# Rat Lung Cu,Zn Superoxide Dismutase

## Isolation and Sequence of a Full-Length cDNA and Studies of Enzyme Induction

**Michael A. Hass, Jawaid Iqbal, Linda B. Clerch, Lee Frank, and Donald Massaro**

*Calvin and Flavia Oak Asthma Research and Treatment Facility, Pulmonary Research Laboratories, Department of Medicine, University of Miami School of Medicine, Miami, Florida 33136; and the Veterans Administration Medical Center, Miami, Florida 33125*

## Abstract

**The synthesis of Cu,Zn SOD by rat lung increases spontaneously in the fetus in late gestation and during exposure of neonatal and adult rats to > 95% $O_2$. To explore the regulation of these increases, we measured rat lung Cu,Zn SOD synthesis and activity. We also cloned and sequenced a rat lung Cu,Zn SOD cDNA that was used to measure Cu,Zn SOD mRNA concentration. We found that (*a*) under normal gestational and postgestational conditions the synthesis of this enzyme was regulated pretranslationally; (*b*) the increased synthesis that occurs under hyperoxia (> 95% $O_2$), was pretranslationally mediated in otherwise unmanipulated neonatal rats but translationally controlled in hyperoxic adult rats; and (*c*) in lungs of rats made tolerant to > 95% $O_2$ by allowing 24 h rest in air after an initial 48 h in > 95% $O_2$, the increased Cu,Zn SOD synthesis that occurred during the second period of hyperoxia was regulated pretranslationally. We conclude Cu,Zn SOD gene expression in the lung is developmentally regulated under normal conditions and in response to an oxidant challenge. Tolerance, whether endogenous or induced, appears to require the accumulation of increased amounts of Cu,Zn SOD mRNA.**

## Introduction

The sequential four-electron reduction of oxygen, which occurs during normal oxidative metabolism, results in the production of highly reactive, potentially tissue damaging, oxygen species including superoxide, the hydroxyl radical, and hydrogen peroxide ($H_2O_2$) (1, 2). The antioxidant enzymes SOD, catalase, and glutathione peroxidase reduce the cellular concentration of these toxic moieties and therefore are important components of the cell's antioxidant defenses (1–4). The cellular production of oxygen radicals and $H_2O_2$ is increased during exposure to hyperoxia (5, 6) and survival during prolonged exposure to hyperoxia seems to require an increase of antioxidant enzyme activity in the lung (7–10), in particular, of Cu,Zn SOD (10). Furthermore, at birth the lungs are abruptly exposed to an oxygen concentration that is much higher than in utero, and, in late gestation, presumably in preparation for birth, the rate of Cu,Zn SOD synthesis by fetal lung increases markedly (11).

The ability to survive prolonged exposure to hyperoxia (tolerance) can be an endogenous property as occurs in the neonates of some species (12), can be pharmacologically induced by treating adult rats with endotoxin (9, 10), and can be produced in adult rats by exposure to ~ 85% $O_2$ before exposure to > 95% $O_2$ (7). Recently, remarkable tolerance to > 95% $O_2$ was induced in adult rats by providing a short rest period in air, or in 50–75% $O_2$, between periods of exposure to > 95% $O_2$ (13). Tolerance in this new model is associated with an increase in the activity of SOD, catalase, and glutathione peroxidase in the lung during reexposure to > 95% $O_2$ (Frank, L., J. Iqbal, M. Hass, and D. Massaro, unpublished observations).

The mechanisms that might bring about the elevation of lung antioxidant enzyme activity in response to hyperoxia and in preparation for birth have been explored only for Cu,Zn SOD; in these cases the increase is due to a faster rate of Cu,Zn SOD synthesis (11, 14, 15). However, the level of gene expression at which the increased synthesis is regulated is not known. We now report the isolation and sequence of a full-length cDNA for rat lung Cu,Zn SOD and its use, with measurements of Cu,Zn SOD activity and synthesis, to explore the level at which the expression of gene(s) for Cu,Zn SOD are regulated in the lung during development and in response to hyperoxia.

