل من خلال nitrosylation، أو glutathionylation، أو acylation، أو sulfhydration، أو التناسق مع المعادن.وعلى خلاف سلاسل الإشارات النوعية، تنظّم شبكات استشعار الأكسدة والاختزال على نطاق واسع معدلات وأنشطة شبكات الإشارات عالية الطاقة، والقنوات الأيونية، وتفاعلات البروتين–البروتين، وذلك من خلال تنظيم الحساسية والتوزيع والتجمع الجزيئي الكبير. وبما أن بقايا السيستئين غير المشاركة مباشرة في مسار نقل الإشارة تتوسط هذه التأثيرات، فإن التحكم باستشعار الأكسدة والاختزال يمتلك تأثيرًا عموديًا أو متعامدًا على الإشارات. ويتيح هذا التحكم المتعامد تكامل الإشارات وفقًا لمرحلة دورة الخلية والحالة الفسيولوجية للخلية، دون تعديل الجوانب المركزية لمسارات الإشارات. وتشير الأدلة الحديثة إلى أن تجمعات الثيول/ثنائي الكبريتيد في الإنسان قد تصبح أكثر أكسدة مع التقدم في العمر، والتعرّضات البيئية، والحالات المهيئة للإصابة بالأمراض، مما يدل على أن الثيولات المستشعرة للأكسدة والاختزال قد تمثل واجهة آلية رئيسية في بدء المرض وتطوره في العديد من الأمراض البشرية.
الكلمات المفتاحية :الاستماتة، الغلوتاثيون، علم الأشعة، الأكسدة والاختزال، الثيوريدوكسين، دورة الخلية، الإجهاد التأكسدي.

Redox Signaling: Orthogonal Regulation of Cell Cycle and Programmed Cell Death

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Abstract
Three main mechanisms are utilized in cellular communication by the biological system: the paths that rely on the presence of high-energy molecules, the ones relying on the cellular redox state, and the ion influx and efflux through cellular membranes via gated channels. Integrated cell signaling has to be thought of in conceptual terms involving all three types. Recent advances in redox biology have shown that thiol/disulfide networks are regarded as non-equilibrium dynamic states, become steadily more oxidized throughout the lifespan of a cell, and have distinctive redox potentials across various subcellular organelles. These observations have been used to form a distinct difference between redox sensing - broad biological systems that regulate and maintain redox balance - and redox signaling, which entails the transmission of particular activational or inhibitory signals. Both utilize sulfur-based molecular switches, especially the cysteine amino acid residues (Cys) in the proteins that can be reacts with nitrosylation,

glutathionylation, acylation, sulfhydration, or metal coordination. Unlike specific signaling cascades, redox sensing networks worldwide regulate rates and activities of high-energy signaling networks, ion channels and protein-protein interactions by regulating sensitivity, distribution and macromolecular assembly. Since Cys residues not directly involved in a signal transduction pathway mediate these effects, redox sensing control has a perpendicular effect on signaling. This orthogonal control enables signal integration upon the cycle phase and physiological state of the cell without modifying the central aspects of the signal pathways. Recent evidence that human thiol/disulfide pools can get increasingly oxidized with age, environmental exposures and disease-prone conditions is indicative that redox-sensing thiols may be a key mechanistic interface of disease initiation and progression in many human diseases.**Keywords:** Apoptosis, glutathione, radiology, redox, thioredoxin, cell cycle, oxidative stress

1. Introduction

Proteins are unique biological macromolecules which contain particular amino acids (cysteine, methionine and selenocysteine) that can undergo reversible oxidation-reduction reactions as part of their normal operation. Early biochemical studies emphasized the roles of protein cysteine (Cys) residues in a range of catalytic reactions, in the maintenance of protein structure by disulfide bonds and zinc (Zn(2+)) binding, and as protective against reactive species in cells. More recently we have expanded our knowledge to understand that reversible, covalent changes on specific Cys residues are important in cellular communication. Although there has been a tremendous progress especially with with regard to kinase pathways and the oxidative basis of illness, a complete definition of what can be termed as cannonical redox signaling pathways is somewhat elusive. A good example is the Nrf2 transcription factor mechanism of controlling the expression of antioxidant genes [1]. In this case, Keap-1 protein sensor thiols are oxidized or alkylated, which activates the release of Nrf2. Nrf2 subsequently translocates into the nucleus, interacts with small Maf proteins and triggers gene transcription by binding to an Antioxidant Response Elements (AREs) [1].

A large number of cellular processes have come to be referred to as redox signaling simply because they respond to such generals' oxidants as H 2 O 2 or reductants as N - acetylcysteine. This sensitivity in itself does not constitute a formal redox signaling pathway such as Nrf2 or well-known kinase cascades. Although kinetase reactions to oxidants and reductants are common as far as kinase reactions are concerned [2], these reactions are hardly characterized as redox signal pathways. The paper will review the literature on thiol/disulfide systems and how they vary during the cell cycle and in the various cellular compartments. These data lead to the conclusion that universal redox control mechanisms have specific biological functions, independent of specific signal transmission. I would therefore suggest redox sensing to characterize the universal thiol-based control systems and redox signals to characterize mechanisms in which a particular redox element transmits an activation or deactivation signal and which is a clear signaling pathway. Even though this distinction will be difficult to apply to some scenarios, this practice will contribute towards the construction of integrated models of cell signaling that

explain the wide varieties of functional outcomes that are associated with redox compartmentalization and cell cycle. The sections below initially give a concise description of protein Cys and covalent and non-covalent changes on which thiol-based regulation is based. I then recapitulate variations in steady-state redox potential throughout the cell cycle and its association with biological activity. This inspires the notion of redox sensing as opposed to redox signals, where redox sensing orchestrates cellular signals based on cell type, cycle phase, and metabolic state. The last part presents the role of redox sensing in structure-function relationships of proteins, with a call to undertake systematic investigations to understand Cys-containing proteins, in order to construct comprehensive models to explain cellular responses to stress.

