Tyrosine Phosphorylation Regulates Manganese Superoxide Dismutase (MnSOD) RNA-Binding Protein Activity and MnSOD Protein Expression

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ABSTRACT: All cells tested contain a cytosolic protein that binds to a defined region in the 3′ untranslated region of manganese superoxide dismutase (MnSOD) RNA; both the MnSOD RNA-binding protein (MnSOD-BP) and the cis element are required for efficient translation of MnSOD RNA [Chung, D. J., Wright, A. E., and Clerch, L. B. (1997) *Biochemistry 37*, 16298−16306]. This study was designed to test the hypothesis that MnSOD-BP activity is regulated by phosphorylation. When cell extracts from whole rat lung or a rat lung fibroblast cell line, RFL-6, were treated in vitro with a protein tyrosine phosphatase, there was a 4-fold increase in MnSOD-BP activity indicating that MnSOD-BP activity was upregulated by tyrosine dephosphorylation. RFL-6 cells treated in cell culture with herbimycin A or genistein, inhibitors of protein tyrosine kinase, had significantly more MnSOD-BP activity than cells treated with diluent. In RFL-6 cells treated with herbimycin A, the increase in MnSOD-BP activity was associated with an increase in the level of MnSOD protein without a change in MnSOD mRNA concentration. We propose that the modulation of MnSOD protein expression by the tyrosine phosphorylation state of MnSOD-BP is a potential therapeutic target for increasing MnSOD activity during periods of oxidative stress.

Manganese superoxide dismutase (MnSOD)[1] is a mitochondrial antioxidant enzyme whose mRNA is encoded by nuclear DNA and translated in the cytoplasm. A leader peptide targets MnSOD protein to the mitochondrial matrix where it catalyzes the conversion of superoxide radical to H_2O_2 and O_2 and is critical in protecting cells against oxidant stress (*1*−*3*). In several animal models, the activity of MnSOD, which protects the lung against superoxide radical and the damaging effects of high concentrations of oxygen, appears to be appreciably regulated at a posttranscriptional level (*4*). In animal models in which the lung is protected against the damaging effects of hyperoxia (tolerant models), MnSOD activity increases due, at least in part, to an increase in the rate of lung MnSOD protein synthesis and translational efficiency measured as the rate of MnSOD protein synthesis per RNA (*5*, *6*). In animal models in which the lung is damaged by oxygen toxicity, exposure to oxidant stress significantly decreases MnSOD protein synthesis in the lung despite a large oxygen-induced increase in the concentration of lung MnSOD RNA (*7*, *8*). This decrease in translational efficiency (amount of MnSOD protein synthesized per RNA) is responsible, at least, in part, for pulmonary oxygen toxicity. To understand the mechanism(s) responsible for MnSOD posttranscriptional regulation and gain insight into possible therapeutic approaches to protect the lung against oxidative damage, we began to search for a MnSOD RNA-binding protein that might have a regulatory effect on MnSOD mRNA stability or translation. We found there is a protein that binds to a 41-base element located in the 3′ UTR of MnSOD RNA (*9*) and that in a cell-free system both the binding protein and cis element are required for superior translation (*10*). These prior studies in a cell-free translation system indicated that the MnSOD RNA-binding protein is a positive regulator of MnSOD protein expression and that the MnSOD RNA 3′ cis element is a translational enhancer. When the MnSOD 3′ UTR response element, designated MnSOD-RE, was placed 3′ of the coding region of chloramphenicol acetyltransferase, translation of the reporter in cell culture was enhanced, providing further support for the hypothesis that MnSOD-RE performs the function of a translational enhancer (*11*). The control of gene expression at a posttranscriptional level through the action of cytoplasmic RNA-binding proteins is an increasingly active area of research (*12*, *13*). However, in only a few cases have detailed mechanisms been explicated.

