

## SUPEROXIDE DISMUTASE MULTIGENE FAMILY: A COMPARISON OF THE CuZn-SOD (SOD1), Mn-SOD (SOD2), AND EC-SOD (SOD3) GENE STRUCTURES, EVOLUTION, AND EXPRESSION

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**Abstract**—Superoxide dismutases are an ubiquitous family of enzymes that function to efficiently catalyze the dismutation of superoxide anions. Three unique and highly compartmentalized mammalian superoxide dismutases have been biochemically and molecularly characterized to date. SOD1, or CuZn-SOD (EC 1.15.1.1), was the first enzyme to be characterized and is a copper and zinc-containing homodimer that is found almost exclusively in intracellular cytoplasmic spaces. SOD2, or Mn-SOD (EC 1.15.1.1), exists as a tetramer and is initially synthesized containing a leader peptide, which targets this manganese-containing enzyme exclusively to the mitochondrial spaces. SOD3, or EC-SOD (EC 1.15.1.1), is the most recently characterized SOD, exists as a copper and zinc-containing tetramer, and is synthesized containing a signal peptide that directs this enzyme exclusively to extracellular spaces. What role(s) these SODs play in both normal and disease states is only slowly beginning to be understood. A molecular understanding of each of these genes has proven useful toward the deciphering of their biological roles. For example, a variety of single amino acid mutations in SOD1 have been linked to familial amyotrophic lateral sclerosis. Knocking out the SOD2 gene in mice results in a lethal cardiomyopathy. A single amino acid mutation in human SOD3 is associated with 10 to 30-fold increases in serum SOD3 levels. As more information is obtained, further insights will be gained. © 2002 Elsevier Science Inc.

**Keywords**—Superoxide dismutase, Superoxide, Antioxidant, Gene family, Transcription, Polymorphism, Free radicals

### INTRODUCTION

The evolution of aerobic organisms that can survive in oxygen-rich environments requires an effective defense system against reactive oxygen species (ROS), which are produced following single electron reductions of molecular oxygen. While physiological concentrations of ROS in aerobic organisms are beneficial and involve cell signaling pathways and survival from invading pathogens, an unbalanced, elevated concentration of ROS may contribute to the development of various diseases, such as cancer, hypertension, diabetes, atherosclerosis, inflammation, and premature aging. The superoxide dismutases (SODs) are the first and most important line of antioxi-

dant enzyme defense systems against ROS and particularly superoxide anion radicals. At present, three distinct isoforms of SOD have been identified in mammals, and their genomic structure, cDNA, and proteins have been described. Two isoforms of SOD have Cu and Zn in their catalytic center and are localized to either intracellular cytoplasmic compartments (CuZn-SOD or SOD1) or to extracellular elements (EC-SOD or SOD3). SOD1 has a molecular mass of about 32,000 Da and has been found in the cytoplasm, nuclear compartments, and lysosomes of mammalian cells [1–4]. SOD3 is the most recently discovered and least characterized member of the SOD family. The enzyme exists as a homotetramer of molecular weight 135,000 Da with high affinity for heparin [5]. SOD3 was first detected in human plasma, lymph, ascites, and cerebrospinal fluids [6,7]. The expression pattern of SOD3 is highly restricted to the specific cell type and tissues where its activity can exceed that of SOD1 and SOD2. A third isoform of SODs has manganese

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## SUPEROXIDE DISMUTASE GENE FAMILY