2. The Cysteine Proteome

The human genome encodes approximately 214,000 Cys residues within its proteins [3]. Research by Miseta and Csutora [4] investigated the correlation between Cys prevalence in proteins and organismal complexity. They discovered that Cys is underrepresented in proteins compared to random expectations based on tRNA abundance, even after adjusting for GC content (ranging from 0.41% in archaea to 2.26% in mammals, versus a random probability of 3.28%). Their analysis also revealed that 92% of mammalian proteins contain at least one Cys, compared to only 50% of archaeal proteins, indicating an increase in Cys usage with evolutionary complexity. This was further supported by analysis of ten ribosomal proteins from five species, showing a rise in Cys content from archaea to humans. Ribosomal proteins generally contained about half the Cys of the species' overall proteome, suggesting selection against Cys. Other amino acids like phenylalanine, tyrosine, or isoleucine did not show this pattern. Cys distribution was non-random, with a notable enrichment of adjacent dithiol motifs ($C-X-X-C$ occurring 1.5 times more often than C-X-C or C-X-X-X-C), found in 20% of human proteins studied. While 21% of archaeal Cys reside in such motifs, yeast (5%) and plants ($j5\%$) showed lower incidence. These findings indicate that Cys content increased during evolution alongside organismal complexity.

Such an increase is probably indicative of the development of Cys-based signaling and regulatory roles. There are many roles related to Cys covalent and non-covalent modifications (Figure 1). The reversible redox reactions are single Cys (forming mixed disulfides with glutathione, cysteine or other proteins) or pairs of Cys (forming internal disulfide, or forming metal clusters). Sulfenic acid intermediates are typically unstable, but they are common and tend to resolve to disulfide. Oxidation states (sulfinic and sulfonic acids) are mostly irreversible in mammals, although there are a few exceptions such as peroxiredoxins. Cys is also involved in S -nitrosylation (bythe NO), S-sulfhydration (bythe H 2 - S), as well as reaction with aldehydes (to thiohemiacetals) or acyl-CoAs (to thioesters). These additions regulate protein activity, stability, assembly with other proteins, localization in sub-cellular locations via cytoskeleton or membrane binding, and, by secretory pathways or into organelles and the nucleus.

Polar amino acid like cysteine may be freely present on the surface and can be ionized to a thiolate anion in the presence of cationic residues to bind or react with metals and

oxidants, or reactive electrophiles. These features form the basis of its action in controlling protein action, architecture, location and functions.

The trends in Cys conservation of ribosomal proteins during evolution (Figure 3) 2A-D) indicate the same trend in cytoplasmic and mitochondrial ribosomes though they operate differently. The Cys percentage is lower, consistently than a purely chance codon usage would suggest and this indicates an evolutionary pressure against non-functional Cys. Nonetheless, those Cys residues which exist are more likely to be preserved in complex organisms, indicating conserved functions. This is in stark contrast with mitochondrial targeting sequences (MTS, Figure 2E-F) in which Cys percentages are nearly randomly distributed, but less conserved. Therefore, it can be expected that the protein structures of animals do not use indiscriminate Cys, but rather it is a functional structure that the residues of Cys are carefully placed and otherwise conserved during evolution, which is a characteristic of functional significance.

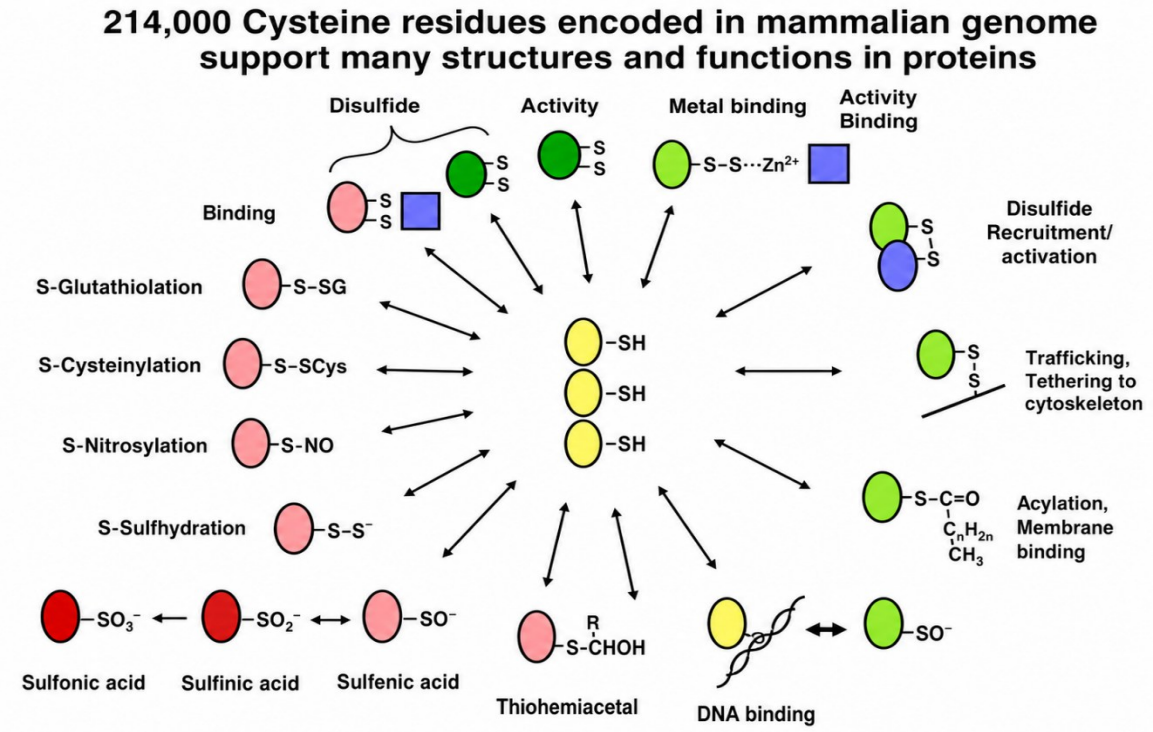
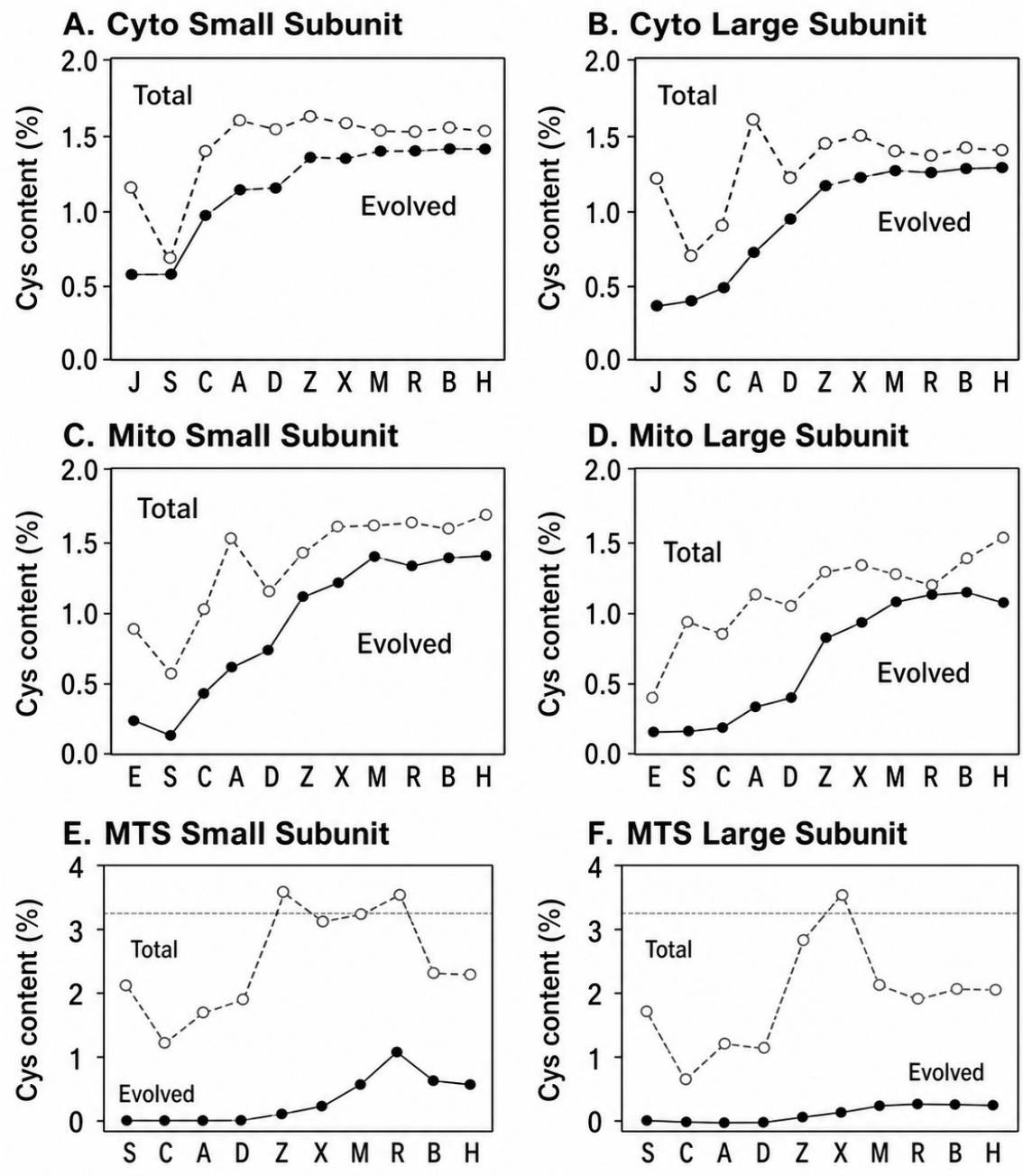