## Methods

*Animals.* Adult Sprague-Dawley albino rats were purchased from Charles River Breeding Laboratories (Wilmington, MA) and subsequently maintained in the Animal Care Facility of the University of Miami. They were allowed rodent laboratory chow (model 5001; Ralston Purina, St. Louis, MO) and water ad lib. Lighting was provided from 7 a.m. to 7 p.m. daily. We bred the fetal and neonatal rats used in these experiments in our Animal Care Facility. This was accomplished by placing one male with two females overnight for 12 h. Probable pregnancy was determined the next morning by the presence of sperm in a smear made of vaginal contents. The time of conception was assumed to be 2 a.m., the midpoint of the cohabitation period. Preterm fetuses were delivered by hysterotomy after anesthetizing the dam with sodium pentobarbital (~ 60 mg i.p./kg body wt). The time of natural birth was determined by frequent observation of the gravid female during the day or was assumed to be 2 a.m. if birth occurred during the night. The size of each litter was adjusted to 10 pups within 12 h of birth, with the day of birth considered day 1. Rats were killed by cutting the great vessels of the abdomen after the animals were anesthetized by the intraperitoneal injection of sodium pentobarbital (~ 60 mg/kg).

*Exposure to hyperoxia.* Rats were exposed to > 95% $O_2$ at 1 atm in 3.5-$ft^3$ exposure chambers constructed from modified clear plastic nursery isolettes (model 86; Air Shields, Hatboro, PA). The conditions of $O_2$ exposure (> 95% $O_2$, < 0.5% $CO_2$, 22–25°C, and 40–60% humidity) were monitored four times each day. For air exposure, rats were maintained in room air in cages adjacent to those housing $O_2$-exposed rats. Exposures to hyperoxia were continuous except for a daily 10- to 15-min period when the chambers were opened for animal

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Address reprint requests to Dr. Donald Massaro, Pulmonary Research R120, University of Miami School of Medicine, P. O. Box 016960, Miami, FL 33101.

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maintenance purposes and to allow dams from $O_2$ litters and air litters to be switched to prevent the development of $O_2$ toxicity in the nurturing dams. In some experiments on adult rats, continuous exposures to > 95% $O_2$ were interrupted after 48 h for a 24-h rest period in air before reexposure to > 95% $O_2$.

*Cloning of Cu,Zn SOD cDNA.* We used an adult rat lung cDNA library previously constructed in this laboratory (16). Recombinant clones ($1.5 \times 10^5$) in λgt11 (17) were screened after inducing the synthesis of cDNA-β-galactosidase fusion proteins with isopropyl β-D-thiogalactopyranoside. The fusion proteins were blotted onto nitrocellulose filters (Millipore Corp., Milford, MA), and incubated with goat anti-rat Cu,Zn SOD (17) plus rabbit anti-goat IgG-horseradish peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA). Four rounds of screening yielded 11 plaque-purified Cu,Zn SOD cDNA clones (frequency = 0.01%).

*Sequencing of Cu,Zn SOD cDNA.* The Cu,Zn SOD cDNA was excised from λgt11 and subcloned into PGEM-Blue (Promega Biotec, Madison, WI), using Eco RI. Two restriction fragments of Cu,Zn SOD cDNA were generated with Pst I, and were also subcloned into PGEM Blue. The PGEM Blue cDNA constructs were sequenced by the dideoxynucleotide method (18) using a kit (Promega Biotec) with T7 and SP6 promoter primers to sequence from both ends of each insert. The site of each base in the sequence was determined at least three times with an average of eight determinations per base. Approximately 85% of the Cu,Zn SOD cDNA coding sequence was determined on both strands.

*Tissue analysis.* Poly(A)$^+$ mRNA was isolated from lungs of adult and 1-d-old rats by homogenization in guanidine isothiocyanate-containing buffer, centrifugation through cesium chloride, and elution from oligo dT cellulose (19, 20). Poly(A)$^+$ mRNA was separated by electrophoresis through formaldehyde-agarose (1.5%) gels in 10 mM 3-[*N*-morpholine]propanesulfonic acid, 1 mM EDTA, 5 mM sodium acetate, and transferred to nitrocellulose. Filters containing lung poly(A)$^+$ mRNA were hybridized with the nick-translated, $^{32}$P-labeled, 5′ Pst I fragment (236 bp) of Cu,Zn SOD cDNA (21) for 16 h at 42°C in 0.6 M sodium chloride, 75 mM sodium citrate, 65 mM sodium phosphate, 5 mM EDTA, 0.02% Ficoll, 0.02% polyvinylpyrolidine, 0.02% BSA, 50 μg/ml transfer (t) RNA, and 50% formamide. The filters were washed in 0.5× SSC and subjected to radioautography at −70°C.