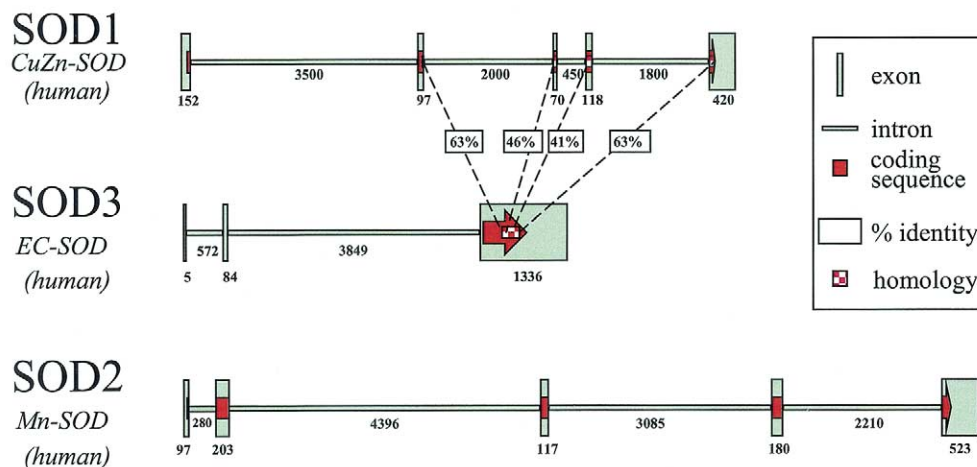


Fig. 1. Genomic organization of the three known members of the human SOD enzyme family. SOD3 was placed in the middle in order to demonstrate areas of amino acid sequence homology between SOD1 and SOD3. SOD2 has no significant amino acid sequence homology with either SOD1 or SOD3. The size of each exon and intron, in base pairs, is shown in association with that fragment. Data for this figure was extracted from the following sources: SOD1 [21], SOD2 [26], and SOD3 [32].

(Mn) as a cofactor and has been localized to mitochondria of aerobic cells (Mn-SOD or SOD2) [8]. It exists as a homotetramer with an individual subunit molecular weight of about 23,000 Da [9]. SOD2 has been shown to play a major role in promoting cellular differentiation and tumorigenesis [10] and in protecting against hyperoxia-induced pulmonary toxicity [11]. The numerous studies on the physiological function of SOD1 and SOD2 and their role in protection against ROS are summarized in several excellent reviews [12–17]. However, the available information related to SOD3 has not been reviewed in a comparative perspective along with the other two isoforms. This review focuses on comparative characteristics of all three SOD genes, their evolution and ontogeny, and their transcriptional regulation by various intra- and extracellular stimuli.

### GENE STRUCTURE

#### *SOD1*

The genomic sequence for SOD1 has been identified in the rat [18,19], mouse [20], and human [21]. The genomic organization of SOD1 gene shows striking similarity among species and has five exons and four introns (Fig. 1). The TATA and CCAAT boxes, as well as several highly conserved GC-rich regions, have been localized in all three species with a similar pattern in the proximal promoter region. Such a high level of homology in the 5' flanking sequence suggests that intense

evolutionary factors have preserved key regulatory regions for this gene. The 3' end of SOD1 gene possesses several poly(A) signal sequences that terminate the mRNA species with different lengths. The consensus sequences YGTGTTY and a G/T cluster required for efficient formation of 3'-termini have also been located downstream from the polyadenylation signal in the rat SOD1 gene. The promoter region of the human SOD1 gene has been studied and several putative binding sites for NF1, Sp1, AP1, AP2, GRE, HSF, and NF- $\kappa$ B transcription factors have been found [22]. The role of Sp1 and Egr-1 transcription factors in basal and inducible expression of human SOD1 has been confirmed [23].

#### *SOD2*

The complete genomic structure for SOD2 has been determined for the human [24,25], rat [26], and mouse [27,28]. Partial identification and characterization of a bovine SOD2 gene has been described [29]. All of these species show marked conservation of structure and sequence. The physical structure of the SOD2 gene is composed of 5 exons and 4 introns (see Fig. 1). Genomic southern blotting supports the existence of one SOD2 gene for human [25] murine [28], and bovine species [29], whereas two genes per haploid genome has been described in the rat [26].

The promoter regions in all four species share common features. There are no upstream TATA or CAAT

box elements identified. However, GC-rich regions are present in all four species. Such features can be typical of "housekeeping" genes [30,31]. The human and mouse genes each contain putative NF- $\kappa$ B transcription regulatory element. For humans, it is located in the 3'-flanking region of the gene [25] while the mouse contains two potential elements in the 5'-flanking region [28]. Also present in the promoter region of all four species are multiple copies of Sp-1 and AP-2 consensus sequences.

### *SOD3*

The genomic structure for human SOD3 has been determined [32]. A partial genomic clone encoding the complete open reading frame for mouse SOD3 has been reported [33]. Currently, SOD3 cDNA clones for the human [34], rat [35,36], mouse [37], and rabbit [38] have been isolated and sequenced. The SOD3 gene shares 40–60% similarity with the SOD1 gene at the exon level, but shows no similarity with SOD2 (Fig. 1). The mouse SOD3 gene consists of two exons separated by a 4 kb intron while in human three exons have been found. The promoter region of human and mouse SOD3 apparently lacks classical TATA or CCAAT boxes [32]. In humans, several putative transcriptional response elements have been identified and include a metal regulatory element, an AP-1 site as well as two potential antioxidant response elements [32]. In contrast, the mouse proximal promoter, characterized by unusually GA-rich sequence, has multiple putative binding sites for Krüppel-like and Ets-family transcription factors. The functional importance of these sites is not clear at this time.

