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REVIEW ARTICLE

From Top-Down to Bottom-Up: Computational Modeling Approaches for Cellular Redoxin Networks

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Abstract

Significance: Thioredoxin, glutaredoxin, and peroxiredoxin systems play critical roles in a large number of redox-sensitive cellular processes. These systems are linked to each other by coupled redox cycles and common reaction intermediates into a larger network. Given the scale and connectivity of this network, computational approaches are required to analyze its dynamics and organization. ***Recent Advances:*** Theoretical advances, as well as new redox proteomic methods, have led to the development of both top-down and bottom-up systems biology approaches to analyze the these systems and the network as a whole. Top-down approaches have been based on modifications to the Nernst equation or on graph theoretical approaches, while bottom-up approaches have been based on kinetic or stoichiometric modeling techniques. ***Critical Issues:*** This review will consider the rationale behind these approaches and focus on their advantages and limitations. Further, the review will discuss modeling standards to ensure model accuracy and availability. ***Future Directions:*** Top-down and bottom-up approaches have distinct strengths and limitations in describing cellular redoxin networks. The availability of methods to overcome these limitations, together with the adoption of common modeling standards, is expected to increase the pace of model-led discovery within the redox biology field. *Antioxid. Redox Signal*. 18, 2075–2086.

Introduction

The cellular redox environment is a complex network consisting of metabolic and signaling pathways, redox metabolites, enzymes, and redox-sensitive transcription factors. Within this network, the thioredoxin, glutathione (GSH)-glutaredoxin, and peroxiredoxin thiol systems play key roles in DNA synthesis, redox homeostasis, antioxidant defense, redox signaling, and metabolism (72). These systems are present in most living cells and their cytosolic organization is similar in *Escherichia coli*, *Saccharomyces cerevisiae*, and human cells (30, 72). However, additional mitochondrial, nuclear, and/or chloroplast redoxin systems are found within higher eukaryotic cells that can dramatically increase the size of this network (30, 43, 72). Further, a number of organisms, including several human pathogens, have networks that show significant organizational differences to these canonical systems (34).

In the canonical cytoplasmic systems, reducing equivalents from NADPH are transferred to thioredoxins and GSH, which in turn reduce a number of redox partners, including the peroxiredoxins and glutaredoxins. Upon reduction, the redoxins reduce a number of cellular redox partners using either dithiol or monothiol mechanisms. The redox partners for these systems have been identified through biochemical, genetic, and proteomic methods and include a large and diverse set of protein and nonprotein targets (Fig. 1) (39, 71, 72).

The large number of processes influenced by redoxins has spurred the development of a number of distinct top-down and bottom-up systems biology approaches to model these systems. In addition, the involvement of redoxin systems in a wide variety of pathologies such as cancer (31), together with the increasing use of systems biology approaches in medicine (5, 26, 75), has added further impetus to the development of computational models of redoxin systems. The computational approaches to modeling these systems have all aimed to integrate information about the components of these systems so that the functional organization and in some cases, the dynamics of these systems could be understood from a network perspective.

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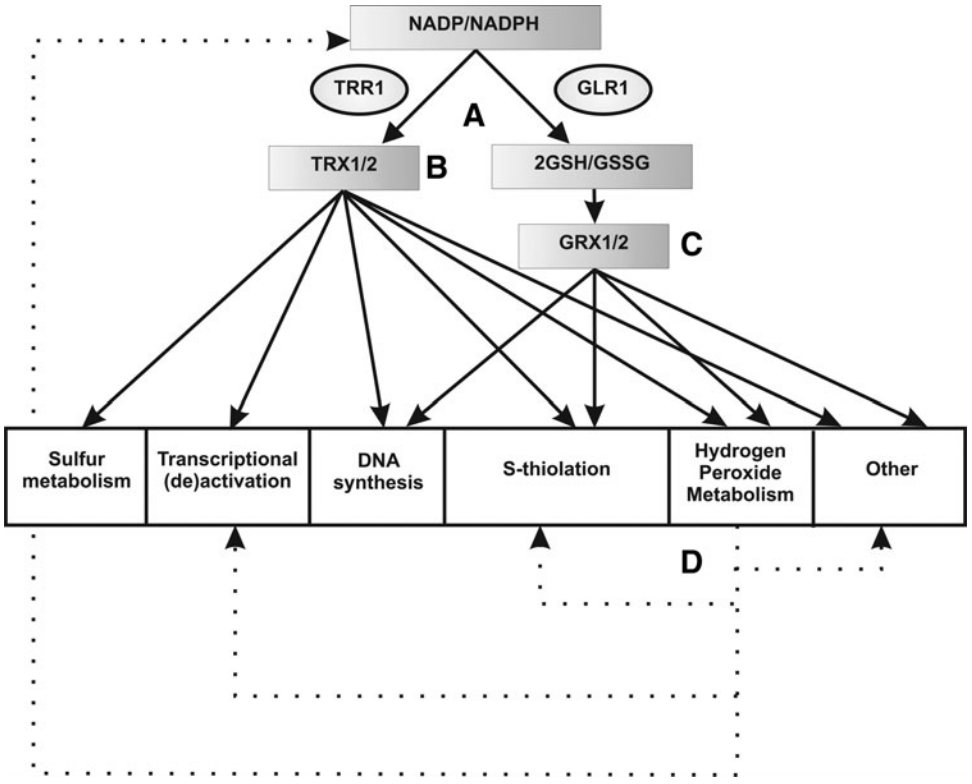

