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Systemic Redox Regulation of Cellular Information Processing

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Abstract

Receptor-mediated signaling leads to transient changes in redox state, resulting in reversible oxidation of protein cysteine thiols. Numerous signaling proteins have been identified as being redox sensitive; however, to date, most investigations have focused on the ramifications of isolated protein modifications on cellular phenotypes. We propose that reversible thiol oxidation of proteins in a signaling network and their systemic interactions introduce features in the dynamics and control of cellular responses that are unique compared with isolated oxidative protein modifications. Simulations of dynamic redox regulation in different cellular contexts reveal feasible regulatory features for future experimental investigation. We suggest that location within a network, compartmentalization, and the degree of connectivity between redox proteins can dramatically modulate cellular information processing. *Antioxid. Redox Signal.* 16, 374–380.

Introduction

With systems biology maturing as a discipline, it is natural that different approaches have emerged which study the complexity of biological systems. One subdiscipline that has arisen is known as cellular information processing. This community borrows from engineering to treat the cell as a "black box" with a number of extracellular inputs (*e.g.*, soluble ligands, matrix interactions) being interpreted, integrated, and processed to elicit outputs (*e.g.*, change in phenotype, apoptosis, and transcription). This subdiscipline generally focuses on small- to medium-sized network modeling (rather than on large genome-wide or proteomic-wide data analysis) to enrich the understanding of regulatory mechanisms.

Oxidative modifications of protein thiols are emerging as important regulatory mechanisms, operating in combination with phosphorylation events to propagate signals. As more proteins are identified as being redox sensitive, it becomes imperative to ask how thiol oxidation within a signaling network modulates the interpretation of extracellular cues. We constructed and analyzed computational models of common kinase/phosphatase motifs in response to transient cellular oxidation in order to examine the systemic behavior that emerges due to redox sensitivity of signaling proteins. While we focus on redox control in the presence of hydrogen peroxide, the dynamic regulation of intracellular oxidants allows the principles to extend to other species.

Reversible Inactivation of Phosphatases

One of the most prevalent, conserved roles of protein thiol oxidation in signaling is the inactivation of active-site cysteines across the protein tyrosine phosphatase (PTP) family. An open question in redox biology is how the reactivity of phosphatases with hydrogen peroxide can explain the large degree of thiol modification observed in cells; postulated mechanisms include locally enhanced regions of oxidation or indirect oxidation mediated by other thiol proteins (9). Although the consequences on enzymatic function are uniformly inhibitory, the susceptibility of PTP isoforms to oxidation varies, as shown by both *in vitro* kinetic experiments and cellular studies. Furthermore, some serine/threonine

Innovation

Decades of research have led to a cumulative knowledge base of proteins that undergo thiol modification during receptor-mediated signaling; however, the systemic consequence of redox-sensitive proteins on cellular responses is still an open question. The computational modeling results presented here indicate that location within a network, compartmentation, and the degree of connectivity between redox proteins can dramatically affect signaling dynamics and reveal plausible regulatory features for future experimental investigation.

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phosphatases, such as calcineurin and PP2A, have cysteine thiols susceptible to oxidation, while other members of this protein family do not. Examination of signaling networks requires accounting for the different degrees of inhibition of phosphatases during receptor-initiated cellular oxidation.

We investigated general properties of systemic redox regulation through phosphatase inactivation during dynamic cellular oxidation using a computational model that can account for previously published data (1). Chen *et al.* showed that platelet-derived growth factor (PDGF) stimulation of human lens epithelial cells activates the ERK signaling cascade with concomitant production of hydrogen peroxide. Catalase-mediated scavenging of the peroxide resulted in severely ablated ERK signaling. Furthermore, exogenous addition of H_2O_2 was sufficient to activate ERK signaling. PDGF has been reported to oxidize and inactivate multiple PTPs such as SHP2 in fibroblasts (5); however, the identities of all phosphatases affected during this mitogenic stimulus are unknown. We hypothesized that these observations can be explained by a model of ERK signaling that takes into account reactive oxygen species (ROS)-mediated, reversible oxidative inactivation of multiple phosphatases. The opposing activities of kinases and phosphatases counterbalance each other in the basal state. When the cells are stimulated with PDGF, the ERK pathway receives an upstream activating signal originating from the receptor. Simultaneously, ROS-mediated inactivation of phosphatases skews the enzymatic balance in favor of the kinases, thereby activating the signaling cascade. Over time, the input signal decays and ROS production ceases, allowing the phosphatases to be reduced back from their reversible inactive state. The signaling pathway is then restored to its basal state.