One of the central means of regulating gene expression is through signal transduction pathways involving kinase/phosphatase cascades (*14*). At the level of transcriptional control, this may result in phosphoregulation of DNA-binding transcription factors; at the level of translational control, this may result in phosphoregulation of initiation or elongation factors involved in protein synthesis. The experiments described in this paper were designed to test the hypothesis that MnSOD-BP activity is regulated by a phosphorylation

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[1] Abbreviations: DMSO, dimethyl sulfoxide; DU, relative densitometry units; MnSOD, manganese superoxide dismutase; MnSOD-BP, manganese superoxide dismutase RNA-binding protein; MnSOD-RE, manganese superoxide dismutase response element; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; REMSA, RNA electrophoretic mobility shift assay; RFL-6, rat lung fibroblast cell line; ROS, reactive oxygen species; RPA, ribonuclease protection assay; SE, standard error of the mean; UTR, untranslated region; YOP, Protein Tyrosine Phosphatase from *Yersinia enterocolitica*.

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switch mechanism. Reagents that alter the phosphorylation state of proteins were used to test the effect of phosphorylation on MnSOD-BP activity both in vitro and in cell culture. To assess the functional consequence of MnSOD-BP phosphoregulation, the concentrations of endogenous MnSOD protein and mRNA were determined in RFL-6 cells in culture after treatment with an inhibitor of protein tyrosine kinase. The results from the in vitro experiments indicate that MnSOD-BP is posttranslationally modified by tyrosine phosphorylation and that the dephosphorylated protein is more active in binding to the MnSOD 3′ UTR cis element. In the cell culture studies, data show that inhibition of tyrosine kinase not only increases MnSOD-BP activity but also causes an elevation in the level of MnSOD protein without a change in MnSOD RNA concentration.

EXPERIMENTAL PROCEDURES

Animals. Sprague Dawley rats were obtained from Taconic Farms, Germantown, NY, and were maintained in the animal care facility at Georgetown University Medical Center. All procedures were in accordance with USDA and NIH guidelines. Rats were killed by exsanguination after anesthesia with intraperitoneal injection of xylazine (∼10 mg/kg) plus ketamine (∼75 mg/kg). Lungs were excised, frozen in liquid nitrogen, and stored at −70 °C until use.

Measurement of RNA-Binding Activity. Cell extracts were prepared as previously described (*10*). Briefly, rat lung tissue or cultured cells were homogenized in 25 mM Tris buffer, pH 7.4, containing 0.5 mM phenylmethanesulfonyl fluoride, 0.2 units/mL aprotinin, 10 μg/mL leupeptin, 0.5 mM EDTA, 1% Triton X-100 and 40 mM KCl. Supernatant material after centrifugation at 100 000 g (S100) was used for all RNA-binding assays described in this paper.

The RNA probe was transcribed from RMS-12-Cl-16 plasmid as previously described (*10*). Briefly, after linearization of RMS-12-Cl-16 plasmid with Asp I, a 132 base-long ^{32}P-labeled transcript was prepared with the SP6 riboprobe system (Promega) for synthesis of high specific activity radiolabeled RNA probes according to the manufacturer's instructions. This probe is designated RMS-NA.

An RNA electrophoretic mobility shift assay (REMSA) was used to assay for MnSOD-BP activity as previously described (*9*, *10*). For quantification, autoradiographs of the gels were scanned by laser densitometry (Molecular Dynamics) using Image Quant Software; the data are expressed as relative densitometry units.

To perform a cross-linking assay, after treatment with heparin in the standard RNA-binding assay (*9*, *10*), ^{32}P-labeled RMS-NA probe was bound to protein by cross-linking with ultraviolet light irradiation for 30 min and assessed as previously described (*15*). To leave very small RNA oligonucleotides on the protein, the ribonucleoprotein complex was treated both with RNase T1 that cleaves 3′ of the G residues and with RNase A that cleaves after C and U residues. On the basis of the sequence of the RNA labeled probe and the 41-base region, we have identified as the region of binding, we estimate that the contribution of the RNA to the molecular mass is ≤2%. The molecular mass of the binding protein was measured by calibration against ^{14}C-labeled protein standards (Amersham).