FIG. 1. The yeast cytosolic redoxin network is both complex and interconnected. Even in a relatively simple organism like the yeast cell, the cytosolic redoxin network has multiple components that are capable of cross talk. In this network the pentose phosphate pathway provides reducing equivalents in the form of NADPH that are transferred *via* thioredoxin reductase (TRR1) and glutathione reductase (GLR1) to the thioredoxins (TRX1, TRX2) and glutathione (GSH) (A), which in turn reduces the cytosolic glutaredoxins (GRX1, GRX2). Thioredoxins and glutaredoxins reduce a large number of protein and nonprotein targets involved DNA synthesis, hydrogen peroxide metabolism, S-thiolation, sulfur metabolism, transcriptional regulation, and several other processes (B, C). In the yeast redoxin network, both thioredoxins and glutaredoxins can reduce glutathionylated proteins. At lower concentrations, hydrogen peroxide acts as a signaling molecule and, among other interactions, can affect the steady-state NADP/NADPH ratio (D). However, at higher concentrations hydrogen peroxide causes the nonspecific oxidation of cellular components (D). For clarity, the reactions involving hydrogen peroxide are shown by a dashed line and reactions involving other reactive oxygen and nitrogenous species and antioxidant molecules are not shown. This figure was generated using references (9, 19, 28, 54, 62, 72).

In this review we consider the various modeling approaches used, the challenges associated with these approaches and the emerging trends in modeling these systems. We have limited this review to the modeling of redoxin systems as opposed to the entire cellular redox network for a number of reasons. First, the complexity and in some cases the sizes of some redoxin networks are significant (30, 43, 72), and these networks have been modeled independently of the greater cellular redox network (61). Second, while the methods to model certain components of the cellular redox network are well established [see for example (53, 55, 80)], the correct modeling approach for redoxin systems has been less clear (41, 60). Finally, we limited this review to redoxin systems to allow for sufficient clarity and depth, but the concepts highlighted here may also be applicable to the modeling of other redox systems.

Why Model Redoxin Networks?

The biggest impetus for modeling redoxin networks has come from an improved understanding of the scale and the complexity of these networks. At the molecular level, a susceptible cysteine residue within a target protein can be oxidized into a spectrum of reversible oxidation states from the disulfide to glutathionylated form, each of which may be reduced by a distinct thiol redox system (10, 12, 25, 38, 71). At the systems level, biochemical and redox proteomic studies have shown that the thiol redox systems reduce a vast array of targets and therefore affect a large number of cellular processes (39, 71, 72). Further contributing to complexity at this level is the cross-talk within and between the thiol redox systems within a given network (Fig. 1). In *S. cerevisiae*, for example, the thioredoxin system nonreciprocally affects the redox state of the GSH/glutaredoxin system and can reduce gluathionylated targets (28, 73), while in mammalian cells, oxidation of peroxiredoxins by hydrogen peroxide triggers thioredoxin oxidation, which in turn can trigger apoptosis signal-regulating kinase 1 (Ask1) activation (20). Given this complexity, computational modeling of these systems is currently the best, and perhaps only viable method, to understand the organization and regulation of these systems as an integrated whole.

Another important goal for modeling thiol redox networks is to match model construction and behavior with current theories on the organization of these systems. Changes in the

oxidation status of a number of cellular redox couples are correlated with a number of pathological conditions that led to the proposal of a disrupted balance between oxidants and antioxidants in oxidative stress-associated pathologies. However, the situation *in vivo* appears to be more complex than a simple redox balance and select nodes within a given cellular redox network may be oxidized under the oxidative stress conditions, resulting in the physiological responses associated with certain pathologies (36). It is also believed that redoxin systems participate in redox signaling (20, 37) although the nature and properties of these signals are still being clarified (17). Computational models of redoxin systems offer useful platforms to test, refine, and provide mechanistic details for these theories, which could then guide wet-laboratory analyses and eventually therapeutic strategies.

A final important goal for modeling redoxin systems is that digitizing these systems will allow for comparative system analysis, which may be able to answer fundamental questions about the organization and regulation of these networks. For example, a comparison of the structural and dynamic properties of the redoxin networks in fast and slow replicating cells may give an indication of the optimal network configuration for dNTP synthesis, which may be rate limiting in some cells (40). Similarly, a comparison of the redoxin networks of healthy and diseased cells may yield information on flux and concentration control distribution (16) within these networks and point to potential therapeutic targets.

However, before these goals can be realized, the most appropriate approaches to modeling these systems must be adopted. Currently, both top-down and bottom-up modeling approaches have been used to describe these systems. A detailed description of the differences between these approaches may be found elsewhere (7, 77). In general, top-down approaches are inductive, in which molecular datasets are interrogated to find the underlying connections and relationships between the nodes within a given network, while bottom-up approaches are mechanistic in that the interactions between the nodes in a network are explicitly modeled with the aim to describe the emergent properties of that network (7).

Top-Down Modeling of Redox Thiol Networks

There are two major top-down approaches to modeling redoxin systems. In the first of these approaches, a number of definitions for the cellular redox potential were proposed and subsequently used to identify the key redox couples involved in redox homeostasis within the cell. These definitions have drawn from studies on the *in vitro* redox regulation of proteins [reviewed in Ref. (24)] and on genetic studies that indicated that redoxin systems operate as electron flow pathways [reviewed in Refs. (58, 63, 72)]. These studies were subsequently extrapolated and cellular redox potentials, based on the Nernst equation, were determined using several low-molecular-weight redox couples found *in vivo*. Schafer and Buettner (64) modified the Nernst equation to quantify the contribution of several low molecular weight redox couples to the cellular redox state. To account for differences in the concentrations of these redox couples, the half-cell potentials (E_h) of these redox couples were multiplied by the concentration of their reduced species and the cellular redox environment (*RE*) was defined as the sum of these products:

$$RE = \sum_{i=1}^{n} E_{hi} \times [reduced]_i \quad (1)$$

Using this expression, Schafer and Buettner determined that 2GSH/GSSG couple was the major cellular redox buffer. Modifications to this proposal were forwarded by Hancock *et al.* (29) and Martinovich *et al.* (51) because there was no clear physical meaning for the units (mV·mM) of the redox environment expression (Equation 1) (51) and, that when using this expression, the calculated cellular redox potential would be more negative than the redox potentials of the individual redox couples within the cell (29). Hancock *et al.* (29) therefore modified Equation (1) to include the contribution of the oxidized species and normalized against the total concentration of a given redox couple (Equation 2), while Martinovich *et al.* (51) proposed an effective reduction potential (E^{eff}) as a measure of the total reductive capacity of the cellular redox environment (Equation 3):