We adopted the Raf/MEK/ERK cascade from (6) and modified it by adding ROS-mediated reversible inactivation of phosphatases (Fig. 1A). While this canonical cascade has

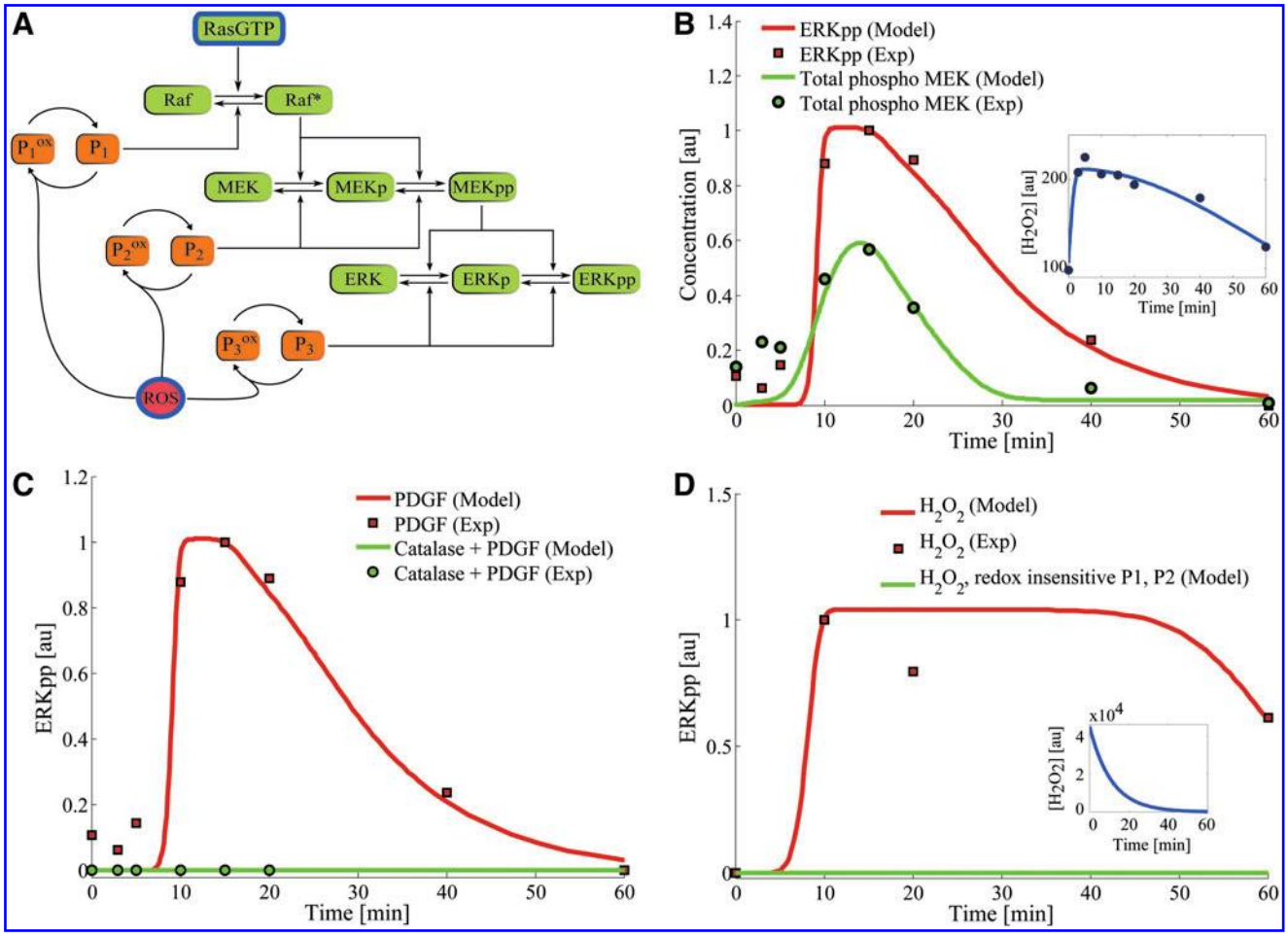

FIG. 1. Computational model of redox regulation in lens epithelial (HLE B3) cells. (A) ERK signaling pathway with two-step phosphorylation was modeled. Phosphatases were reversibly inhibited by oxidation. Species highlighted in blue (RasGTP and ROS) were supplied as input functions to the model. **(B)** Parameters of the model were optimized to fit experimental measurements of MEK and ERK phosphorylation after PDGF stimulation of HLE B3 cells (1). The ROS input function (inset) was obtained by fitting a curve to measured DCF fluorescence. **(C)** Catalase pretreatment was simulated using the model fitted in **B** by setting ROS level to 0. **(D)** Exogenous bolus addition of H_2O_2 was simulated using the fitted model in **(B)** with an exponentially decaying ROS curve (inset), and RasGTP was not allowed to change from its basal level. Optimization of the ROS curve alone was sufficient to fit the experimental data. ERKpp levels fail to increase substantially if phosphatases P1 and P2 are not sensitive to oxidation. (Further details regarding Figure 1 may be viewed as Supplementary Data; available online at www.liebertonline.com/ars). ROS, reactive oxygen species; ERKpp, phosphorylated ERK; PDGF, platelet-derived growth factor.

been extensively modeled in growth factor signaling, kinetic description of phosphatases has been limited to a flat dephosphorylation rate rather than considering phosphatase activity as variable. ROS dynamics were simulated using a curve fitted to the PDGF-induced H_2O_2 time course as measured in (1) (Fig. 1B, inset). A transient RasGTP signal was used as an input to the model and was optimized to fit the data. All three phosphatases (P1, P2, and P3) were assumed to be susceptible to oxidation but with varying sensitivities (Fig. 1A). The oxidation rates were optimized to fit experimentally measured MEK and ERK phosphorylation time courses reported in (1) (Fig. 1B). The variation in oxidation across the cascade may be representative of (a) differences in proximity to the ROS source and, thus, the effective concentration of H_2O_2; (b) differences in the oxidation rates of the proteins; (c) differences in the reduction rates due to structural disulfide bond formation, or (d) the specificity for a reducing enzyme (glutaredoxin *vs*. thioredoxin). Although a previous modeling study demonstrated how inhibition of phosphatases can