Figure 1: A schematic representation of various covalent modifications acting on protein cysteine residues, influencing their structure and biological function.

Advances in redox proteomics allow identification of proteins undergoing redox changes in vivo. Targeted redox Western blotting shows that proteins in various compartments (mitochondria, cytoplasm, nucleus, ER, plasma membrane) are partially oxidized under normal conditions [5]. Cellular H_2O_2 production can oxidize $\sim 0.5\%$ of all thiols per minute [3], and modeling suggests protein oxidation quantitatively consumes low H_2O_2 levels [6]. Mass spectrometry (2-D LC-MS/MS) [5] has identified over 2,000 peptideassociated Cys residues (Y.-M. Go and D.P. Jones, unpublished). Results show hundreds of specific Cys residues are partially oxidized and responsive to cell physiology. Since current methods only capture $\sim 1\%$ of the proteome, the actual number of redox-sensitive Cys is likely far greater.

The redox-sensitive Cys residues identified under normal physiological conditions belong to a diverse array of protein classes. For instance, 34 actin-associated cytoskeletal proteins in aortic endothelial cells show altered oxidation states in response to the extracellular Cys/CySS redox potential [5]. Other oxidized cytoskeletal proteins include tubulin, filamin, and desmin. Signaling proteins (Ras, phosphatases, 14-3-3), translation factors (eIFs, ribosomal subunits), stress proteins (HSPs), detoxification enzymes (peroxiredoxins, GSTs), mitochondrial proteins (ATP synthase, Complex I subunits), metabolic enzymes (involved in fatty acid oxidation), and pro-inflammatory signaling proteins (IL-1β pathway) are all represented. The key conclusion is that a wide variety of proteins harbor Cys residues that are partially oxidized under routine physiological conditions.

Figure 2: Comparative analysis of total and



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utionarily conserved cysteine residues in ribosomal proteins, indicating that a substantial proportion of Cys serve essential biological roles.

3. Patterns of Steady-State Redox Potential Variation in Cells

3.1 Variation with the Cell Life Cycle

Changes in thiol status during the cell cycle have been extensively studied, particularly concerning cancer cell killing. Figure 3 summarizes key observations. Thioredoxins are typically more reduced in proliferating and differentiated cells than in cells undergoing apoptosis. Depletion of metabolic energy sources promotes oxidation. Severe, prolonged oxidation due to energy loss, oxidants, metal ions, or electrophiles triggers cell death via apoptosis or necrosis.Because simple thiol/disulfide ratios (e.g., GSH/GSSG) do not accurately represent twoelectron reaction stoichiometry, data are presented as redox potential (E_h) calculated using the Nernst equation. E_h represents the reducing potential of a disulfide/thiol couple relative to a standard hydrogen electrode [84] (e.g., for glutathione: GSSG $+2H^+ + 2e^- \rightarrow 2GSH$). Figure 3 shows E_h for thioredoxin and GSH/GSSG couples during the cell cycle (right of double lines) and the plasma Cys/CySS couple in vivo (left). Proliferative cells maintain a more reduced environment compared to differentiated cells, reflected by their thiol/disulfide-based control systems (Trx-1, Trx-2, and GSH). Loss of Trx-1 or GSH function reduces growth; severe loss triggers apoptosis or necrosis. Differentiated cells have a more oxidized GSH/GSSG potential. Mild oxidation activates stress gene transcription (including antioxidant defenses). Broad oxidation induces apoptosis or necrosis. Apoptosis, regardless of trigger, causes extensive oxidation of the GSH/GSSG couple [21]. These findings are supported by limited in vivo studies [3, 89], human data showing age-related and pathology-associated oxidation of plasma thiol/disulfide [89,90], and cell culture experiments using the "redox synapse" model.