$$RE = \frac{\sum E_h \cdot [reduced + oxidised]}{\sum [total]} \quad (2)$$

$$E^{eff} = \sum_{i=1}^{k} a_i E_i \quad (3)$$

where E_i is the reduction potential of a given redox couple and a is the specific charge transferred in a given reaction, defined as the charge transferred in a single reaction normalized against the total charge transferred in all reactions. It is calculated from the molar concentration of the reduced species in a given couple and the number of electrons transferred in a given redox reaction (z_i):

$$a = \frac{c_i z_i}{\sum_{j=1}^{k} c_j z_j} \quad (4)$$

Compared to other modeling approaches described below, the use of cellular redox potentials offers a number of advantages. First, this approach follows directly from studies looking at the redox regulation of proteins *in vitro*, and in some cases a clear relationship could be made between changes in the cellular redox potential of a given couple and the activity of a protein *in vivo*. Second, a number of well-established methods are available to assay the low-molecular-weight metabolites and redoxins that are used in these approaches. Finally, in some cases at least, changes in the cellular redox environment have indeed been correlated to physiological changes within cells and tissues (36, 44).

There are, however, a number of significant limitations associated with this approach. The Nernst equation and the modified equations proposed above only provide a measure of the thermodynamic potential but give no indication of whether reactions actually occur or the rates of these reactions within a given system (Fig. 2A, B). Indeed, the biological relevance of using the Nernst equation at all for the GSH and other cellular redox couples has been provocatively questioned (18). Further, while these equations may have some correlative value to physiological changes, they have shown no predictive value thus far. A final major limitation with this approach is that by only taking into account low-molecular-weight electron carriers, the links between redox metabolism and other cellular processes are ignored. Thus, for example,

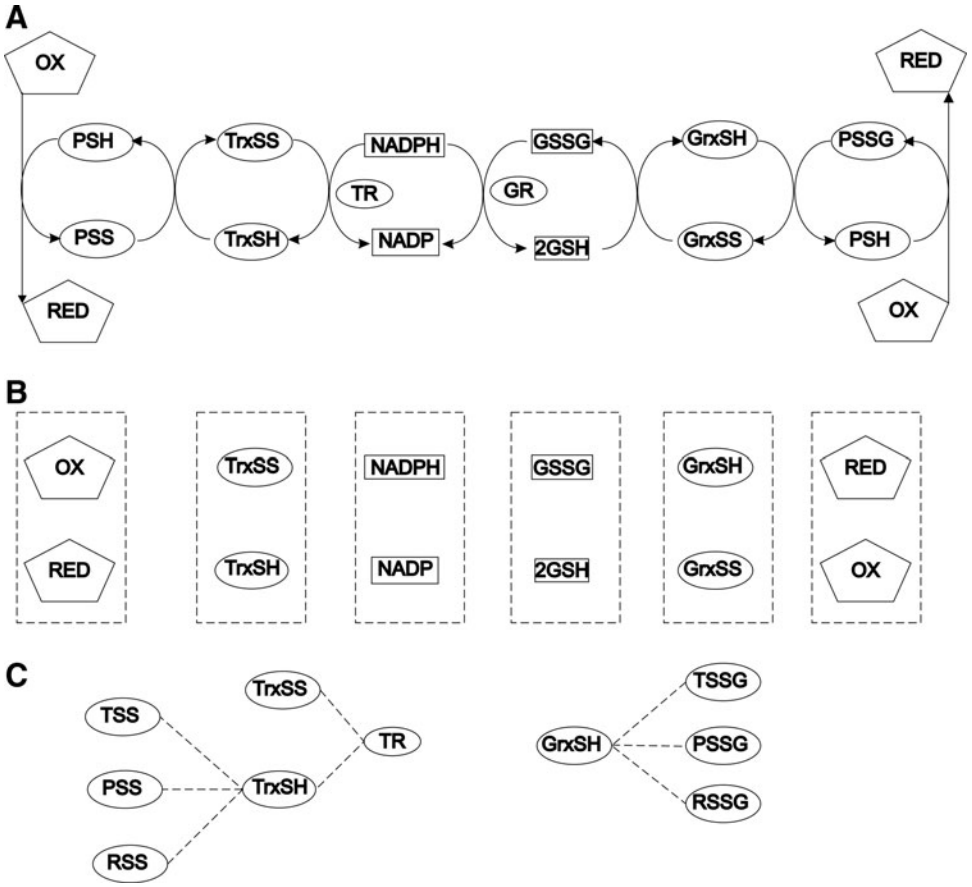

FIG. 2. The cellular redoxin network viewed from the kinetic modeling, redox potential, and redox proteomic perspectives. Bottom-up kinetic models of redoxin networks may incorporate enzymes (thioredoxin reductase [TR] and/or GSH reductase [GR]), redox couples, and, depending on the modeling approach, detailed kinetics of the individual reactions **(A)**. However, these models typically incorporate just a subset of the available protein targets for the thioredoxin and glutaredoxin systems (PSH and PSSG, respectively). Top-down models based on cellular redox potentials only include information on the redox potentials of select redox couples **(B)**, while models based on redox proteomic technologies are derived from protein–protein interaction datasets **(C)**. These latter models tend to include a more exhaustive list of disulfide (TSS, RSS) and glutathionylated (TSSG, RSSG) targets than the bottom-up models but do not include detailed kinetics or nonprotein redox couples (see text for details).

the dynamic redistribution of flux into the pentose phosphate pathway under oxidative stress conditions in *S. cerevisiae* could not be modeled with this approach despite the importance of this pathway in regulating steady-state NADPH levels under oxidative stress conditions (45, 62).