influence mitogen-activated protein kinase (MAPK) dynamics through both duration and amplitude of signal (4), the authors adhered to the convention of a flat rate of dephosphorylation for their simulations. Our added description of time-dependent variation in phosphatase activity due to dynamic oxidative modifications provides regulatory features for fine tuning the response to a given input, depending on which phosphatases in a cascade are affected.

The model was able to reproduce experimentally measured ERK and MEK phosphorylation levels due to PDGF treatment (Fig. 1B). Catalase pretreatment was simulated in the model by setting ROS levels to zero at all times. The model predicted attenuated ERK phosphorylation consistent with published data (Fig. 1C). Furthermore, an exogenous bolus addition of H_2O_2 was simulated using a high initial value followed by a first-order decay (Fig. 1D, inset). Optimization of two parameters, H_2O_2 initial value and decay rate, was enough to capture the experimental observations. We note that the fit in Figure 1B was obtained with very low oxidation rates for P1 and P2 and a much higher rate for P3. Thus, P3 was the most susceptible to oxidation with the pool very quickly oxidized and remaining so for the period of simulation. P2 had the slowest oxidation rate, and less than 1% of total P2 was oxidized throughout the simulation. P1 was maximally oxidized at 16% near 30 min and then monotonically decreased. Interestingly, the model suggests that the slow oxidation could be physiologically important, as making P1 and P2 completely insensitive to oxidation resulted in abrogated ERK phosphorylation even with the exogenous addition of a large amount of H_2O_2 (Fig. 1D).

This observation suggested that the location of P1 and P2 in the signaling network may have a important role in their ability to influence the signaling. To test this idea, we performed further simulations with the optimized model. Different sets of phosphatases were defined to be oxidation sensitive, and dually phosphorylated ERK (ERKpp) was monitored as the output. The results suggest that phosphatases with substrates further upstream of ERK are progressively more potent in influencing the signaling dynamics (Fig. 2A). Furthermore, when pairs of phosphatases are redox sensitive, their individual effects add up qualitatively (Fig. 2B). These results suggest that topologically conserved MAPK networks can produce a variety of outputs—perhaps even to the same input signal—as the dynamics of phosphatase oxidation/reduction change in a context-dependent manner.