## CHROMOSOMAL LOCALIZATION AND POLYMORPHISMS

### *SOD1*

The SOD1 gene has been localized to chromosome 21 (region 21q22) in humans [21], chromosome 1 (1q12 → 14) in bovine species [39], and chromosome 16 (region 16B4 → ter) in the mouse [40]. Human chromosome 21 has been studied intensely because of the association between Down's syndrome and trisomy 21. Although patients with Down's syndrome show a 50% increase in SOD1 activity due to higher levels of SOD1 protein, the role of this enzyme in pathology associated with this disease remains questionable. The increased dosage of SOD1 gene associates with some symptoms of Down's syndrome, such as the pathological abnormalities of tongue neuromuscular junctions [41,42] but has no obvious implication in the development of the major symptoms [43]. On the other hand, more than 90 different

mutations in the SOD1 gene have been associated with amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. This fatal disorder causes degradation of motor neurons in the corticospinal tracts and brain stem. Although only 2% of patients with ALS and 10–15% with familial ALS have mutations in the SOD1 gene, the discovery of these mutations by Rosen et al. in 1993 provided the first molecular insight into the pathogenesis of this disease [44]. Since this discovery, several theories have been proposed to explain the mechanism of motor neuron damage caused by mutations in SOD1. One hypothesis is that mutations in the SOD1 gene may impair antioxidant enzyme activity that in turn could lead to accumulation of toxic superoxide anions. This theory was dismissed experimentally when SOD1 bearing the G93A mutation was overexpressed in mice, resulting in motor neuron disease despite the elevated SOD1 activity [45]. Moreover, complete inactivation of SOD1 in "knock-out" mice does not cause any motor neuron abnormalities [46], although they exhibit increasing embryonic lethality and reduced fertility in females [47].

The opposite gain-of-function theory has been proposed based on the assumption that mutations in the SOD1 gene change the affinity of enzyme to the natural and abnormal substrates [48], impair ability of enzyme to bind zinc [49] or increase the enzyme aggregation in neurons [50,51]. Either way, the dominant mutations in SOD1 play a key role in the pathogenesis of familial ALS.

### *SOD2*

Using enzymatic analysis of mouse/human hybrids, the SOD2 gene was initially localized to chromosome 6 [52]. Later, the SOD2 gene was sublocalized to region 6q25 by fluorescence in situ hybridization and somatic cell hybrid mapping [24]. The importance of SOD2 function in mammalian organism was confirmed by disruption of the SOD2 gene, which turns out to be lethal for mice due to neurodegeneration and damage to the heart [53]. Several genetic variations have been described for the human SOD2 gene. The substitution of Ala-9 to Val in the mitochondria targeting sequence causes premature aging or progeria [54] and is associated with an increased risk of sporadic motor neuron disease, especially in females [55] and with nonfamilial idiopathic cardiomyopathy [56] but has no effect on the occurrence of Parkinson disease [57] or ALS [58,59]. Recently, the Ala-9 Val polymorphism has also been associated with a 1.5-fold increase in the risk of breast cancer in a Finnish population [60]. While it is assumed that this mutation may impair subcellular localization of SOD2, there is no experimental evidence that supports this hypothesis. Another substitution, Ile 58 to Thr, elic-

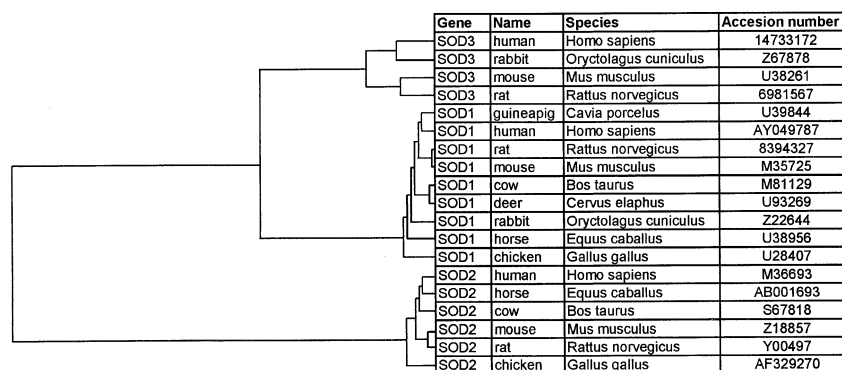


Fig. 2. The evolutionary tree of SOD gene family. The tree shows the calculated evolutionary relationships of known vertebrate SOD genes based on an unweighted-pair-group method analysis. The length of horizontal lines connecting one sequence to another is proportional to the estimated genetic distance between the sequences.