To perform the filter-binding assay, rat lung extract was incubated for 30 min at 30 °C with 50 units of YOP or buffer D (50 mM Tris-HCl, pH 7.0, 100 mM NaCl, 2 mM EDTA, 5 mM DTT, 0.01% Brij 35, and 50% glycerol). This incubation was followed by the standard RNA-binding assay (*9*, *10*) protocol with the following modification: after treatment with T1 RNase and heparin, the samples were not subjected to electrophoresis; instead they were immediately filtered under vacuum through a 0.45 μm nitrocellulose filter (Schleicher & Schnell). Unbound RNA probe passed through the filter and RNA−protein complexes were retained on the filter. Filters were dried and radioactivity was quantified by liquid scintillation counting. Background was calculated from a binding reaction containing ^{32}P-labeled RMS-NA without the addition of protein. The percent of probe bound to protein was calculated as follows: % bound = (dpm bound − background)/total initial probe × 100.

In Vitro Phosphatase Treatment. Cell extracts were incubated for 30 min at 30 °C with protein tyrosine phosphatase from *Yersinia enterocolitica* (YOP) (Calbiochem) or an equal volume of buffer D. After treatment with the enzyme, RNA-binding activity was assessed by REMSA (*9*, *10*).

Cell Culture. Rat lung fibroblast cells, RFL-6 (ATCC, CCL-192) were maintained in Ham's F12K medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.15% sodium bicarbonate, and 50 ng/mL selenium. For treatment with protein tyrosine kinase inhibitors, cells were seeded into 60 mm tissue culture dishes and allowed to achieve ∼75% confluence. Cells were washed with phosphate buffered saline and then treated with herbimycin A (Calbiochem), genistein (Calbiochem), or an equal concentration of the diluent, DMSO, at 37 °C in the same medium described above without the addition of serum.

Measurement of MnSOD Protein and mRNA. MnSOD protein was assessed by western analysis as previously described (*5*) with the following modification: MnSOD protein was detected by enhanced chemiluminesence (Amersham) using the protocol outlined by the manufacturer. To test for specificity of the response, actin protein was measured by western analysis using the chemiluminesence technique with goat anti-actin antibody (Santa Cruz). The protein bands visualized on the autoradiographs were quantified by laser densitometry and the data were expressed as relative densitometry units. MnSOD mRNA was quantified by ribonuclease protection assay using the RPA II kit (Ambion) as previously described (*11*); the data are expressed as densitometry units of MnSOD RNA per 18s RNA as an internal standard.

Statistical Analysis. The values for individual samples were averaged per experimental group, and the standard error of the mean was calculated. The significance of the difference between two groups was obtained using a two-tailed *t*-test analysis assuming unequal variance. The significance of the difference between more than two groups was determined by analysis of variance and Duncan's multiple range test.

RESULTS

Treatment with Protein Tyrosine Phosphatase Increases MnSOD RNA-Binding Protein Activity in Vitro. MnSOD-BP activity was measured by REMSA in rat lung extracts treated with the protein tyrosine phosphatase, YOP. Compared with buffer treated controls, there was a 2.8-and 4-fold