*cRNA synthesis.* The PGEM-Blue construct containing the 5′-Pst I fragment of Cu,Zn SOD cDNA was used to synthesize $^{32}$P-labeled or $^{35}$S-labeled cRNA complementary to the 5′ end of the coding strand and unlabeled cRNA equivalent to the coding strand, according to the Promega Biotec instructions. The radiolabeled cRNA was used as a probe to quantitate lung Cu,Zn SOD mRNA by solution hybridization (22); the unlabeled cRNA was used as a standard with which we defined the range in which hybridization of the radiolabeled probe increased proportionally with added mRNA. A PGEM-Blue construct containing the 3′-end of an α actin cDNA was a gift from Rudolf K. Werner (Department of Biochemistry and Molecular Biology, University of Miami School of Medicine) and was used to synthesize the [$^3$H]cRNA equivalent of the coding strand for use as a mRNA recovery marker.

*Cu,Zn SOD mRNA quantitation.* To isolate total nucleic acids (22), 200–500 mg of rat lung tissue was homogenized in buffer containing 0.2 mg/ml Proteinase K and 3,000 dpm [$^3$H]α actin cRNA. The homogenate was treated with phenol, and nucleic acids were precipitated with ethanol; the nucleic acid pellet was dissolved at 68°C in 1–2 μl $H_2O$/mg of starting lung tissue and the radioactivity in a portion was measured to determine mRNA recovery. Samples of the nucleic acid solution were exposed in triplicate to the radiolabeled-cRNA probe (at 68°C for 16 h) to quantitate Cu,Zn SOD mRNA by solution hybridization (22); 8 U/ml $S_1$ nuclease and 40 μg/ml RNAse A were subsequently added to digest unhybridized probe. The assay had a linear range from 1 to 9 pg standard cRNA and was able to detect differences of 5% (not shown).

*Cu,Zn SOD synthesis and activity.* We measured rates of Cu,Zn SOD synthesis as previously described in detail (14). In brief, ~ 1.0-mm-thick lung slices were incubated in Krebs-Ringer bicarbonate medium, with 5.5 mM glucose, adult rat plasma concentrations of 19 amino acids, and 0.7 mM [$^3$H]phenylalanine. At this concentration of medium phenylalanine, the specific radioactivity of tRNA-bound phenylalanine equals that of medium phenylalanine within 15 min of the start of the incubation, and remains equal for at least 1 h, enabling us to use the medium-specific radioactivity to calculate absolute rates of enzyme synthesis (23). The flasks were shaken at 120 oscillations per min at 37°C for 2 h with a gas phase of 95% $O_2$/5% $CO_2$. After incubation the lung tissue was homogenized, a sample taken for assay of DNA (14), and the remainder used to measure phenylalanine incorporation into Cu,Zn SOD (14). Cu,Zn SOD activity was measured as previously described (14).

*Statistical analysis.* For each parameter measured or calculated from measurements, the values for individual animals were averaged per experimental group and the SE of the group mean was calculated. The significance of the difference between two groups was obtained using an unpaired *t* test analysis (24). Multiple group comparisons were made by analysis of variance and Duncan's multiple range test (25); Kramer's extension (26) of Duncan's test was used to identify intergroup significant differences.

## Results and Discussion

*Cu,Zn SOD cDNA.* The rat lung Cu,Zn SOD cDNA clones we isolated each contained a single Eco RI-excisable insert of 600–700 bp. The Cu,Zn SOD cDNA sequenced contained 55 bp 5′ to the full-length 459-bp coding region and 104 bp at the 3′ end of the insert, for a total of 618 bp (Fig. 1). Fig. 2 shows the position of restriction site sequences, including the Pst I site at position 236, that we used in our sequencing strategy.