its a 3-fold decrease in enzymatic activity of SOD2 and reduces the tumor-suppressive effect of the enzyme [61, 62]. At least three heterozygous mutations in the proximal promoter of human SOD2 have been identified and linked to the reduced transcriptional activity in transient transfection experiments [63].

### SOD3

The SOD3 gene has been localized to chromosome 4 (region 4p-q21) of the human [64] and in the middle of chromosome 5, tightly linked to the QDPR locus in mouse [37,65]. To date, only one mutation located in the center of the carboxyl-terminal cluster of positively charged amino acid residues, which defines the heparin-binding domain, has been described for human SOD3. Substitution of arginine in position 213 to glycine causes an 8–15-fold increase in concentration of plasma SOD3 levels [66–68]. The effect of this SOD3 polymorphism, which has been found in 4% of Swedish [69], 3% of Australian [70] and 6% of Japanese [67] subjects studied, is not entirely clear but early studies suggest that this amino acid mutation impairs affinity for heparin and endothelial cell surface and may reduce susceptibility to trypsin-like proteases. Two additional polymorphisms have been identified in the human SOD3 gene; a transition mutation of A to G at position 241 resulting in a Thr to Ala (T40A) substitution and a silent transition mutation of C to T at position 280 [71]. While the substitution of nucleotide A to G at position 241 creates a new BssHII restriction site, the T40A amino acid change does not seem to affect heparin binding capacity or the specific activity of EC-SOD [71]. SOD3 null mutant mice show enhanced sensitivity to hyperoxia [33], worsened outcome from focal cerebral ischemia [72], and have dramatic impairments in spatial learning [73].

### EVOLUTION

The appearance of SOD enzymes was triggered by the proliferation of photosynthetic organisms that began to produce oxygen about 2 billion years ago. A variety of antioxidant enzymes evolved to neutralize the toxic effects of subproducts of oxygen utilization. Two major kinds of superoxide dismutase appeared in prokaryotes at that time, copper/zinc-containing SODs and iron/manganese-containing SODs. Is it possible that all forms of SOD originated from a single protein whose function was to protect primitive organisms from a relatively new toxin, oxygen? Although both types of enzymes carry out the same function, their completely different crystal structures, utilization of different metal cofactors, and distinctive catalytic mechanism strongly argue against a common ancestor. The evolutionary tree for CuZn containing SOD, based on multiple sequence alignments with structural superimpositions of crystal structures, shows that extracellular SOD diverged from the cytosolic form at early stages of evolution, before the differentiation of fungi, plants, and metazoa [74]. Our own phylogenetic analysis of all known vertebrate SOD genes show close similarities between SOD1 and SOD3 with very low homology to SOD2 (Fig. 2). The structural core of SOD1 exists as a Greek key  $\beta$ -barrel motif, consisting of eight  $\beta$ -barrels [75]. The amino acid substitutions, as well as deletions and insertions, occur mostly outside of this structural motif. These data support the theory that CuZn-SOD evolution involved gene duplication and fusion with subsequent addition of exons I and III. Interestingly, the evolutionary rates of CuZn- and Mn-SOD differed considerably during the last billion years. While Mn-SOD proteins have evolved at a relatively constant rate, CuZn-SODs evolved unusually slowly at the beginning and erratically quickly in the most recent 100 million years [76,77]. Why such an abnormal evolutionary

rate took place remains unclear; one possible explanation is that CuZn containing SOD was caught in “folding-block” when most changes in amino acid composition were deleterious [76]. The accumulation of silent mutations finally led to an escape from this “evolutionary hibernation” and a return to the faster evolutionary rate. While the plausibility of this theory remains questionable, the existence of aerobic life on Earth proves that SOD successfully evolved as a potent protective enzyme against oxygen toxicity.

### TRANSCRIPTIONAL REGULATION

Transcriptional regulation of all three isoforms of superoxide dismutase are highly controlled based on extra- and intracellular conditions. In this section we will describe only well-documented and reproducible stimulation or repression of individual SOD gene transcription. An overall summary of factors regulating SOD mRNA levels are summarized in Table 1.