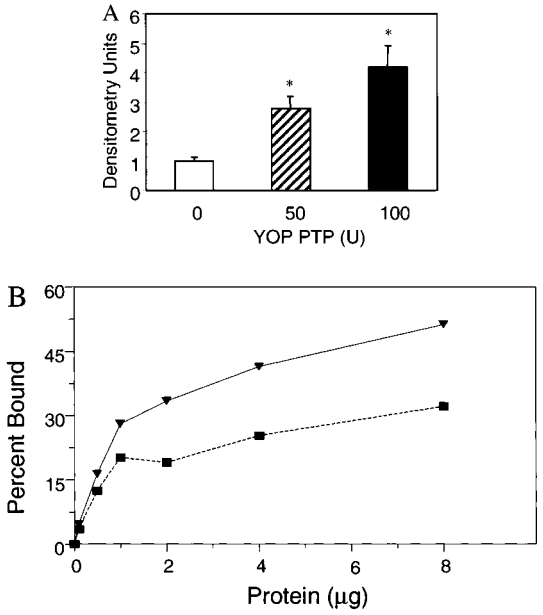

FIGURE 1: Effect of protein tyrosine phosphatase treatment on MnSOD-BP activity in rat lung extract. (A) Rat lung protein extracts (2 μg) were treated with buffer, 50 U YOP or 100 U YOP for 30 min at 30 °C and RNA-binding activity was assayed by REMSA. Autoradiographs were quantified by densitometry; the data are expressed as relative densitometry units and analyzed using a two-tailed paired t-test. Data are given as mean ± SE, $n = 4$ for each group, (*) $P < 0.04$ compared with buffer-treated extract. (B) Increasing amounts of rat lung protein extracts (abscissa) were treated with 50 units of YOP or buffer for 30 min at 30 °C and analyzed by a filter-binding assay. The triangles are the data for YOP-treated extract. The squares are the data for the buffer-treated control.

increase in MnSOD-BP activity in extracts treated with 50 and 100 units, respectively (Figure 1A). Measurements of binding activity with a filter-binding assay confirmed that protein tyrosine phosphatase increased MnSOD-BP activity; a representative binding graph is shown in Figure 1B. At lung extract concentrations greater than 1 μg of protein, a higher percentage of ^{32}P-labeled MnSOD 3′ UTR RNA was bound by extracts that had been treated with 50 units of YOP compared to control. These results indicate that MnSOD-BP activity was modulated by tyrosine phosphorylation in a dose−response manner and that the intracellular pool of MnSOD-BP in all probability includes both tyrosine phosphorylated and dephosphorylated forms of the protein. Dephosphorylation increased the ability of the protein to bind the MnSOD RNA 3′ UTR cis element previously shown to be a translational enhancer (*10*, *11*).

RFL-6 Cell Culture Is an Appropriate Model to Study Phosphoregulation of MnSOD-BP. To substantiate that a nontransformed rat lung fibroblast cell line, RFL-6, is an appropriate model to examine the in vivo regulation of MnSOD-BP by phosphorylation, we ascertained whether RFL-6 cells contained MnSOD-BP with the same size, sulfhydryl group requirement, and in vitro response to protein tyrosine phosphatase as found in rat lung tissue. Figure 2A depicts a REMSA in which extracts from both neonatal rat lung tissue and RFL-6 cells showed MnSOD-BP activity with a retarded complex of the same electrophoretic mobility. For both lung and RFL-6 extracts, RNA-binding activity was eliminated when the extract was treated with either 100 mM diamide or 10 mM *N*-ethylmaleimide. These results are in agreement with our previous findings indicating MnSOD RNA-binding activity requires that the protein contain a free sulfhydryl group (*9*). When tested in vitro, YOP treatment increased the MnSOD RNA-binding activity in extracts from RFL-6 cells to approximately the same degree (4-fold) as in cell extracts from rat lung tissue. Figure 2B is a representative REMSA showing the effect of YOP treatment on RFL-6 cell extract. Using laser densitometry to quantify the increase, MnSOD-BP activity in control extract was 1509 ± 245 SE, MnSOD-BP activity in RFL-6 extract treated with 100 units YOP was 6312 ± 1116 SE, $n = 4$ for each group, $p < 0.04$. When examined by ultraviolet light covalent cross-linking of the radioactively labeled phosphorus of the RNA to the protein, the autoradiograph showed one major band at ~90 kDa in both lung and RFL-6 cell extracts with or without PTP treatment (Figure 2C). Although a small change in apparent molecular mass cannot be ruled out, it appears that RFL-6 cells and rat lung contain the same size MnSOD-BP and that treatment with protein tyrosine phosphatase does not activate a different binding protein but rather increases the activity of the ~90 kDa MnSOD RNA-binding protein.