The nucleotide sequence of the rat lung Cu,Zn SOD coding region (annotated bases 1–459 in Fig. 1) is 88% homologous to

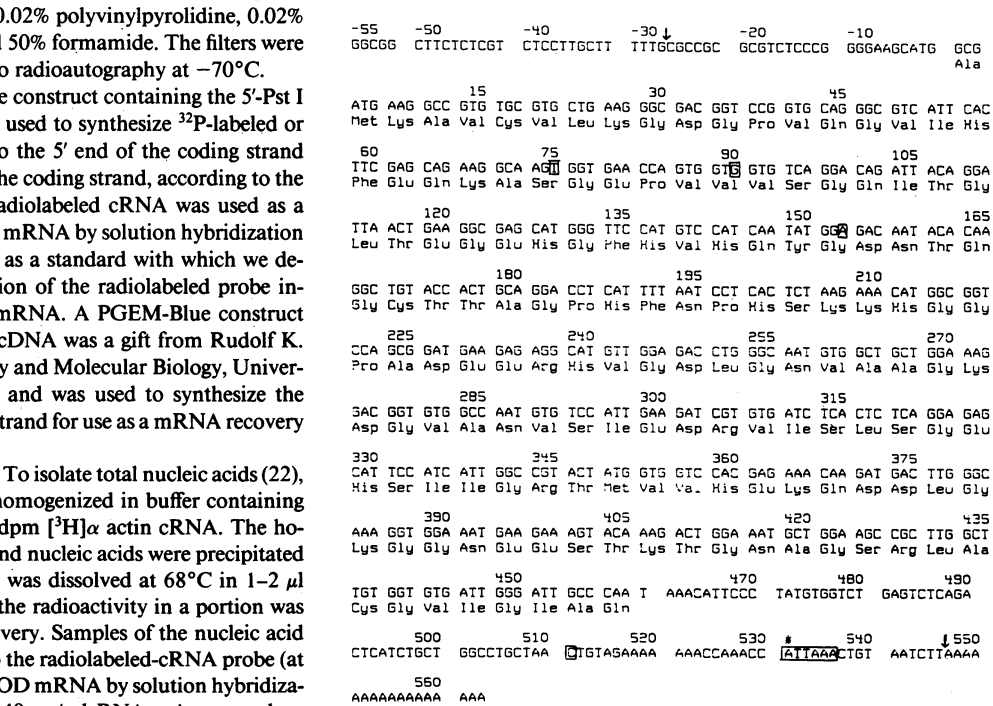


*Figure 1.* Sequence of Cu,Zn SOD cDNA. Bases different from those reported for rat liver Cu,Zn SOD cDNA (24) are boxed. *Arrows* indicate the position of insertions in the liver cDNA: 19 bases between lung bases -27 and -26, 5 bases between lung bases 546–547. The putative poly A addition signal is boxed with an asterisk.

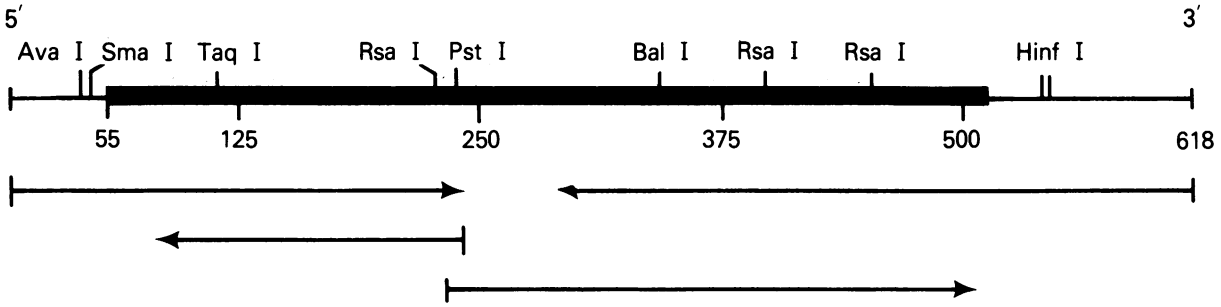


*Figure 2.* Restriction map and sequencing strategy. The coding region is represented by the heavy black line, with the positions of restriction site sequences indicated above. Lower in the figure is the strategy used for determining the cDNA sequence, with sequencing performed from both ends of each insert.