An alternate top-down approach to modeling redoxin systems has resulted from the development of redox proteomics technologies that have allowed for the global identification of redox-regulated proteins, the "redoxome" [reviewed in Refs. (39, 49, 71)]. These technologies have already generated interesting data sets which could yield insights into the scale and connectivity of redoxin networks and highlight potential hubs and bottlenecks within these networks (14, 39). However, there are some significant limitations associated with both the datasets generated by proteomics methods as well as the computational analyses undertaken on such datasets (52).

A critical area of concern with all proteomic methods is the reliability of the datasets generated by these methods. A comparison of the yeast two-hybrid assay, mass spectrometry of purified complexes, correlated mRNA expression, and *in silico* interaction prediction technologies showed that the protein interactome data sets generated by these approaches suffered from low coverage and/or low accuracy (76). In particular, all the methods tested generated a high number of false-positive interactions, which would clearly bias any downstream analyses. Since then there have been further attempts to determine the accuracy of protein–protein interactomes (74) and a number of curated molecular interaction databases have become available (3, 48) although there are no high-quality protein interactome databases available for plants (14). To the best of our knowledge, the datasets generated by current redox proteomics technologies have not been validated by independent approaches, and therefore the results emanating from the computational analyses of these datasets must be considered in this light.

An additional limitation with redox proteomic datasets is that dynamic information, such as reaction rates and directions, as well as non-protein redox couples such as GSH and NADP/NADPH, are excluded in these analyses (Fig. 2C). Further, most redox proteomic studies undertaken thus far have not followed changes in the redoxome with time, which

has consequently constrained their application to bottom-up quantitative models of redoxin networks (below). Taken together, these issues have limited the general applicability of the computational models developed using only these redox proteomic datasets.

Assuming that an appropriate proteomic dataset is obtained, the properties of this dataset can be analyzed using tools from graph theory. Graph theory is a large field of mathematics and excellent reviews on its application to biological datasets may be found elsewhere (4, 5, 52). In these analyses individual proteins are referred to as nodes which linked by "edges" or interactions forming a graph, which may be directed with edges associated with a particular direction (*e.g.*, signal cascade) or undirected. The topological properties of these graphs can be described using a number of key analytical measures, including the degree distribution, centrality, network diameter, and clustering (52). Unfortunately, none of these measures have yet been reported for the redoxin subnetwork, and it is therefore not known how the structure of this network compares to the global and other protein–protein interaction networks. Such an analysis could also yield information about the structural motifs found within the redoxin networks. Motifs are recurring patterns of association between nodes that confer specific properties to reaction networks (17) and are usually over-represented in biological networks when compared to a random Erdos-Renyi network (3).

Of the graph theoretical measures listed above, the degree distribution of the redox network has been used to identify "hubs" within these networks. The degree distribution of a network simply measures the number of nodes in a network (n_k) that have a degree k:

$$P(k) = \frac{n_k}{n} \tag{5}$$

Analysis of several real-life networks, including protein–protein interaction networks, has shown that their degree distributions could be described with a so-called power law and these networks are commonly referred to as being scale-free. In such networks, a small number of hub nodes are connected to a disproportionally high number of edges. In graph theoretical terms, these hub nodes have a relatively high degree and this scale-free organization ensures that these networks are more resistant to random deletions of nodes within the network when compared to randomized networks. Conversely, the deletion of a hub can have a serious effect on the overall connectivity within a network (35). Analyses of protein–protein interaction datasets showed that many essential genes in *S. cerevisiae* and other organisms were indeed hub nodes, which has prompted a search for hub nodes within redox and other proteomic datasets (14, 39). Another important topological measure within protein networks is "betweenness," which is a measure of the centrality of a network. A node with a high betweenness centrality is a node that has many shortest paths going through it and therefore represents a bottleneck within a network. In regulatory networks, these bottleneck nodes were also likely to be essential genes (81). Thus far, no bottleneck nodes have been identified for the redoxin subnetwork of the total protein interactome.

While scale-free networks appear to be ubiquitous in nature, there are some cautions that should be exercised when drawing broad generalizations from the results of proteomic and graph theoretical studies. Surprisingly, it has been shown for several real-life networks, including metabolic networks, that system controllability, which is the ability to move a dynamic system from an initial state to a given final state, was determined by "driver" nodes, which were usually not hub nodes (50). This suggests that the current focus on identifying hub nodes within redox proteomic datasets needs to be expanded. Second, many of the graph theoretical analyses on proteomic datasets have been based on only a sample of the total protein interactome and the sampling regime used can dramatically affect the apparent properties of these networks (52, 68). Finally and most importantly, many of the purported power laws that have been used to describe scale-free networks lack appropriate statistical support, and in many cases there is no mechanistic basis for the reported scale-free nature of these networks (67).

Bottom-Up Modeling of Redoxin Networks

Bottom-up models can be developed using quantitative or constraint-based modeling techniques (Fig. 3) [reviewed in Refs. (13, 16, 70)]. In both these techniques, the species (s) within a reaction network are described by a series of differential equations that are captured in a stoichiometric matrix (N). Each row in the matrix corresponds to a species, while each column represents the reactions involving that species (Fig. 3C). The coefficients within this matrix reflect the stoichiometry of reactions, while their signs, either positive or negative, indicate whether a species is produced or consumed by a given reaction (13, 16, 70). The stoichiometric matrix is multiplied by a rate vector (v) to give a general kinetic model for a series of reactions (Equation 6):

$$\frac{ds}{dt} = Nv \tag{6}$$

At a steady state, changes in the species concentrations are set to zero (*i.e.*, $Nv = 0$) and the system can solved using numerical approaches. However, a major difference between quantitative and constraint-based techniques is that the fluxes within constraint-based models are assigned upper and lower bounds in the rate vector, while in quantitative models, the rates within the system are described by kinetic expressions (70). Thus, quantitative models can offer greater insights into the dynamics of a given network of reactions, as bifurcation, stability, and control analytical methods can be used to analyze these models (13, 16). Constraint-based models on the other hand, can offer greater coverage of the total reaction network within a cell and genome-scale mathematical models of the complete or near-complete metabolic networks of several organisms have been developed using this approach [reviewed in Refs. (15, 70)]. This modeling framework has shown a high predictive power, especially for metabolic engineering (6), despite not explicitly modeling important regulatory reactions such as signal transduction cascades and gene regulation networks (13).