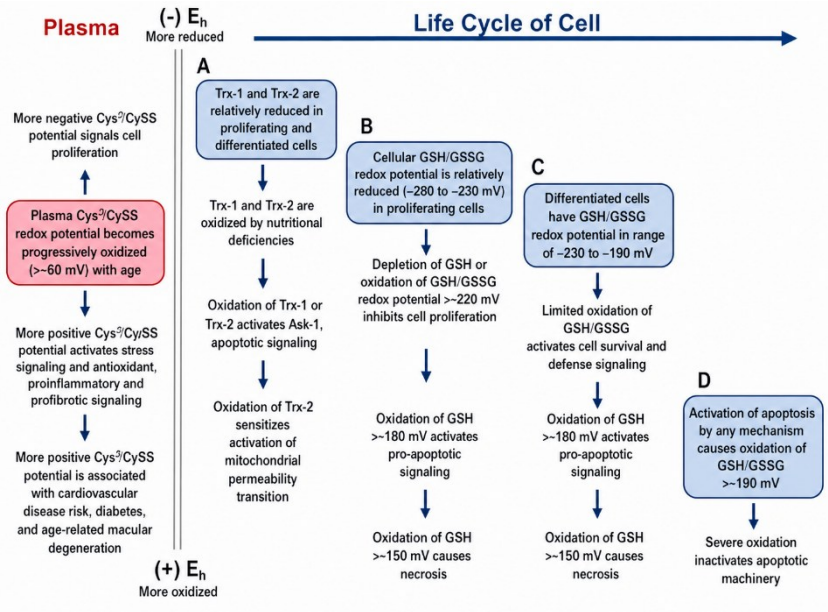

Figure 3: Generalized scheme of thiol/disulfide redox changes during the cell life cycle and associated functional characteristics.

Proliferative cells exhibit higher levels of both GSH and thioredoxin [7-10]. Depleting GSH or inhibiting thioredoxin impairs proliferation, which is restored by replenishing GSH [7-10]. Similar observations exist for normal cells; intestinal crypt cells (proliferative) have higher GSH than villus cells (non-dividing) [11]. Conditions limiting GSH availability in vivo also limit cell division, and proliferation resumes upon GSH restoration and can be enhanced by GSH precursors [12-14]. Recent work highlights that maintaining nuclear GSH is critical for proliferation, as various GSH-depleting agents affect nuclear redox status [15].

Studies measuring the GSH/GSSG redox potential (E_h^{GSSG}) confirm that proliferating cells maintain a relatively low (more negative) E_h. For instance, E_h^{GSSG} in rapidly dividing HT-29 colon cancer cells is approximately -260 mV , shifting to -200 mV upon differentiation [16]. Similarly, Caco-2 cells show E_h^{GSSG} of -245 mV , which becomes -205 mV after confluence and spontaneous differentiation [17]. In vivo rat colon studies show similar redox changes with proliferation/differentiation [18], as do NIH3T3 fibroblasts [19].

Less is known about the intracellular Cys system's role in proliferation, but Cys is essential for protein synthesis. Due to metabolic links between Cys and GSH, Cys availability may sometimes limit growth under conditions previously attributed to GSH restriction. E_h^{CySS} becomes more oxidized with differentiation, though changes are smaller than for E_h^{GSSG} [20]. Importantly, the extracellular E_h^{CySS} is a critical proliferation determinant for many cell types [21], including monocytes [22], endothelial cells [23], retinal pigment epithelial cells [24], and colon cancer cell lines [25]. As Cys/CySS is the primary low-molecularweight thiol/disulfide system in most extracellular fluids, it acts as a key extracellular counterpart to the intracellular GSH/GSSG system in regulating proliferation.

Oxidation of thiol systems is known to induce cell death by disrupting Ca^{2+} homeostasis, activating the mitochondrial permeability transition, and stimulating ASK-1. During apoptosis, GSH efflux is activated [26], and a common feature regardless of the apoptotic trigger (e.g., staurosporine [27], glucocorticoids [28], growth factor withdrawal [29], terminal differentiation [17]) is oxidation of E_h^{GSSG} to approximately -170 mV . Further oxidation to around -150 mV leads to necrosis.

Oxidized extracellular E_h^{CySS} activates inflammatory signaling in endothelial cells, monocytes, and neutrophils [23,30]. It also triggers stress and antioxidant responses in monocytes [22], endothelial cells [23], colonic epithelial cells [31], and lung fibroblasts [32]. Although direct mechanistic links to human disease are still emerging, studies have linked oxidative E_h^{CySS} to persistent atrial fibrillation [33] and adverse cardiovascular outcomes [34]. While more research is needed, current evidence strongly suggests that thiol/disulfide redox potential is a key parameter regulating molecular activities throughout the cell cycle.

3.2 Subcellular Redox Compartmentalization

Recent reviews [21] detail the compartmentalization of redox potential, supporting its role in regulating molecular functions. Early GSH studies missed mitochondrial-cytoplasmic differences partly due to pH variations; correcting for pH reveals a lower (more negative) E_h^{GSSG} in mitochondria. Early reports of high nuclear GSH [35] were questioned as artifacts [36] but later vindicated. Studies on AP-1 transcription factor activation showed low-level oxidation promotes, while high-level oxidation inhibits, activation [37-39], attributed to distinct redox events in cytoplasm versus nucleus. NF- κB and Nrf2 show similar compartment-specific redox sensitivity [40-42]. Subsequent research confirmed nuclear GSH is distinct from cytoplasmic GSH [15], and thioredoxin-1 (Trx-1) translocates to the nucleus under oxidative stress [43]. The endoplasmic reticulum (ER) maintains a relatively oxidized environment, essential for processing secretory proteins, with E_h^{GSSG} measured using ER-targeted probes [44,45].

Figure 4 depicts subcellular thiol/disulfide redox potential compartmentalization (conceptual, as different couples were measured in different compartments). Using measured reduction states and standard potentials for active-site dithiols, steady-state E_h for Trx-2 in mitochondria is highly reduced ($\sim -330\text{mV}$), while nuclear Trx-1 is $\sim -300\text{mV}$ and cytoplasmic Trx-1 is $\sim -270\text{mV}$. Among GSH/GSSG pools, mitochondria are most reduced ($\sim -300\text{mV}$). Nuclear E_h^{GSSG} is likely $< -260\text{mV}$, based on higher GSH concentration and lower protein glutathionylation compared to cytoplasm. ER values are around -185 mV [44], falling between cytoplasmic (-260 to -200 mV) and plasma (-140 mV) values.