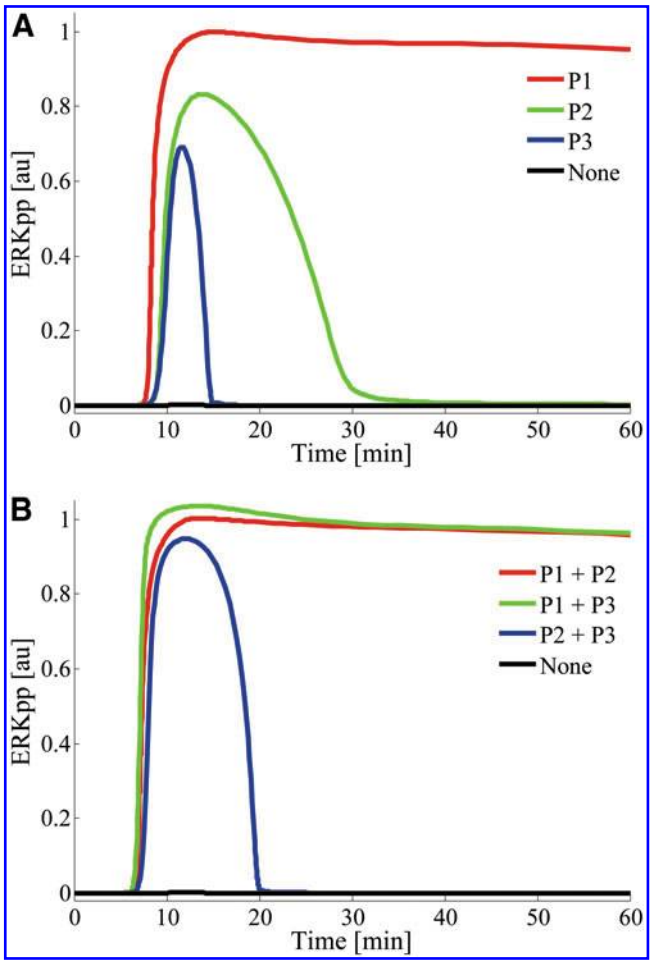

FIG. 2. Topological features influence the contribution of phosphatases. Simulations were performed by setting different combinations of phosphatases as redox sensitive. **(A)** ERKpp levels when only a single phosphatase is redox sensitive. The same oxidation and reduction rates were assigned to each phosphatase for consistency. Oxidation of phosphatases with substrates further removed from ERK leads to greater amplification of the response. **(B)** ERKpp response when pairs of phosphatases are redox sensitive. The output for two phosphatases appears to be a qualitative sum of the outputs when individual phosphatases are redox sensitive. (To see this illustration in color the reader is referred to the Web version of this article at www.liebertonline.com/ars). (Further details regarding Figure 2 may be viewed as Supplementary Data; available online at www.liebertonline.com/ars).

Oxidative Control Through Compartmentalization

Apart from punctuate distribution of ROS sources, differences in redox potential in the cellular environment are also created by organellar compartmentalization. The redox microenvironments faced by proteins in a signaling cascade can be quite different as they move across compartments. In particular, the nucleus is a more reducing microenvironment than the cytosol by approximately a 20 mV difference in thioredoxin potential (2). Oxidative modification can be reduced or enhanced by the transport process; consequently,

the dynamic location of a protein can impact its oxidation state and, hence, catalytic activity.

We modeled the impact of translocation of a redox-sensitive protein from cytosol to a relatively more reducing nucleus. MAPKs can translocate to the nucleus to perform their functions as transcription factor regulators (7). Phosphatases of MAPKs present in the nucleus are exposed to a more reduced environment as compared with the cytosolic phosphatases and are, hence, likely to be more active. As a consequence, the cytosolic activity of MAPK may not correspond exactly with the nuclear activity of MAPK. To assess the effects of compartmentalization of redox state between the cytosol and nucleus, we used the optimized ERK signaling model shown in Figure 1 and extended it to include nucleo-cytoplasmic shuttling of ERK and its phosphatase P3 (Fig. 3A). When the nucleus is assumed to be at the same redox state as the cytosol with protein oxidation and reduction occurring at the same rate in both compartments, the cytosolic and nuclear fractions of active MAPK follow each other very closely (Fig. 3B). However, when the nucleus is modeled as more reduced than the cytosol and free of ROS, then the nuclear fraction of active MAPK is significantly attenuated (Fig. 3C). Moreover, the overall signal strength is also reduced by the nuclear compartmentalization. Observations of ERK oscillations between the nucleus and cytosol have an unexplained mechanistic basis in signaling regulation (7). The ability to regulate the active form of the kinase by redox regulation of its corresponding phosphatase may be an explanation for the shuttling process.