that of human fibroblast Cu,Zn SOD (27). The rat lung sequence differs from the sequence of a full-length rat liver Cu,Zn SOD cDNA by only three bases, these probably due to different interpretation of the sequence gel within a region of G band compression (28). The amino acid sequence derived from each of these Cu,Zn SOD cDNA nucleotide sequences is the same as that determined for rat liver Cu,Zn SOD using classical amino acid sequencing methods (29). The lung's 3′ noncoding sequence (bases 460–563) is 95% homologous with that of the rat liver (28), and 76% homologous with that of human fibroblasts (27). None of these 3′ sequences contain the typical polyadenylation signal (AATAAA); rather, all three have ATTAAA 10–15 bp before the polyadenylation site (bases 531–536 in this sequence) (27, 28).

One major difference is evident between the noncoding regions of the rat lung and liver cDNAs. The lung 5′ noncoding cDNA sequence contains 55 bp that are exactly homologous with the 5′ noncoding sequence of the rat liver cDNA, but the latter (28) has an additional 19 bases inserted between bases -26 and -27 of the lung cDNA. 8 of the 19 bases could form G-C pairs, but 40% homology within this stretch would appear too low to have caused misreading of the Cu,Zn SOD mRNA by reverse transcriptase during cDNA synthesis. Alternatively, the liver and lung mRNA might differ due to differential splicing of the nascent mRNA; the 19 bases are a direct repeat of the proposed consensus sequence for acceptor splice junctions (30, 31).

| | 5′ | 3′ |
|---|---|---|
| | CCCCCC C | G |
| Consensus | X AGG | |
| | TTTTTT T | T |
| Bases 1–10 | CTCTCCCAGG | |
| Bases 11–19 | TTCCCAGGG | |

Another possibility is that different Cu,Zn SOD genes are expressed in rat lung and liver.

Northern blot analysis of mRNA from lungs of 1-d-old and adult rats showed a single band of ~ 0.7 kb that hybridized with Cu,Zn SOD cDNA (Fig. 3), as was reported in several adult rat tissues, including lung (32). The presence of only one mRNA species is consistent with, though not proof of, the existence of a single Cu,Zn SOD gene in the rat.

*Lung Cu,Zn SOD gene expression from late gestation to adulthood.* The activity of Cu,Zn SOD per milligram DNA in rat lung increases from late gestation to adulthood; this is brought about by a rate of synthesis that slightly exceeds degradation of the enzyme (11). The rate of synthesis peaks one day before birth (gestation day 22) and decreases thereafter to adult values (11). We have now found the concentration of Cu,Zn SOD mRNA in rat lung to also be highest in late gestation (days 18–22), with a subsequent postnatal decrease to adult values (Table I). The similarity between the time-course of changes in the concentration of Cu,Zn SOD mRNA (Table I)

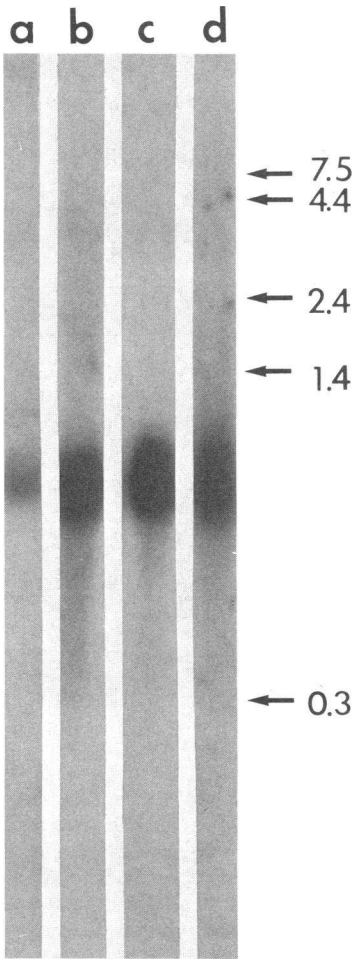


*Figure 3.* Northern blot analysis of lung poly(A)$^+$ mRNA. Poly(A)$^+$ was isolated from lungs of adult and neonatal (1-d-old) rats. The RNA was electrophoresed through a formaldehyde-agarose (1.5%) gel, transferred to nitrocellulose and hybridized with nick-translated $^{32}$P-labeled Cu,Zn SOD cDNA at 42°C. Filters were washed with 0.5× SSC and autoradiographed at −70°C. The size of RNA standard (Bethesda Research Laboratories, Gaithersburg, MD) is indicated in kilobases. (*a*) 5 μg adult RNA, (*b*) 15 μg adult RNA, (*c*) 25 μg adult RNA, (*d*) 10 μg neonatal RNA.