Despite their advantages, there have been very few quantitative redoxin system models published in the literature although the GSH/oxidized GSH couple has been included in some models (23, 33). A major reason for this has been the inconsistent descriptions of redoxin activity with redoxins described both as redox couples and as enzymes (see (60) for a detailed discussion). If redoxins were treated as redox

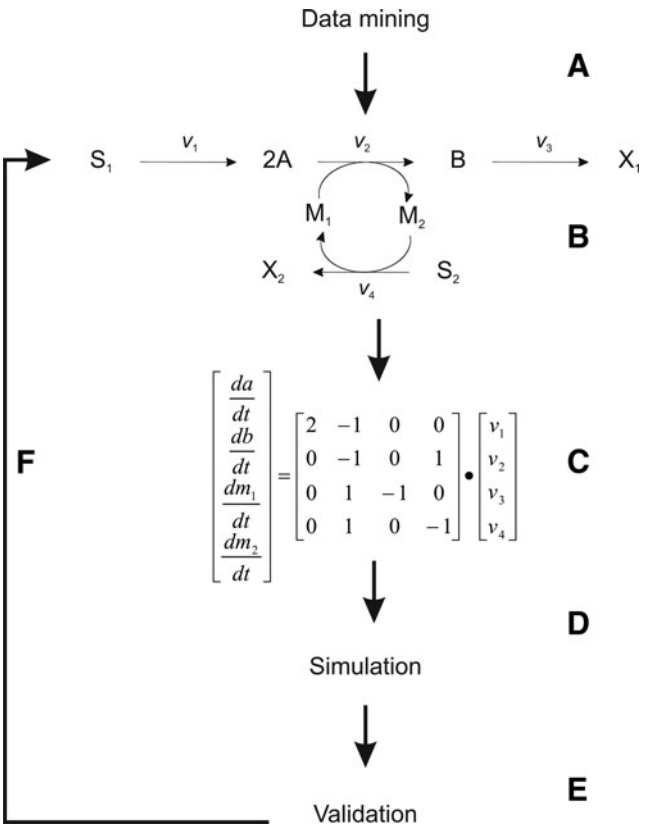

FIG. 3. The steps involved in building a bottom-up computational model. The first step in building a bottom-up computational model is searching the literature and specialized databases for information on the network being modeled (A). For genome-scale models this process can be automated, but manual curation is still undertaken to ensure accuracy (11, 70). Once the network and its parameters have been obtained (B), computational modeling software (42) is used to create a kinetic model of the system (C). In this simple reaction network, v_i represents the rates through the individual steps while the source (S_x) and sink (X_x) pools represent species concentrations which are kept constant during the time-scale described by the model. In constraint-based models, the rate vector defines the direction and upper and lower rate limits for a given reaction within the network, while in quantitative models kinetic rate laws are used to describe individual reactions (16). The model may be simulated (D) and the results obtained compared to realistic datasets (E), which may lead to further cycles of model refinement (F).

couples, then the couples would be variables within computational models of these systems and each member of a couple would be described with an ordinary differential equation. On the other hand if redoxins were modeled as enzymes, then their rate constants and concentrations would be parameters within the rate vectors of these models [cf. Equation (6), Fig. 3C].

A solution to the discrepancy between the descriptions of redoxin activity was recently proposed. Using kinetic modeling it was shown that the enzyme-like behavior attributed to thioredoxins and glutaredoxins was due to the saturation of the redox cycles within these systems and that mass action kinetics was sufficient to describe thioredoxin and glutaredoxin activity (60). Further, this study showed that the ratios of oxidized to reduced redoxin which had been used to describe cellular redox potentials (see above) actually reflected the relative rates of redoxin oxidation and reduction. Since then, two articles have been published that have used this approach to model redoxin systems. The first article published by Adimora *et al.* (1) provided a detailed quantitative model of hydrogen peroxide consumption by Jurkat T-cells. In contrast to previously published models, this model considered hydrogen peroxide reduction by catalases, GSH peroxidases, peroxiredoxins, and protein thiols. Analysis of the model showed that protein thiol groups play a significant role in reducing hydrogen peroxide and that the thioredoxin rather than the GSH system plays a larger role hydrogen peroxide detoxification (1).

While the modeling results allowed these researchers to determine intracellular fluxes and sensitivities for this system that could not be determined using current wet-lab technologies, it was the approach used by these researchers to construct the model that was significant for the field. The authors hypothesized that the thiol pool either reacted in bulk with hydrogen peroxide ("slow model") or a subset of the pool reacted more rapidly with hydrogen peroxide ("fast model"). Both models were then constructed using parameters from the literature and were fitted to four independent datasets which showed that the fast model was more accurate. The fast model was validated by comparing the model output to independent datasets in which the redox ratios of thioredoxin and GSH were determined (1). The use of these redox ratios to validate the model circumvented the need to measure the fluxes within the system and is similar to the approach used to validate kinetic models of phosphorylation-dependent signal transduction pathways (32). Furthermore, the use of independent datasets to fit and then validate the model ensured that potential missing parameters or reactions were not "fitted away" (7, 66), giving the model very good predictive power.