Oxidative Control Through Kinases

Unlike the phosphatases, the reported examples of other signaling molecules are much more varied in the nature and consequences of their thiol modifications, ranging from affecting enzymatic activity, cellular location, or binding in macromolecular complexes. Redox sensitivity of kinases can also potentially influence cell signaling. There is recent evidence that the MAPK p38 can be reversibly oxidized (8). It was found that under prostaglandin-stimulated oxidative conditions or with exogenous H_2O_2 addition, phosphorylation of p38 is maintained, while its activity is reversibly inhibited by oxidation at multiple cysteine sites. To investigate this scenario, we modified the model shown in Figure 1 to include ROS-mediated reversible oxidation of a dually phosphorylated MAPK (Fig. 4A). In the absence of kinase oxidation, most of the dually phosphorylated MAPK is available as free (*i.e.*, unbound to other proteins) active MAPK, assuming that the dually phosphorylated form is the enzymatically

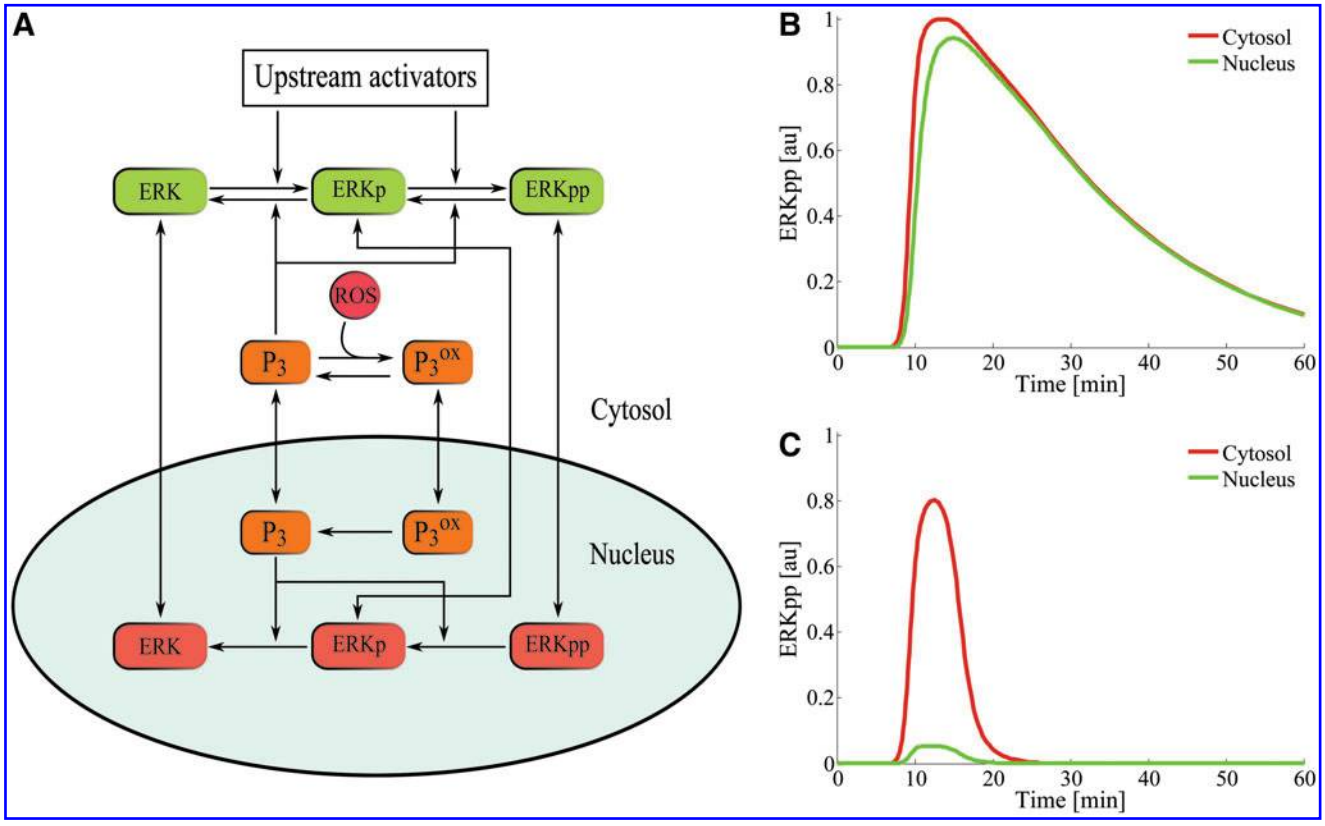

FIG. 3. Compartmentalization of redox potential affects signaling. (A) The model optimized to HLE B3 cell data (Fig. 1) was modified to include nucleo-cytoplasmic shuttling of ERK and its phosphatase P3. Nuclear entry and exit were modeled as first order reactions with equal rate constants and were assumed to be the same for all proteins concerned. **(B)** The nucleus was assumed to be at an identical oxidative state as the cytosol. Cytosolic and nuclear levels of ERK were predicted to be similar. **(C)** The nucleus was modeled as a more reduced compartment so that there was no protein oxidation in the nucleus. Protein reduction rates were taken to be the same in both compartments. Both the nuclear and cytosolic signals are attenuated compared to *panel* **(B).** (To see this illustration in color the reader is referred to the Web version of this article at www.liebertonline.com/ars). (Further details regarding Figure 3 may be viewed as Supplementary Data; available online at www.liebertonline.com/ars).