Many links exist between compartment-specific redox potential changes and cell function [21]. Cytoplasmic activation of AP-1, NF- κ B, and Nrf2 pathways, and their sensitivity to nuclear oxidative disruption, is well-documented [39, 41, 43]. EGF-induced growth signaling causes selective oxidation of cytoplasmic Trx-1 without affecting GSH, nuclear Trx-1, or mitochondrial Trx-2 [46]. TNF- α signaling is linked to mitochondrial Trx2 oxidation without altering cytoplasmic Trx-1 or GSH [47]. Similarly, inflammatory signaling from oxidized extracellular E_h^{CySS} in endothelial cells involves mitochondrial Trx-2 oxidation, but not cytoplasmic Trx-1, nuclear Trx-1, or GSH [5]. Such selective compartment oxidation suggests independent redox control, allowing tailored modulation of protein functions.

Combined with data showing cell-cycle-dependent thiol/disulfide changes and the reversible modifications of Cys residues, these findings indicate redox elements both support specific signals and coordinate broader signaling functions in cell cycle control and apoptosis.

4. Distinguishing Between Redox Sensing and Redox Signaling

A previous review on the non-equilibrium thermodynamics of thiol/disulfide systems highlighted the need for quantitative models to accurately describe redox signaling and control pathways [18]. A major challenge is distinguishing sulfur switches that are integral components of a redox signal pathway from those that regulate pathway activity. "Redox signaling" is often used broadly when an oxidant like H_2O_2 triggers a response. The Nrf2 pathway is a prototypical example [1,48]. Keap-1 Cys oxidation or alkylation signals Nrf2

release, nuclear translocation, and ARE-mediated gene activation. While many other redox-sensitive transcription factors exist [49], few have defined pathways where a specific oxidation event transmits a direct signal to a specific response.

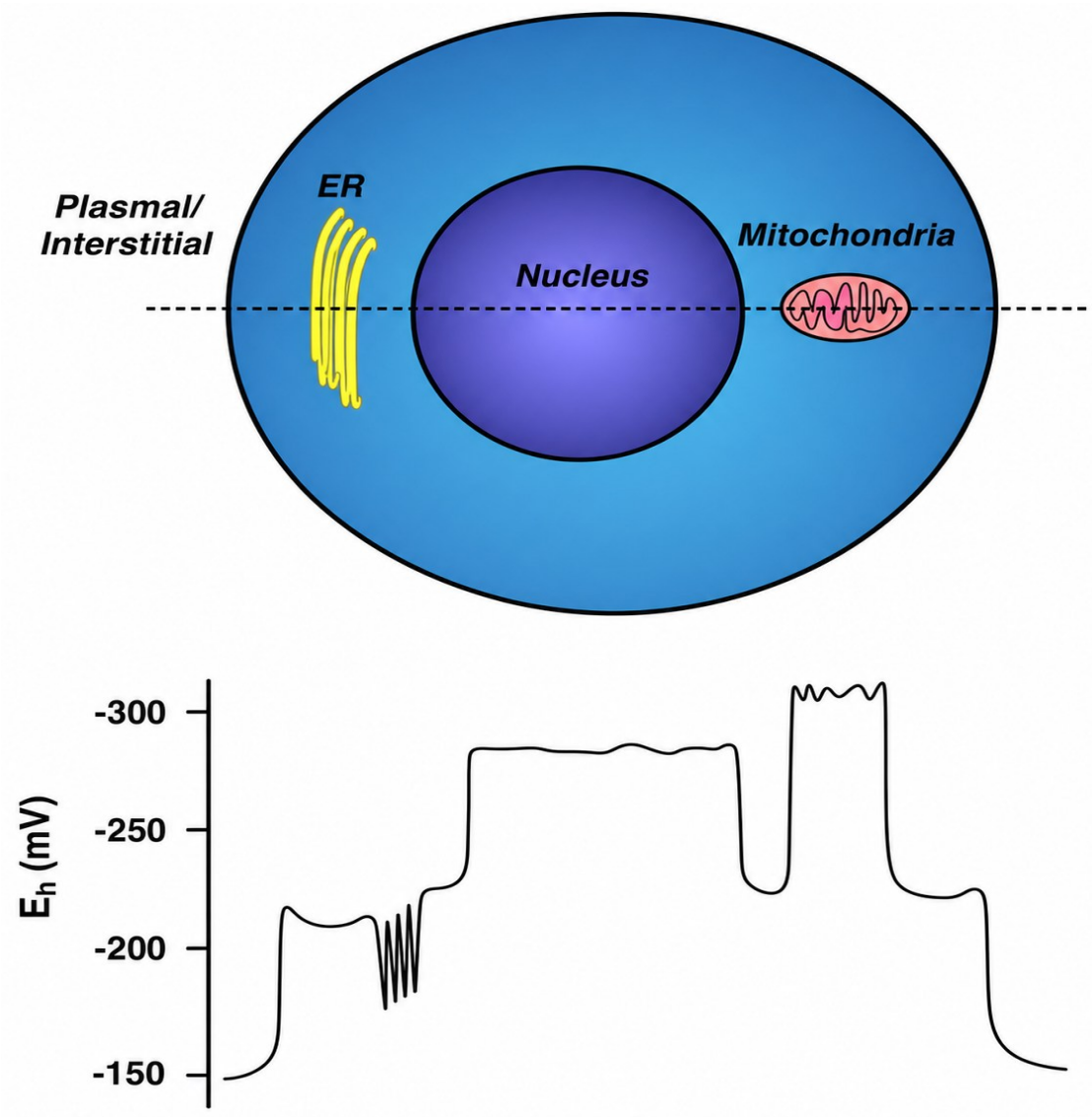

Figure 4: Schematic diagram illustrating the distinct steady-state redox potentials (E_h) measured across various subcellular compartments, including the extracellular space, cytoplasm, endoplasmic reticulum (ER), nucleus, and mitochondria.

Conversely, numerous examples show kinase pathways are redox-sensitive. Wright et al. [2] found low-level oxidative stress increased the phosphorylation of 85% of detectable phosphoproteins. Oxidizing conditions often stimulate kinases while simultaneously inhibiting phosphatases [50,51]. The scarcity of defined discrete redox pathways and the extensive crosstalk between redox and kinase systems necessitate distinguishing signal pathway elements from pathway regulators. This is crucial when interpreting "redox signaling" based on experiments using non-specific agents like high-dose H_2O_2 or NAC, which globally challenge cellular thiols (typically $< 100\mu M$). While such experiments

may not define specific pathways, the observed responses can be valuable evidence for regulation via redox-sensing (non-signal) thiols.