*Table I. Developmental Changes in Concentration of Lung Cu,Zn SOD mRNA*

| Age | Cu,Zn SOD mRNA |
|---|---|
| | *molecules · mg DNA$^{-1}$ × 10$^6$* |
| 18 d gestation | 617±42 (6) |
| 21 d gestation | 780±111 (5) |
| 22 d gestation | 611±39 (4) |
| 10 d old | 323±67 (3)* |
| Adult (*~60 d*) | 203±6 (4)* |

Rat lungs were obtained at the indicated ages and assayed for Cu,Zn SOD mRNA by solution hybridization using $^{32}$P-labeled cRNA. Mean±SE are given. Numbers in parentheses indicate the number of rats.
* $P < 0.05$ vs. 21 d of gestation.

*Table III. Cu,Zn SOD mRNA Concentration in Lungs of Neonatal Rats Exposed to Air or > 95% $O_2$*

| Age | Gas | Cu,Zn SOD mRNA |
|---|---|---|
| | | *molecules · mg DNA$^{-1}$ × 10$^6$* |
| 8 d | Air | 451±24 (5) |
| | >95% $O_2$ | 674±79 (5) |
| *P* | | <0.05 |
| 11d | Air | 186±19 (3) |
| | >95% $O_2$ | 254±10 (3) |
| *P* | | <0.05 |

Otherwise unmanipulated neonatal rats were allowed to breath air or > 95% $O_2$ for 72 h. Their lungs were then removed and assayed for Cu,Zn SOD mRNA by solution hybridization using $^{35}$S-labeled cRNA. Means±SE are given. Numbers in parentheses indicate the number of rats.

and the rate of Cu,Zn SOD synthesis (11) indicates that under normal steady-state conditions, the rate of Cu,Zn SOD synthesis by the lung is regulated at a pretranslational level.

*Cu,Zn SOD gene expression in lungs from otherwise unmanipulated neonatal and adult rats exposed to hyperoxia.* Lungs from adult rats exposed to > 95% $O_2$ for 24 h exhibit a rate of Cu,Zn SOD synthesis that is 25–30% faster than it is in lungs from air-breathing rats; these differences disappear by 48 h of exposure because of a decrease in the synthesis rate of the enzyme in hyperoxic rats (15). We have now found the increased rate of Cu,Zn SOD synthesis was present by 12 h of in vivo hyperoxia and occurred without an elevation in the concentration of Cu,Zn SOD mRNA or in the activity of the enzyme (Table II). The rate of Cu,Zn SOD synthesis in lungs from $O_2$-exposed neonatal rats is faster than the rate in lungs from air-breathing pups (15), and, unlike in adult rats, this difference occurred with an increase in the concentration of Cu,Zn SOD mRNA (Table III). Hence, some time between the neonatal period and adulthood, otherwise untreated rats switch from pretranslational to translational regulation of Cu,Zn SOD synthesis in response to continuous exposure to > 95% $O_2$. It is possible this conversion occurs concomitantly with the loss of tolerance to hyperoxia that also takes place between those ages.

*Table II. Cu,Zn SOD Synthesis, mRNA Concentration, and Activity in Lungs of Air- or $O_2$-exposed but Otherwise Unmanipulated Adult Rats*

| Exposure in vivo | Cu,Zn SOD | | |
|---|---|---|---|
| | Synthesis | mRNA | Activity |
| | *pmol · mg DNA$^{-1}$ · h$^{-1}$* | *molecules · mg DNA$^{-1}$ × 10$^6$* | *U · mg DNA$^{-1}$* |
| 3 h | | | |
| Air | 8.1±0.8 (3) | NM | 39.8±1.5 (3) |
| $O_2$ | 9.1±0.5 (3) | NM | 42.8±0.4 (3) |
| *P* | NS | | NS |
| 6 h | | | |
| Air | 7.9±0.9 (3) | NM | 44.2±0.4 (3) |
| $O_2$ | 9.8±1.0 (3) | NM | 47.5±2.1 (3) |
| *P* | NS | | NS |
| 12 h | | | |
| Air | 9.0±0.4 (3) | 274±31 (3) | 38.0±1.2 (8) |
| $O_2$ | 11.5±0.4 (3) | 226±16 (6) | 39.2±1.4 (8) |
| *P* | <0.05 | NS | NS |