Inhibition of Tyrosine Kinase Increases MnSOD-BP Activity in RFL-6 Cells in Culture. To test whether tyrosine phosphorylation would alter MnSOD-BP activity in cell culture, RFL-6 cells were treated with herbimycin A, an inhibitor of tyrosine kinase. A dose response study showed 1 μM of herbimycin A resulted in the greatest increase in MnSOD-BP activity as measured by REMSA; increasing doses of herbimycin A did not further increase MnSOD-BP activity (data not shown). A time course study indicated the maximal effect occurred at 1 h (Figure 3). MnSOD-BP activity in RFL-6 cells 1 h after treatment with 1 μM of herbimycin A was increased 1.7-fold compared to the diluent control (Figure 3).

In separate experiments, genistein, a different inhibitor of tyrosine kinase, was used to confirm that an inhibition of tyrosine kinase elevated MnSOD-BP activity. A dose−response curve indicated MnSOD-BP activity was maximally elevated by treatment with 50 μM genistein; a time course study demonstrated that the effect was maximal at 3 h of treatment (data not shown). In RFL-6 treated with 50 μM genistein for 3 h, MnSOD-BP activity was increased 2.3-fold compared with the DMSO control. MnSOD-BP activity (DU) was 23.2 ± 6.5 SE and 53.7 ± 10.7 SE in control versus genistein-treated cells, respectively, $n = 4$, $P < 0.02$. Treatment with either herbimycin A or genistein did not induce a different RNA-binding protein; one major band with an apparent molecular mass of 90 kDa was observed by cross-linking analysis (data not shown). The results of these studies in RFL-6 cells treated with tyrosine kinase inhibitors support the hypothesis formulated from the in vitro studies, namely that MnSOD-BP is posttranslationally modified by tyrosine phosphorylation and that the dephosphorylated protein is more active in binding to the MnSOD 3′ UTR cis element than the phosphorylated protein.

Inhibition of Tyrosine Kinase Increases MnSOD Protein Expression without a Change in MnSOD RNA Concentration. To determine if the herbimycin A-induced increase in MnSOD-BP activity affected MnSOD expression, MnSOD protein and mRNA concentrations were measured in RFL-6