Broadly, cell signaling and metabolic regulation are linked to three interconvertible energy currencies: phosphorylation energy (ATP), transmembrane gradients (ion/solute concentration), and redox energy (Figure 5). Each underlies major signaling types: kinase signals, ion signaling, and redox signals, respectively. However, as these pathways are themselves regulated by mechanisms also based on these currencies, distinguishing signal pathways from their regulatory systems is difficult. A complete systems biology description of redox signals must ultimately incorporate all three signal types.

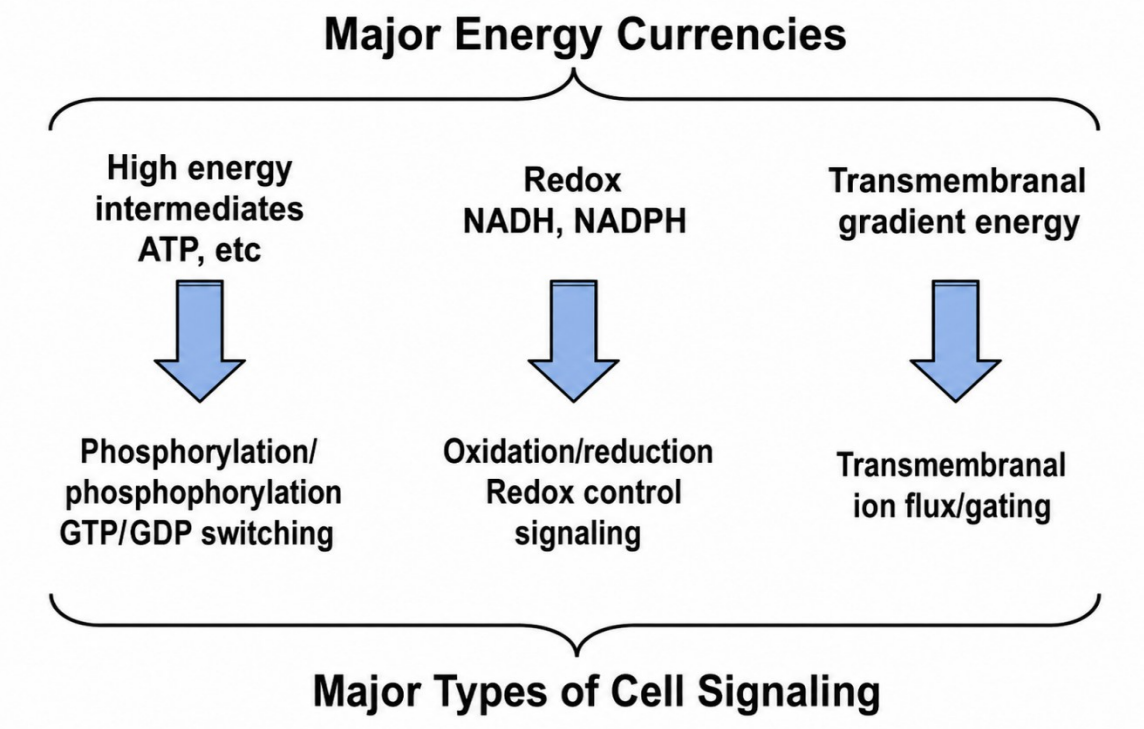

Figure 5: The three fundamental energy currencies of biological systems-redox potential, transmembrane ion gradients, and high-energy phosphate bonds (ATP)-serve as the basis for the major classes of cellular signaling mechanisms.

In view of the data described above: (1) there are a great number of Cys residues, (2) quite a large proportion of them are subject to reversible oxidation or other modification, (3) redox potential varies in the intracellular compartments, (4) the Cys residues may be classified by their use, so it can be of much benefit. Terminology needs to be clarified to support global redox changes (e.g. during differentiation or apoptosis) and special signal transmission. I suggest the use of redox signals to represent Cys residues as key specific components of any given redox signaling pathway and redox sensing to represent Cys residues that modulate or combine signal functions regardless of the pathway involved. This diagram depicts the discrete signal pathways (in the down-right arrows) and the redox regulation systems that operate in an orthogonal manner with them (in the bottom-left arrows). It also incorporates kinase and ion channel signaling to highlight the fact that all the types of redox sensing are regulated. As an example, in ASK-1-mediated apoptotic signaling through Trx-1, Cys32/35 oxidation is a direct signal of the pathway [52], and

Cys62/69 oxidation controls such a pathway activation by modulating the rate of Cys32/35 disulfide reduction [53]. The activation of kinases is controlled by numerous redox sensations [2,54], and NMDA. A redox-perceiving thiol on the outside also facilitates receptor ion flux [55].