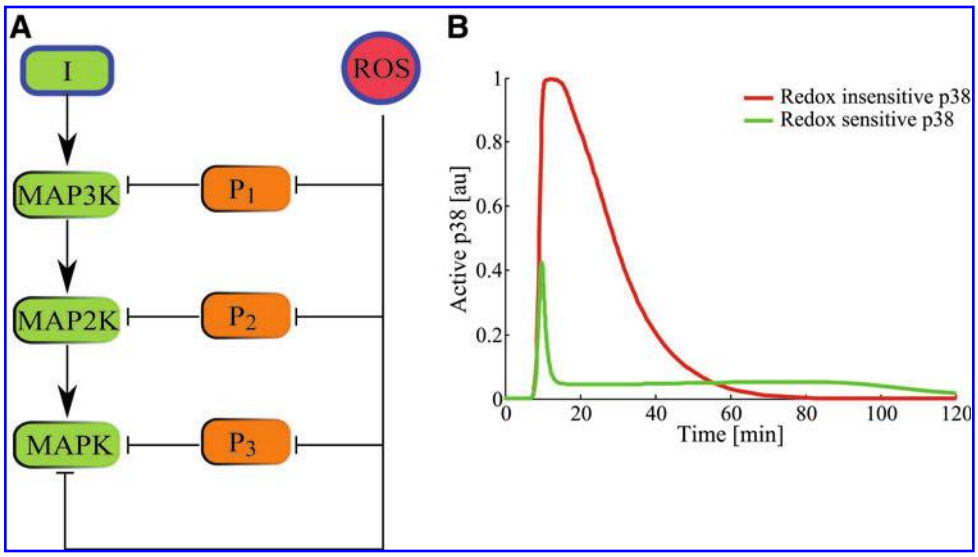

FIG. 4. Oxidative inhibition of MAPK. (A) Reversible oxidative inhibition of MAPK was modeled by modifying the model in Figure 1. The dual-phosphorylated form of MAPK was assumed to be redox sensitive, and the oxidation was reversible. **(B)** Taking the MAPK cascade as corresponding to the p38 signaling pathway, simulations were performed with dually phosphorylated p38 being sensitive or not to oxidation. Reduced, dually phosphorylated p38 unbound to other proteins was assumed to be the enzymatically active form, and the oxidized form was catalytically inactive. Inclusion of reversible oxidation resulted in low p38 activity sustained over a longer period of time. (To see this illustration in color the reader is referred to the Web version of this article at www.liebertonline.com/ars). (Further details regarding Figure 4 may be viewed as Supplementary Data; available online at www.liebertonline.com/ars). MAPK, mitogen-activated protein kinase.

active form (Fig. 4B). When the kinase is sensitive to oxidation, only a fraction of the phosphorylated MAPK is available in its active form, the rest being in the oxidized state (Fig. 4B). These results agree qualitatively with the data reported by (8). In addition, the oxidation of phosphorylated MAPK serves as a sequestration mechanism for the active kinase. The oxidized form is reduced slowly back to the active form allowing the active MAPK to be maintained at an almost steady, low level for a longer period of time. This modeling result illustrates how oxidation of the kinase may be a mechanism to trap the rapidly generated active kinase and release it slowly over time.