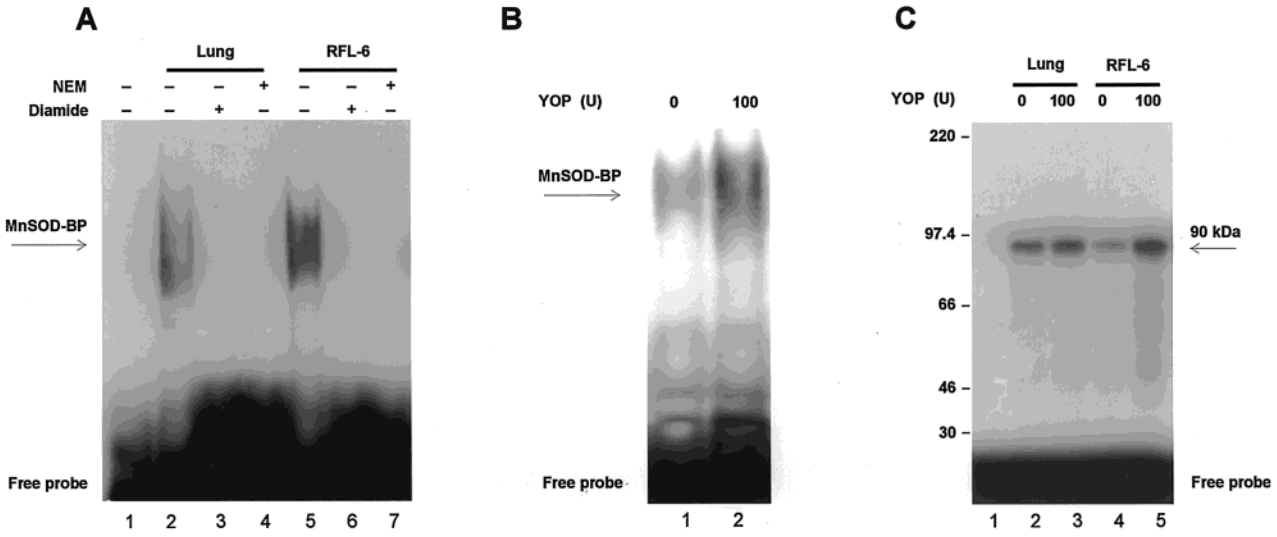

FIGURE 2: MnSOD-BP characterization in rat lung and RFL-6 cells. (A) REMSA using protein extracts (2 μg) from rat lung (lanes 2−4) and RFL-6 cells (lanes 5−7). Lane 1 shows the reagent blank when no protein is present. The arrow points to the major RNA−protein complex seen in lanes 2 and 5. Treatment of extract with either 100 mM diamide (lanes 3 and 6) or 10 mM *N*-ethylmaleimide (NEM) (lanes 4 and 7) eliminates the formation of the RNA−protein complex. The migration of the free probe is indicated at the bottom of the autoradiograph. (B) Representative REMSA of MnSOD-BP activity in RFL-6 protein extracts (5 μg) treated with buffer (lane 1) or 100 units of YOP (lane 2). (C) Ultraviolet light cross-linking analysis of rat lung (lanes 2 and 3) and RFL-6 cell (lanes 4 and 5) extracts after treatment with buffer (0 U YOP) or 100 U YOP as indicated at the top of the gel. Lane 1 is the result of a control reaction in which no protein was added. The arrow indicates one major MnSOD-BP band at ~90 kDa detected in both lung and RFL-6 cell extract. The migration of the free probe is indicated at the bottom of the autoradiograph.

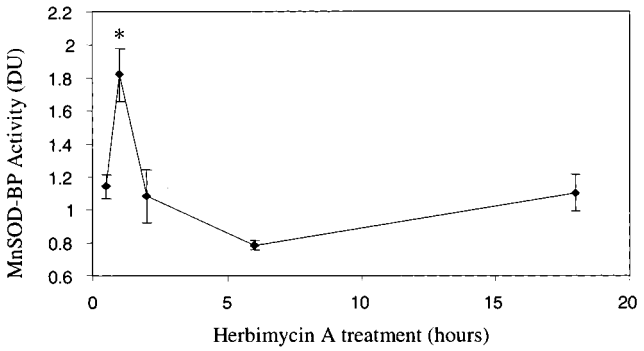

FIGURE 3: Effect of inhibition of tyrosine kinase activity on MnSOD-BP in RFL-6 cells. Time course of MnSOD-BP activity (assayed by REMSA) beginning at 0.5 h in RFL-6 cells treated with 1 μM herbimycin A at 37 °C. MnSOD-BP activity is given as mean ± SE densitometry units (DU) normalized to diluent DMSO-treated control cells. The data were analyzed by an analysis of variance, $n = 7$ at 0.5 and 2 h, $n = 8$ at 1 h, $n = 4$ at 6 and 18 h, (*) $P < 0.05$ compared with all other time points.

cells that were treated for 1 h with 1 μM of herbimycin A. MnSOD protein was increased 2.2-fold in herbimycin A-treated cells compared with DMSO-treated control cells (Figure 4). To examine the specificity of this response, the concentration of actin protein was assessed in the same samples; treatment with herbimycin A did not change the amount of immunoreactive actin (Figure 4). Measured by RPA, the concentration of MnSOD mRNA was not altered by treating cells with herbimycin A; expressed as relative densitometry units per 18s RNA, the concentration of MnSOD RNA was 1.00 ± 0.11 SE in DMSO diluent-treated RFL-6 cells and 0.82 ± 0.17 SE after cells were treated with 1μM herbimycin A for 1 h at 37 °C, $n = 6$ in each group, $P = 0.38$. Thus, an increase in MnSOD protein occurred without a concurrent increase in MnSOD mRNA. These results indicate that inhibition of tyrosine kinase not only increases MnSOD-BP activity but also causes a posttranscriptional 2.6-fold elevation in MnSOD protein expression.