A model of the thioredoxin system in *E. coli* was also published last year (61). This system was modeled using both realistic and "core" models to identify and describe the kinetic motifs found in the system. Whereas realistic models use realistic kinetic parameters and rate expressions, core models use default kinetic parameter sets and basic rate expressions and are often useful in studying the generic underlying behavior within systems. Analysis of these models showed that the thioredoxin system was adaptable with the kinetic profiles (fluxes and species concentrations) of thioredoxin-dependent oxidation reactions affected by changes in concentrations of components of the thioredoxin system. The thioredoxin reductase concentration in particular affected the maximal fluxes through thioredoxin-dependent oxidation reactions, suggesting that the fluxes through thioredoxin-dependent processes such as ribonucleotide reduction depend not only on the enzymes involved in these processes but on thioredoxin reductase activity as well (61).

An unexpected behavior shown by the models was the presence of ultrasensitive changes in the reduced thioredoxin concentration with changes in the thioredoxin reductase concentration (Fig. 4A). Ultrasensitive responses are associated with switching behaviors in systems and are usually generated by enzyme moiety cycles in which convertor enzymes are operating under zero-order conditions (27). Using core modeling it was shown that the thioredoxin

ultrasensitive response was mediated by the coupling of the thioredoxin redox cycle to the redox cycle of a peroxiredoxin, Tpx (*E. coli* thiol peroxidase). Interestingly, computational modeling was used in this study to evaluate a theory on the organization of the cellular redoxin network. The results showed that in contrast to electron flow models of the thioredoxin system (41), all thioredoxin-dependent processes are capable of affecting each other through their combined effects on the thioredoxin redox cycle (Fig. 4B, C) (61).

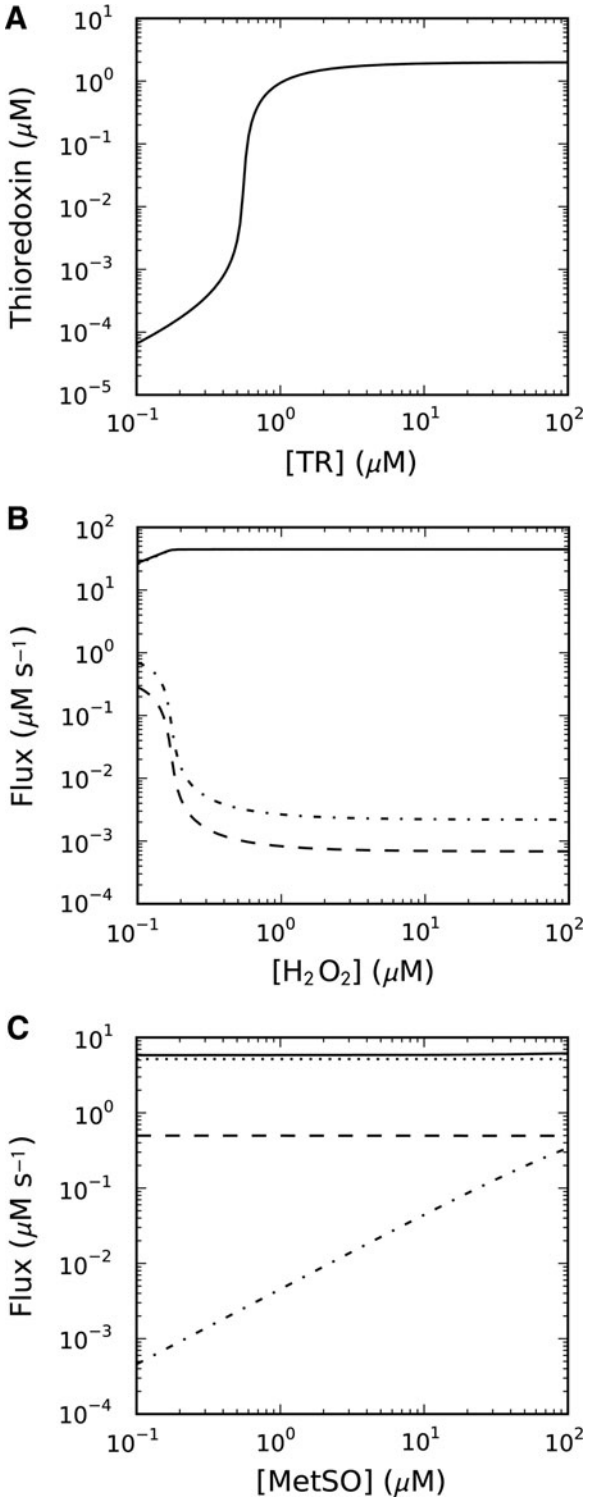

FIG. 4. Computational models provide platforms to study the regulation of redoxin systems as an integrated whole, to generate hypotheses, and to test theories on the organization of these systems. Analysis of a realistic computational model of the *Escherichia coli* thioredoxin system revealed that changes in the thioredoxin reductase concentration triggered ultrasensitive changes in the reduced thioredoxin concentration **(A)** by a novel mechanism (see text for details). It was also revealed that depending on their kinetics, thioredoxin-dependent reactions can be sensitive changes in the other parts of the system. Thus, increases in the hydrogen peroxide concentration decreased the fluxes of bulk protein disulfide (dashes) and methionine sulfoxide (dash-dot) reduction while the fluxes through thioredoxin reductase (solid) and Tpx (dots) were unaffected **(B)**. In contrast, changes in the methionine sulfoxide (MetSO) concentration had a negligible effect on the fluxes through other thioredoxin-dependent reactions **(C)** (61). Note that the parameter scans are plotted in double-log space so that scaled changes in the flux are reported.

The bottom-up models described above have included an important simplification for ease of modeling. Only a fraction of the total number of redoxin reactions were explicitly modeled with most protein substrates combined into an oxidized pool, which was assigned a concentration and reactions involving this pool were described with mass action kinetics. In contrast, most redoxin-dependent reactions are covered by the stoichiometric matrices of genome-scale constraint-based models. While these models are available for a number of model organisms and pathogens and can be freely downloaded from the BioModels (47) and BiGG databases (65), there have been no studies to date comparing *in silico* redoxin gene deletions with the wealth of *in vivo* and *in vitro* biochemical data available on the effect of these mutations on cells (72).