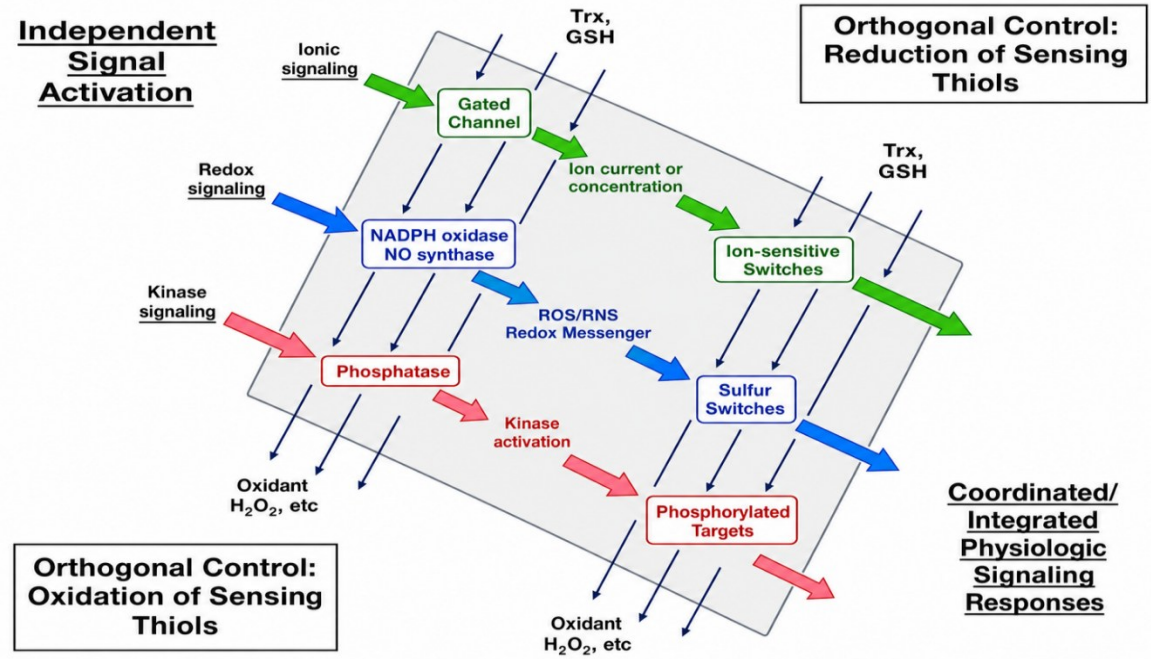

Figure 6: Orthogonal regulation of major signal transduction systems (kinase, redox, ion) by redox sensing mechanisms. Redox sensing thiols perpendicularly modulate the amplitude or context of signaling without altering the core pathway components.

This orthogonal control (Figure 6) means redox-sensing thiols can modulate signals appropriately for the cell state, provided the redox potential change is sufficient. A 60 mV change (observed during HT-29 differentiation [16]) corresponds to a 10 -fold change in the reduced/oxidized ratio for a single Cys and a 100 -fold change for a Cys pair. This shift significantly alters Nrf2 system sensitivity, leading to increased detoxification gene expression in differentiated vs. dividing cells [16]. As summarized in Figure 3, redox potential changes during cell cycle, differentiation, and apoptosis are sufficient for redox-sensing thiols to profoundly impact cellular structure and function. This distinction between redox signaling pathways and redox sensing mechanisms will aid accurate pathway description and facilitate mathematical modeling in redox systems biology.

5. Implications of Cys Redox Sensing in Biological Systems

The Cys underrepresentation in proteins implies that resulted in its reactive properties having a strong property of influence on evolution. This is also enhanced by an assortment of reactions as well as sensitivity to oxidants/electrophiles evidenced in the abundance of toxicology literature. Provided that the concentrations of H_2O_2 or NAC routinely employed to study the phenomenon of redox signaling are enough to oxidize cellular proteins globally, then it must rethink the distinct impact of a particular pathway. Biology has tended to overlook this mismatch or suggest that non-critical, rather, non-critical Cys and Met residues are decoys, preventing the critical active site Cys to be oxidized [56].

Another theory, founded on the separation of redox signals and redox sensing is that much of what is found in mammalian systems is dedicated to redox sensing. Examples include: interference of ER disulfide formation resulting in unfolded protein response [57,58]; Cys oxidation in mucus resulting into high viscosity and barricade activity [59]; the need of balanced GSH/GSSG reaction on platelets activation [60]; the need of high GSH to proliferate [15]; low E_h^{CySS} robustly prompts cell reproduction

These thiol properties are used to form these redox sensing functions. Conserved Cys residues, which are involved in redox processes, are found in the cytoskeleton proteins [62]. Oxidative stress leads to massive oxidation, crosslinking and association of actin with many partners [63,64]. Such oxidation, crosslinking, and binding can under physiological conditions, control the activity and location of interacting proteins. The same case applies to α/beta-tubulin, scaffold proteins (14-3-3), chaperones (HSP60, 90), translation factors (EF1 α, EF2) ubiquitination plant life (E1) and other proteins that are found (partially) oxidized in normal condition [5]. Their dynamic redox responses are probably indicative of redox sensing roles coordinating and regulating larger protein networks. Thiol functions in the processing and trafficking of proteins [45] provide proof-of-principle. The observation of many partially oxidized proteins at physiological conditions, in addition to the non-equilibrium nature of thiol/disulfide systems, essentially requires such regulatory actions, although not all of them may be biologically used.

Mechanistic theory Cys-based redox sensing has its roots in classic work regarding diffusion of Ca2+ in cytoplasm [65,66]. Buffering slows the rate of diffusion of Ca2 + (50 -fold) [65,66]. This may have similar effects on protein diffusion since the exchange of thiol groups with sulfur groups is relatively slow. In low conditions, translational movement is not impeded. but when conditions are conducive to form disulfide bonds between a protein and immobile membrane or cytoskeletal elements then its movement is slowed, regulated by disulfide exchange rates.The possible impact is discussed in figure 7. Having a homogenous cytoplasmic redox state of thiol/disulfide will require homogenous cytoplasmic E. h . The formation of temporary oxidation sites locally (e.g., at the plasma membrane or around mitochondria) might accelerate the diffusion of proteins and amplify local signals by occupation of components at the active site. Therefore, Cys residues may possess biological activities not evident solely using site-directed mutagenesis, and which are hard to monitor studying.purified solutions of proteins. Reductions in H_2O_2 and H_2S Global influences signaling can occur by effects of global reductions of H_2O_2 and H_2S.

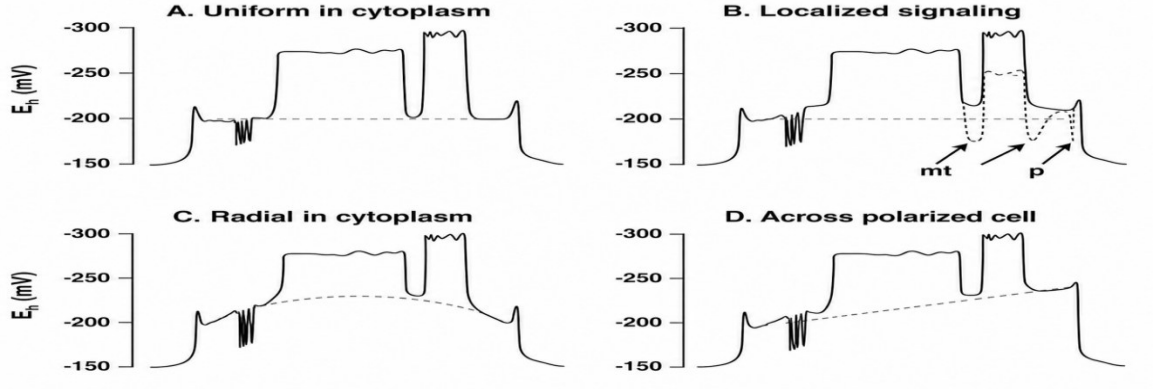

Figure 7: Proposed models illustrating how redox sensing mechanisms could influence cellular signaling organization, including uniform cytoplasmic redox, localized redox microdomains near membranes or mitochondria, and transcellular or radial redox gradients.