DISCUSSION

The findings in this report suggest that the intracellular pool of MnSOD-BP includes both tyrosine phosphorylated and dephosphorylated forms of the protein and that tyrosine dephosphorylation increases RNA-binding activity. In vitro studies showed that treatment of cell extract with protein tyrosine phosphatase YOP increased RNA-binding activity, presumably by increasing the quantity of dephosphorylated RNA-binding protein. In a cell culture model, inhibition of tyrosine kinase induced an increase in MnSOD-BP activity and an increase in MnSOD protein expression. We do not have an antibody to MnSOD-BP that would allow us to perform immunoprecipitation and definitively prove that herbimycin A acted directly on MnSOD-BP; but, taken together with the in vitro experiments, the results suggest that a kinase/phosphatase signal transduction pathway may exert fine control over posttranscriptional regulation of MnSOD expression. A switch in the state of tyrosine phosphorylation of the RNA-binding protein would allow a cellular response to a change in the external environment resulting in a change in MnSOD protein synthesis. From the viewpoint of physiological significance, these pathways are likely to affect the capacity of the cell to rid itself of superoxide and produce hydrogen peroxide because the binding protein and cis element are required for enhanced translation of MnSOD (*10*). Therefore, the phosphorylation state of the RNA-binding protein could affect either the ability of the cell to withstand the damaging effects of reactive oxygen species or perhaps regulate downstream signaling by reactive oxygen species when they act as second messengers; for example in the activation of NFκB (*16*). The findings presented in this paper have led us to propose the model depicted in Figure 5 to explain why some why some

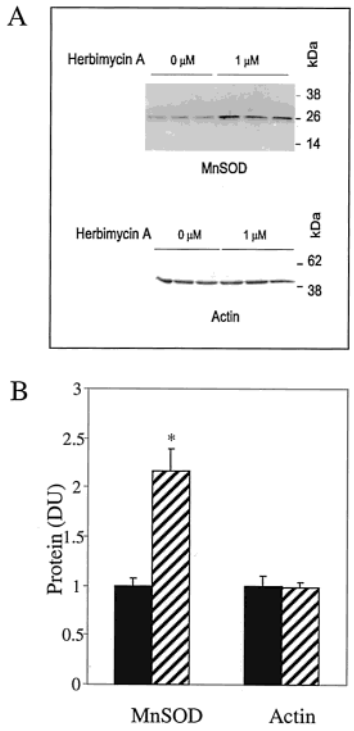

FIGURE 4: Effect of herbimycin A on MnSOD protein and mRNA in RFL-6 cells. (A) Representative western of MnSOD protein (upper gel) and actin protein (lower gel). The photos of the gel show three separate samples for each treatment with DMSO (0 μM herbimycin A) or 1 μM herbimycin A. (B) Quantification of the level of MnSOD protein and actin protein in RFL-6 cells treated with DMSO (solid bar) or 1 μM herbimycin A (crosshatched bar) for 1 h at 37 °C. Autoradiographs of the western blots were quantified by densitometry; the data are expressed as relative densitometry units (DU) and analyzed by an unpaired t-test assuming unequal variance. The data are given as mean ± SE, $n = 10$ for MnSOD, $n = 6$ for actin, (*) $P < 0.001$ compared to diluent-treated cells.

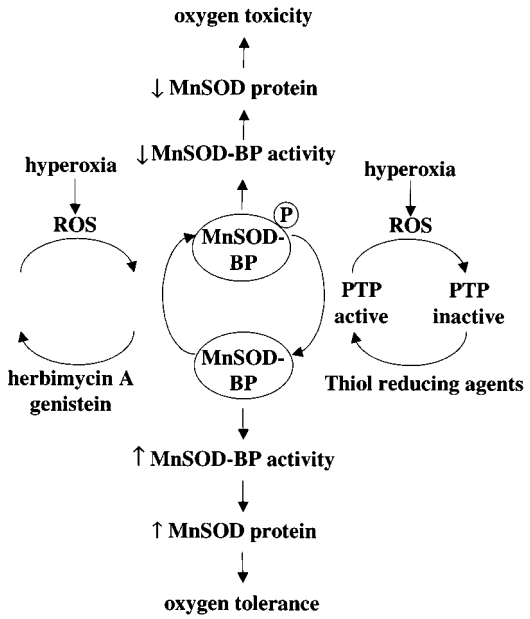

FIGURE 5: Proposed mechanism of the affect of MnSOD-BP phosphoregulation on MnSOD protein expression with the resultant physiological consequence of oxygen tolerance or pulmonary oxygen toxicity.