While bottom-up models have given mechanistic insights into the organization of redoxin systems, there are a number of limitations associated with this modeling approach. Arguably, the most important of these limitations is the uncertainty about the rate expressions used to describe redoxin reactions. While mass action kinetics has successfully been used to describe thioredoxin, glutaredoxin, and peroxiredoxin reactions within computational models (1, 60, 61), a number of questions remain. For example, as glutaredoxins and peroxiredoxins are generally considered to be enzymes, would modeling these redoxins with saturatable kinetic expressions, which require considerably more parameters, lead to more accurate and complete descriptions? It has also been proposed that glutaredoxins can deglutathionylate substrates using either a mono- or a di-thiol mechanism (21), but it is not clear which of these mechanisms should be used in computational models, particularly as the structural properties of models created using each of these mechanisms are different (Fig. 5). Similarly, the experimentally determined second-order rate constants for yeast peroxiredoxins do not agree with their second-order rate constants determined by steady-state kinetics (*i.e.*, k_{cat}/K_m) (56). The reason for this discrepancy is not known and therefore it is not clear whether the kinetic expressions used in models of the peroxiredoxin system are valid.

Another limitation of bottom-up modeling approaches is that the complete parameter sets for many of the reactions

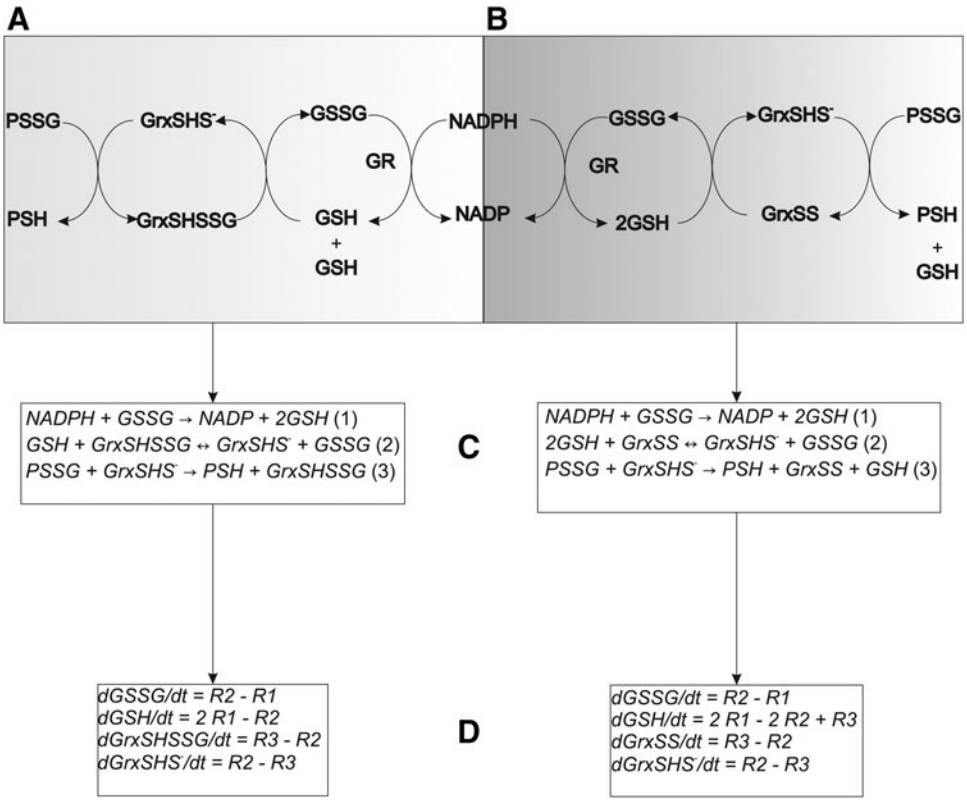

FIG. 5. Kinetic models based on the mono-and di-thiol mechanisms proposed for glutaredoxin activity have distinct structural properties. The glutaredoxin system can be modeled with either a monothiol **(A)** or dithiol **(B)** mechanism. In the monothiol mechanism, deglutathionylation of a substrate (PSSG) results in the formation of a glutaredoxin-GSH mixed disulfide (GrxSHSSG), while in the dithiol mechanism the deglutathionylation reaction results in the formation of oxidized glutaredoxin (GrxSS). Both mechanisms contain a GSH reductase reaction (GR) for the reduction of oxidized GSH and the side reaction associated with the monothiol mechanism is not shown [reviewed in Ref. (54)]. Models based on these mechanisms will include either GrxSHSSG or GrxSS **(C)** and the ordinary differential equations **(D)**, and therefore the stoichiometric matrices, describing these mechanisms are distinct.

within the thiol redox network are unavailable and current models of these systems are therefore expected to be limited. The use of robust fitting routines together with good experimental designs may, however, allow these missing parameters to be estimated (1). A further limitation in these types of studies is that some redoxins are present at concentrations that equal or exceed the concentrations of their substrates and therefore the Michaelis-Menten rate expressions and parameters determined for these molecules *in vitro* may not always be used to accurately describe their activity *in silico* (61, 79). In addition, the concentrations and rate constants for many of the known interactions between the thiol redox systems and their redox partners can vary almost a thousand fold. For example, in *E. coli* the concentration of GSH is in the millimolar range, while thioredoxin (Trx1) concentration is 2 μM (8). Larger models of complete thiol networks are therefore likely to be stiff.