Hypothetical roles for redox sensing are depicted in Figures 7C and 7D. The more reduced nucleus and more oxidized extracellular space relative to the cytoplasm could establish a radial gradient. A $60-120\text{mV}$ radial gradient could support 10- to 100-fold concentration gradients of proteins undergoing redox-dependent association with cytoskeletal elements, enabling protein sorting and signaling localization (Fig. 7C). Such gradients could enhance signaling efficiency. Similarly, transcellular redox gradients exist in transport epithelia [67,68], potentially directing protein distribution and activity across polarized cells (Fig. 7D). While speculative, the prevalence of redox-sensing Cys could explain the vast literature where global oxidation impacts specific signaling pathways.

6. Challenges and Opportunities

Studies with NOX-1-transfected cells show kinase activation without detectable changes in GSH/GSSG or Trx-1 [69], suggesting specific redox signals occur without global thiol system changes. The non-equilibrium nature of Cys/CySS, GSH/GSSG, and protein thiol/disulfide pairs means these systems react slowly, preventing traditional "buffering" of generalized thiol/disulfide. Advances in mass spectrometry reveal hundreds to thousands of specific protein Cys residues are partially oxidized in vivo. A central challenge is identifying the oxidation of a particular thiol among the $\sim 214{,}000$ in the genome. Cumulative databases from multiple experiments will be needed. Current methods can measure $\sim 1\%$ of the protein Cys in a single MS experiment; redox Western blots can measure select proteins [70,71]; fluorescent redox sensors allow live-cell imaging [72]; and nanotechnology offers nanoscale oxidant detection [73].

Accurately measuring short-lived oxidants at low concentrations with spatial resolution remains challenging. Furthermore, distinguishing specific ROS (e.g., H_2O_2, lipid hydroperoxides, superoxide) from other potential oxidants (quinones, epoxides, aldehydes, etc.) is often lacking. Viewing redox-sensing thiols as common components provides an opportunity to correct persistent misinterpretations. The free radical theory of aging often overshadows the functional roles of thiol-based redox reactions, creating experimental conditions where destructive chemistry prevails because cells are dead [75]. Protein oxidation during tissue preparation is often dismissed as an "artifact" without questioning why evolution would produce such reactive proteins. Endogenous Cys oxidation rates are near H_2O_2 turnover rates [3]. Thus, for a redox sensing thiol, oxidation during extraction from a reducing cellular environment is expected. This "artifact" can actually serve as a basis for identifying redox-sensing thiols. Studying this subset of easily, reversibly oxidizable Cys presents a tremendous opportunity to advance understanding of signaling, cell cycle, and apoptosis.

7. Clinical Implications

The awareness of the rich numbers of redox-detecting thiols assists clarify the unconnection between the robust association of oxidative stress and pathology with the failure of extensive antioxidant examinations to exhibit definite advantage [76-83].

Radical and non-radical mechanisms are involved in oxidative stress [3]. Free radical scavengers were used in most clinical trials but free radicals only make a small proportion of biological oxidants [3]. In addition, radical scavengers metamorphose radicals into H 2 O 2 which are more plentiful as non-radical oxidants. The selective damage of thiols by non-radical oxidants instead of the non-selective destruction of molecules indicates that oxidation is more complex as a pathophysiological entity than being bad.

Scientifically, it is known that human thiol/disulfide pools become older, experience oxidative stress conditions (smoking, alcohol) and other risk factors that predispose to chronic diseases (high BMI, endothelial dysfunction) [3]. E_h^{CySS} of oxidized C has the concentration needed to trigger inflammatory cytokines in cell culture which is correlated to the increased levels of cytokines in humans [30]. Oxidative thiol/disulfide changes, which occur as a result of redox-sensing thiols, therefore, provide a mechanistic connection.

between usual oxidative stresses and the disease progression-determining signaling pathways. Recent data demonstrates that dietary sulfur amino acid may have a short-term impact on plasma redox-state [85,86], and oxidation may be prevented by supplements (Zn^{2+}, vitamins $\mathrm{C}+\mathrm{E}$) over 5 years in the elderly population [87,88]. The general oxidation of thiol pairs/disulfides in the system probably influences numerous processes because of the large number of redox-sensing thiols. The process of cell proliferation and cell repair could decrease with age and oxidative stress due to response of cells to oxidized Eh through sensors and shift the balance towards apoptosis. Redox-sensing thiols sensitize platelet activation, fibrosis signaling and inflammation.

The knowledge of redox-sensing thiols will inform the ways of risk and advancement management of diseases. Development of targeted antioxidants will be made possible by new methods of detecting these thiols. Although speculative, advances in the understanding of thiol/disulfide modifications in vascular disease are encouraging in the promise that this emerging direction will result in a high health gain.

8. ConclusionS

Advancements in the NADPH oxidase-produced ROS, Nrf2 signaling technology, and crosstalk with kinase technologies have developed redox signaling into a full-fledged discipline. The argument presented by this review of the distinction between universal redox sensing processes, and specific redox signalings is that it is based on: (1) the characteristic redox potentials of proliferation, differentiation, apoptosis and subcellular compartments; (2) the non-equilibrium maintenance of thiol/disulfide couples; and (3) the presence of reversibly oxidized thiols in physiological Thus, the fundamental rule of regulation of coordination of different biological processes is possibly the redox sensing through thiols. This control operates via special conserved Cys residues, and is orthogonal to cell signaling pathways, such that signals can be modulated without changing the underlying signal machinery. This view suggests that a specific group of Cys activities in redox sensing can offer a general principle of regulation. Though not entirely recognized, mass spectrometry shows that 0-1 percent or more of protein Cys residues are partially oxidized and may play such roles. A variety of reversible changes (oxidation, nitrosylation, acylation, sulfhydration, metal binding) to macromolecular structure and

activity and trafficking can be explained by redox-sensing thiols. Conditional control the participation of cell-cycle-dependent redox changes provides conditional control over signaling activities. The redox compartmentalization offers some specificities to signal molecule concentrations of kinases and ions during cell cycle transitions. Advances in discovering the redox-sensitive proteome and its regulatory networks will enable redox sensing to be integrated with the high-energy chemical and ion gating into complete cellular signaling models. Determinants of human steadfast redox potential have remained unknown, although data are available indicating dietary impact. Therefore, approaches to managing thiol/ disulfide pairs and their sensor thiols, to safeguard them and test their performance in diseases associated with oxidative stress and inflammation, could be developed and evaluated as effective.

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