Otherwise unmanipulated adult rats were allowed to breath air or > 95% $O_2$ ($O_2$) for the indicated time. Lungs were then removed and rates of synthesis of Cu,Zn SOD by lung slices were measured, and the activity of Cu,Zn SOD and concentration of Cu,Zn SOD mRNA determined as described in Methods. Means±SE are given. Numbers in parentheses indicate the number of rats. NM, not measured. NS, $P > 0.05$.

The elevated rates of Cu,Zn SOD synthesis that develop in lungs of adult (Table II) and neonatal rats (15) during exposure to > 95% $O_2$ result in an increase of the enzyme's activity only in the neonatal animals (15). This disparity is partly due to a greater increase in the rate of Cu,Zn SOD synthesis in neonatal compared with adult rats (1.4- and 1.2-fold, respectively) (15). However, a more rapid turnover of Cu,Zn SOD in neonatal than adult lungs (11) also contributes to the elevation of enzyme activity in the neonate because, even for an equal fold increase in synthesis, more rapidly turning over proteins attain a greater relative rise in concentration than more slowly turning over proteins (33). The greater tolerance to hyperoxia by neonatal than adult rats thus is due in part to the more rapid turnover of lung Cu,Zn SOD in neonatal rats.

It is especially interesting that Cu,Zn SOD is degraded much more rapidly in neonatal than adult rat lungs ($t_{1/2}$ = 9 and > 100 h, respectively), in the absence of a difference in the degradation rate of general lung proteins (15). This raises the possibility, among other explanations, that the enzyme in lungs of neonatal rats may be structurally dissimilar from Cu,Zn SOD in adult rats in a manner that results in their not being equally susceptible to proteolysis. This notion is supported by experiments with rat lung homogenates that show the Cu,Zn SOD activity of the neonatal lung is considerably more susceptible to inactivation by heat (80°C) or Cu chelation than is the enzyme activity of the adult (11). These putative differences in structure could be brought about by the expression of different genes for Cu,Zn SOD in neonatal and adult rats, posttranslational modifications of the structure of the enzyme, or by both means.

*Cu,Zn SOD activity, synthesis rate, and mRNA concentration in adult rat lungs: effect of rest period mode of exposure to > 95% $O_2$.* Lungs from rats exposed in sequence to 48 h of

*Table IV. Cu,Zn SOD Synthesis, mRNA Concentration, and Activity in Lung: Rest Period Method of Exposure of Adult Rats*

| Exposure, in vivo | Cu,Zn SOD | | |
|---|---|---|---|
| | Synthesis | mRNA | Activity |
| | *pmol · mg DNA$^{-1}$ · h$^{-1}$* | *molecules · mg DNA$^{-1}$ × 10$^6$* | *U · mg DNA$^{-1}$* |
| 48 h air | | | |
| 48 h $O_2$ | NM | 115±18 (8) | NM |
| | NM | 128±21 (8) | NM |
| *P* | | NS | |
| 72 h air | NM | 254±41 (3) | NM |
| 48 h $O_2$–24 h air | NM | 250±26 (3) | NM |
| *P* | | NS | |
| 144 h air | 8.3±1.0 (3) | 235±25 (4) | 33.8±0.4 (3) |
| 48 h $O_2$–24 h Air–72 h $O_2$ | 13.6±1.0 (3) | 354±15 (4) | 42.0±0.7 (5) |
| *P* | <0.01 | <0.01 | <0.01 |

Adult rats were exposed to air, > 95% $O_2$ ($O_2$) or both as shown. Lungs were then removed and the indicated measurements were made as described in Methods and Materials; mRNA was assayed by solution hybridization using $^{32}$P-labeled cRNA. Mean±SE are given. Numbers in parentheses indicate the number of rats. NM, not measured. NS, $P > 0.05$.