Finally, there are two important assumptions that are usually made in bottom-up models that are particularly relevant to redox biology. First, in most models, intracellular compartments are treated as a well-stirred cells and the spatial segregation of network components is usually not explicitly modeled (13). While this assumption may be valid in some cases (59), in other cases where redox gradients play important physiological roles such as in redox signaling (17), this approach would be very limited. Second, cellular processes *in vivo* show a distinct separation of timescales with redox reactions taking place in the subsecond to the second timescale, while other events such as protein expression can take minutes to hours (2, 16). Bottom-up computational models are usually specific for a given timescale defined by the models' kinetics and do not incorporate events that occur at significantly different timescales. Thus, for example, while a model may include the redox activation of a transcription factor, its effect on gene expression and therefore the redoxin network being described is generally not explicitly modeled. Recently, this limitation has been overcome in a whole cell computational model of *Mycoplasma genitalium* by allowing processes acting at different timescales to exchange information at 1-second intervals (40). However, and somewhat surprisingly, there have been no published computational models on redox-sensitive genetic networks and it is not clear how these networks should be modeled and what effect changes in these genetic networks would have on the dynamics of the redoxin network as a whole.

Standards for Describing Computational Models

Regardless of the modeling approach adopted, it is essential for this developing field that the models produced are

both accurate and accessible to other researchers. Fortunately, the systems biology community has adopted a number of standards for the description of quantitative models that could be used to describe redox systems biology models. The minimum information requested in the annotation of biochemical models (MIRIAM) standard for model description and annotation provides a number of rules that must be satisfied if a model is to be considered compliant (46). Arguably, the most important of these rules is that models must be available in systems biology mark-up language (SBML) or CellML format or failing that must be fully described so that they can be built using another computational modeling platform. However, having models in SBML format is preferred as such models can be immediately interrogated across a variety of computational modeling platforms. Indeed, a number of journals, including *Molecular Systems Biology, FEBS Journal,* and *Microbiology* require that SBML-formatted computational models are deposited in public databases such as BioModels (47) (www.ebi.ac.uk/biomodels-main/) or JWS On-line (57) (http://jjj.biochem.sun.ac.za/) before publication. This requirement is not particularly onerous as the most commonly used commercial and open-source modeling packages (42) have SBML-convertors (http://sbml.org/SBML_Software_Guide/SBML_Software_Summary). For a comparison of the modeling software and databases available for computational systems biology research, the reader is referred to Klipp *et al.* (42).

MIRIAM-compliant models are also required to be able to reproduce the results described in the associated publication. Provided that the modeling was done competently, this may appear to be a superfluous requirement. However, differences in the algorithms used by different modeling software packages can affect the results produced by a model. For this reason, some researchers test the SBML compatibility of their models and their modeling results over more than one platform. In addition, critical parameters can be identified by parameter-sensitivity analysis [see, e.g., Ref. (61)]. While these practices are not standard requirement for researchers building models, in our experience they are often very useful controls in modeling experiments.

Summary: Toward the Promise of Redoxin Systems Biology

Understanding the regulation of redoxin systems from a network perspective could provide insights into the organization of the cellular redox environment as well as pathologies associated with redoxin up- or down-regulation. However, while both top-down and bottom-up computational systems biology approaches have already been used to model human disease (5, 75) and toxicology networks (22, 23), they have not been used to describe the roles of redoxins in pathologies. This is largely due to a number of critical gaps that remain in our understanding of how to model redoxin systems and consequently the top-down and bottom-up modeling approaches used thus far have had varying degrees of success. In general, the top-down approaches used have given broad, but limited descriptions of the state and connectivity of cellular redoxin networks. Surprisingly, Bayesian analysis, which has been used successfully in a number of top-down modeling approaches (78), has not yet been used to describe redoxins networks. The use of this analytical technique, especially when applied to redox metabolomic and proteomic datasets, may provide important insights into the organization of redoxin systems but more importantly, could immediately provide markers or targets for therapeutic intervention.

Bottom-up approaches, in contrast, have included the development of small-scale but detailed quantitative redoxin models incorporating metabolites, protein concentrations, and kinetic information, together with genome-scale, constraint-based stoichiometric models. However, the development of quantitative models has been limited by questions over the accuracy of kinetic expressions used for redoxin activity and missing parameters. These problems are being addressed by reevaluating the kinetic expressions assigned to redoxins and through the use of data-fitting routines to estimate missing parameters for model building. Currently, no studies have been undertaken to validate the modeling of redoxin reactions in genome-scale models and it is therefore not clear what the advantages and limitations are with this approach.

As top-down and bottom-up approaches have distinct strengths and limitations, a combination of these modeling approaches may appear to be an obvious strategy for describing redoxin systems. However, in the short-term, the choice of modeling approach will still depend on the research questions being asked and on the system or network being studied. We expect that top-down modeling approaches will be favored when developing therapeutic interventions or when analyzing systems whose kinetic parameters have not been fully described. Bottom-up approaches, on the other hand, will be favored when the mechanistic details of a given redoxin process needs to be finely interrogated. In the longer term, data generated by redox proteomic studies may well be used to develop increasingly detailed bottom-up models of these networks and the resulting models will be an important resource for both basic and applied researchers. It is therefore essential that these models are accurate and that their limitations are clearly understood. Model integrity is consequently going to be one of the most critical issues facing redox systems biologists going forward. As a first step to ensuring integrity, models should be available for exchange, preferably in SBML-format. This, together with the development of redox-specific databases (69), will encourage the development and adoption of good modeling practices and accelerate model-led discoveries within the redox biology field.

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Abbreviations Used

Ask1 = apoptosis signal-regulating kinase 1
GLR1 = yeast glutathione reductase
Grx = glutaredoxin
GSH = glutathione
MetSO = methionine sulfoxide
MIRIAM = minimum information requested in the annotation of biochemical models
PrSS = oxidized protein
PrSSG = glutathionylated protein
Prx = peroxiredoxin
SBML = systems biology mark-up language
Tpx = *E. coli* thiol peroxidase
TRR1 = yeast thioredoxin reductase
Trx = thioredoxin