Complexity in Signaling Networks

The relative contributions of different redox-sensitive phosphatases can vary from those seen in the linear kinase cascade in Figure 1, depending on the complexity of the system. For example, heregulin stimulation of ErbB4 receptor simultaneously activates the Ras/Raf/MEK/ERK and PI3K/Akt pathways. Apart from a common activating input, the two pathways are connected by two other mediators—cross-talk in the form of Raf inhibition by Akt and a common serine/threonine phosphatase PP2A inhibiting the two pathways at Akt and MEK (Fig. 5A). As with PDGF, EGF receptor ligation has been reported to induce ROS as a necessary component of the signal transduction (1). A computational model of this system was adopted from (3) and was modified to include ROS mediated inactivation of phosphatases. This network presents the interesting feature of ROS playing both an activating and inhibitory role in the ERK pathway at the same time. ROS-mediated inhibition of PP2A and MKP3 directly activates the ERK pathways; however, inhibition of PP2A allows greater activity of Akt, which inhibits ERK signaling through Raf. Simulations show that with the same network structure, either of these effects can dominate depending on the parameters of the model. The presence of ROS could amplify (Fig. 5B) or attenuate (Fig. 5C) ERK phosphorylation in a context-dependent manner. The increased complexity of the network makes it more difficult to predict the role played by ROS in cell signaling and highlights the utility of computational modeling in parallel with experimentation. At the same time, this example also shows that dynamic modification of phosphatase activity can have profound effects on the outcome of signaling.

Conclusion

These simple models are instructive in demonstrating that the degree of cellular oxidation has the ability to modulate the amplitude and duration of signal transduction events in biochemical signaling networks. Furthermore, compartmentalization of redox potential in different cellular organelles can result in different interpretations of the same signaling molecule due to subcellular location. Redox regulation is a powerful mechanism for tuning the cellular response to a given input in a context-dependent manner; thus, a cell may skew toward a particular phenotype based on the interpretation of a cue through altered signaling dynamics. Importantly, hypo- and hyper-responsiveness to receptor ligation can be achieved without changing the topology of a signaling network.

Computational modeling of protein oxidation networks is a promising avenue for interpreting the complex effects of cellular oxidation on cellular information processing. For the model presented in Figure 1, the addition of transient phosphatase oxidation and only slight modification of the kinetic parameters was all that was necessary to explain a number of experimental conditions in which altering redox state affects signaling. While there are numerous aspects of redox signaling ignored in these simulations (spatial gradients, mitochondrial *vs*. Nox ROS production, and additional feedback mechanisms), we can still gain insight into principles of regulatory control by testing network location and differential sensitivities to reversible oxidation. As knowledge builds of redox control mechanisms within signaling networks, it is likely that common regulatory motifs will emerge across the varied receptor families in which oxidative bursts occur. A key question that improved proteomic tools