animals are protected against oxygen toxicity (bottom half of Figure 5) whereas other animals experience severe pulmonary oxidant-induced damage (top half of Figure 5). During oxidative stress, for example during exposure to hyperoxia, the concentrations of the reactive oxygen species, hydrogen peroxide and superoxide anion, increase. These reactive oxygen species inactivate PTP by oxidation to sulfenic derivatives (*17*, *18*). In this scenario, a greater portion of the pool of MnSOD-BP would remain in the tyrosine-phosphorylated form and thus be less active in RNA-binding activity; the result would be a lower level of MnSOD protein (top half of Figure 5). This could explain why lung MnSOD translational efficiency is significantly decreased in animal models that are not tolerant to oxidant stress and experience life-threatening pulmonary oxygen toxicity (*7*, *8*). In adult rats (a nontolerant model), Sheth et al. have demonstrated that rat lung protein tyrosine phosphatase activity was decreased significantly at 60 h of exposure to hyperoxia (*19*). Similarly, any agent that would increase PTK activity would also result in a decreased synthesis of MnSOD and there is evidence that reactive oxygen intermediates cause an increase in PTK activity (*20*). On the other hand, in animal models that tolerate hyperoxia, one would postulate that the protein tyrosine phosphatase activity is increased and the tyrosine kinase activity is decreased (bottom half of Figure 5). Following this scheme, to design a therapeutic approach for increasing MnSOD, one might target MnSOD-BP activity and posttranscriptional MnSOD protein expression by altering the phosphorylation state of MnSOD-BP, that is, by decreasing PTK and/or increasing PTP activity. The results reported in this paper present the challenge of discovering which kinase/phosphatase pathways control MnSOD-BP activity as a possible means of increasing MnSOD protein during periods of oxidative stress.

Kinase/phosphatase signal transduction cascades are critical in regulation of DNA-binding proteins that affect transcription (*21*) as well as in the regulation of the activity of initiation and elongation factors in the control of protein synthesis (*22*). It now appears that the activity of a 3′ UTR RNA-binding protein that has a modulatory function at the level of protein expression is also regulated by its tyrosine phosphorylation state. We propose that MnSOD-BP is a member of a distinct class of 3′ UTR RNA-binding proteins whose phosphorylation state regulates gene expression at a posttranscriptional level. Examples of other cytoplasmic RNA-binding proteins whose binding activity is regulated by phosphorylation include the following (1) adenosine-uridine binding factor (AUBF) that complexes in vitro to several RNAs containing AUUUA rich regions in the 3′ UTR known to be involved in regulating mRNA stability (*23*), (2) iron regulatory protein (IRP) that modulates iron metabolism by binding to transferrin receptor and ferritin mRNAs (*24*), (3) proteins that bind to regions in the 3′ UTR of lactate dehydrogenase A mRNA that are involved in regulating LDH-A mRNA stability (*25*), and (4) AUF1, a protein that binds A+U rich elements located in the 3′ UTR of several highly labile mRNAs coding for cytokines, oncoproteins and growth factors (*26*). In 1997, Sachs and Buratowski suggested that eukaryotic transcription and translation systems have similarities in their organization (*27*). In this paper, we provide support for the notion that a common theme emerges whereby gene expression at both the transcriptional level and posttranscriptional level is regulated by the binding of protein to nucleic acids and this

regulation is controlled by signal transduction pathways involving kinase/phosphatase events.

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