> 95% $O_2$, 24 h of air, and 72 h of > 95% $O_2$ exhibited a 1.6-fold faster rate of Cu,Zn SOD synthesis, increased concentration of Cu,Zn SOD mRNA, and greater Cu,Zn SOD activity than lungs from rats exposed only to air (Table IV). Thus, unlike the increased rate of Cu,Zn SOD synthesis that develops in untreated $O_2$-exposed rats, which is translationally mediated, the increased rate of Cu,Zn SOD synthesis that develops during the rest period mode of exposure to $O_2$ is mediated pretranslationally. $O_2$ exposure and a rest period without subsequent reexposure to $O_2$ is insufficient to increase Cu,Zn SOD mRNA (Table IV) or the antioxidant enzymes. It will be of particular importance to determine the mechanism by which the rest period mode of exposure induces in adult rats the return to the pretranslational regulation of Cu,Zn SOD expression in response to hyperoxia. It will also be necessary to determine if the pretranslational mechanisms responsible for increased Cu,Zn SOD synthesis in hyperoxic neonatal and rest period–treated adult rats are the same.

*Cu,Zn SOD gene expression and regulation in lungs of rats treated with endotoxin.* Only ~ 25% of untreated adult rats survive 72 h of continuous exposure to > 95% $O_2$, whereas virtually all similarly exposed rats treated with a low dose of endotoxin survive (9, 10). This increased tolerance is associated with an elevation of lung Cu,Zn SOD activity that occurs during exposure to > 95% $O_2$ and that is due mainly to an increased rate of Cu,Zn SOD synthesis; rats treated with endotoxin but not exposed to hyperoxia do not exhibit increased lung Cu,Zn SOD synthesis or activity (14). More recent studies have shown the increase in Cu,Zn SOD synthesis by lungs from endotoxin-treated rats is preceded by a slight decrease in its synthesis in air- and $O_2$-breathing rats that occurs at a time when the concentration of Cu,Zn SOD mRNA is elevated ~ 45% in lungs from both groups (34). Continued exposure to > 95% $O_2$ or air results in an increased rate of Cu,Zn SOD synthesis only by lungs from $O_2$-exposed rats. Furthermore, in vitro exposure of lung slices from air-breathing, saline- or air-breathing, endotoxin-treated rats to 95% $O_2$ results in an increased synthesis of Cu,Zn SOD only in slices from endotoxin-treated rats (Frank, L., J. Iqbal, M. Hass, and D. Massaro, unpublished observations). Hence, an oxidant challenge is required (or is one means) to effect an increased rate of Cu,Zn SOD synthesis by lungs with the endotoxin-induced increase of Cu,Zn SOD mRNA concentration.

*Generalizations about Cu,Zn SOD gene expression and regulation.* Consideration of the data presented and reviewed leads us to conclude that under steady-state normoxic conditions, from late gestation to adulthood, Cu,Zn SOD gene expression is regulated mainly at the pretranslational level. In otherwise unmanipulated neonatal and adult rats exposed to hyperoxia the response to this oxidant challenge is developmentally regulated. The response in neonates is a pretranslationally mediated increase in mRNA that leads to tolerance to hyperoxia. By contrast, the increased rate of Cu,Zn SOD synthesis in adult rats is translationally mediated, transient, and does not result in increased enzyme activity or tolerance to 72 h of hyperoxia. A similar pattern of altered gene regulatory mechanisms (but unrelated to hyperoxia) has been identified in cultured mouse fibroblasts at different growth rates, and in rat liver during fetal and postnatal development (35, 36). However, those studies did not offer an explicit mechanism by which the regulation of gene expression may have been switched.

We have studied two methods of inducing tolerance to hyperoxia, endotoxin treatment and the rest period method, and both cause an increase in Cu,Zn SOD mRNA; both methods also result in increases in Cu,Zn SOD synthesis and activity. Based on our present knowledge, it appears that tolerance to hyperoxia requires the presence, or the induction, of a pretranslational increase of Cu,Zn SOD mRNA (and probably the mRNAs of the other antioxidant enzymes). Our findings in otherwise unmanipulated adult rats indicate the response to a transient increase in oxidant stress is mediated translationally.

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