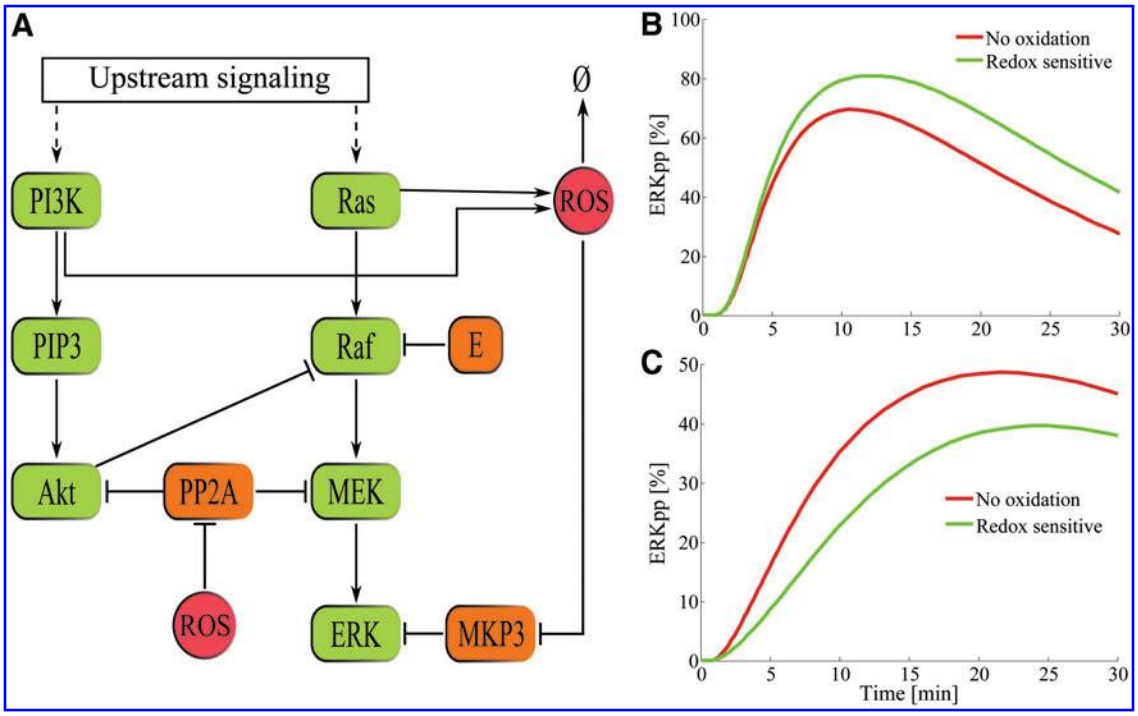

FIG. 5. Increased complexity leads to varied ROS regulation. (A) The Akt-ERK crosstalk model was adopted from (3) and modified to include oxidative inhibition of phosphatases MKP3 and PP2A. ROS production was assumed to be a Nox dependent process requiring the presence of active PI3K and RasGTP for Nox activation. ROS production was, therefore, modeled as a process driven by active PI3K and RasGTP, and the decay was a first-order process. **(B)** Redox-sensitive phosphatases result in amplification of ERKpp compared with a condition where no protein is redox sensitive. **(C)** By strengthening the inhibitory effect of Akt on Raf while at the same time weakening the control of PP2A on MEK dephosphorylation (altering three parameters in all), the qualitative effect of ROS on ERKpp was reversed as compared with *panel* **(B)**. With the altered parameters, the oxidation-sensitive model resulted in signal attenuation compared with the redox-insensitive model. (To see this illustration in color the reader is referred to the Web version of this article at www.liebertonline.com/ars). (Further details regarding Figure 5 may be viewed as Supplementary Data; available online at www.liebertonline.com/ars).

will help answer is whether redox-sensitive proteins are evenly distributed within receptor signaling networks, or clustered at specific nodes for influencing the relay of information.

Notes

The model in Figure 1 was derived from (6) by retaining only the three-step Raf/MEK/ERK module. A skewed Gaussian curve was used as RasGTP input. All parameters of the original model were used unchanged except the initial value of P2, which was increased 10-fold to fit the experimental data. The experimental data were obtained from (1) and represent band intensities of Western blot experiments as quantified in the original publication. The intensities were normalized to loading controls for individual gels. The normalized values for individual proteins were scaled and shifted so that the lowest signal has a value of 0, and the signal at 15 min is 1. The same scaling factor was used for all experimental data in Figure 1A and B. The model outputs were scaled by the computed value of ERKpp at 15 min in Figure 1A and B. The same method was used in Figure 1D; however, the scaling to unity was for the 10 min time point for both experimental and model data. We used a curve fitted to experimentally measured H_2O_2 as ROS input to the models in Figure 1 (see Fig. 1B, inset) to account for multiple sources of ROS (*e.g.*, Noxs, mitochondria) after receptor activation. The same input function was retained for models in Figures 1–4. Since DCF fluorescence intensities cannot be calibrated to true H_2O_2 concentrations, we used DCF fluorescence values from Chen *et al.* (1) as a scaled representation of the concentration. The protein oxidation and reduction rates were defined in this context and do not represent true rates. The fitted phosphatase oxidation rates spanned a wide range with orders of magnitude from 10^{-6} to 10. All proteins were assigned the same reduction rate in Figure 1. The reducing enzymes were assumed to remain constant for the sake of parsimony and were not explicitly modeled. This model was used as the basis for all models in Figures 1–4 with appropriate modifications (see Supplementary Data).

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Abbreviations Used

DCF = dichlorodihydrofluorescein
ERK = extracellular signal regulated kinase
ERKpp = phosphorylated ERK
MAPK = mitogen-activated protein kinase
MEK = MAPK/ERK kinase
MKP3 = MAP kinase phosphatase 3
PDGF = platelet-derived growth factor
PP2A = protein phosphatase 2A
PTP = protein tyrosine phosphatase
ROS = reactive oxygen species

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