#### *SOD1*

SOD1 was found to have a widespread distribution in a variety of cells [3]. The expression of cytoplasmic SOD1 is stable and its activity is often considered as an internal control for SOD1 gene expression.

*Stimuli upregulating SOD1 expression.* Despite the fact that SOD1 is considered to be constitutively expressed, its mRNA levels can be dramatically regulated by various physiological conditions. SOD1 mRNA levels elevate in response to a wide array of mechanical, chemical, and biological messengers such as heat shock [78,79], shear stress [80,81], UVB- and X-irradiation [82–84], heavy metals [85], hydrogen peroxide [79], ozone [86], nitric oxide [87], arachidonic acid [88], and xenochemicals such as  $\beta$ -naphthoflavone, t-butyl-hydroquinone, iodoacetamide [89], 2,3,7,8-tetrachlorodibenzo-p-dioxin [90], and phenobarbital [91]. Analysis of the proximal promoter region reveals Sp1/Egr-1/WT-1 binding sites that are involved in basal and TPA inducible expression of SOD1 [23] as well as C/EBP cis-acting elements [92,93], which are also important for high level expression in rat liver cells [94]. SOD1 expression can also be triggered by ginseng saponins through activation of the AP2 transcription factor [95]. Metal ions are a potent source for the large scale catalysis and production of ROS inside cells. In order to neutralize their harmful effects, the cells increase the synthesis of SOD1 through the metal responsive element located in the 5'-flanking region [85].

*Stimuli downregulating SOD1 expression.* A downregulation of SOD1 has been shown in alveolar type II epithelial cells and lung fibroblasts after exposure to hypoxia [96]. The anticancer drug, mitomycin C also represses the transcription of SOD1 gene in human hepatoma HepG2 cells [97].

#### *SOD2*

Despite the fact that SOD2 is expressed in many cell types and tissues at relatively high levels it is also highly regulated by a variety of intracellular and environmental cues. Characterization of the 5'-flanking genomic region from rat [98], bovine [29], and human [25,99,100] indicates that the SOD2 promoter is TATA and CAAT-less but contains GC-rich sequences immediately upstream from the transcription initiation site. Computer analysis and foot-printing assays reveal a number of putative binding sites for Sp1 and AP2 transcription factors in the proximal promoter of human SOD2. The two proteins have opposite effects on SOD2 expression: while the Sp1 element positively promotes transcription, the AP2 proteins significantly repress the promoter activity [101].

*Stimuli upregulating SOD2 expression.* A wide variety of compounds induce transcription of SOD2. Cytokines such as interleukin (IL)-1 [102–104], IL-4, IL-6 [104], TNF- $\alpha$  [103,105], lipopolysaccharide (LPS) [106], and IFN- $\gamma$  [107] are potent activators of SOD2 in different tissues and cell types. The cytokine inducible enhancer has been localized to the 236 bp sequence within intron 2 of murine [108], rat and human [109] SOD2 genes. The cytokine inducible enhancer regions contain binding sites for NF- $\kappa$ B, C/EBP, and NF-1 transcription factors.

Protein kinase C stimulating agents such as TPA induce human SOD2 expression via activation of a CREB-1/ATF-1 like factor, but not via NF- $\kappa$ B or AP1 [110]. Interestingly, the microtubule-active anticancer drugs, vinblastin, taxol, and vincristine also induce SOD2 expression via activation of protein kinase C [111]. Manganese ions, which at high concentrations are toxic to the cells, induce expression of SOD2 in human breast cancer [112]. Platelet-derived growth factor induces the expression of the SOD2 gene in NIH3T3 cells, and its induction is associated with activation of Egr-1 transcription factor [113].

*Stimuli downregulating SOD2.* The expression of SOD2 in many cancers is decreased due to methylation of particular sequences in the intronic region [114,115] and elevated levels of AP2 transcription factor, which interacts with the 5'-flanking sequences of SOD2 gene [101].

*Post-transcriptional regulation of SOD2.* SOD2 expression is regulated not only at the level of transcription, but

Table 1. Common Intra- and Extracellular Stimuli Affecting Expression of SOD1, SOD2, and SOD3

	SOD1	SOD2	SOD3
Proximal promoter TATA or CAAT box GC-rich region	Yes Yes	No Yes	No Yes
Proinflammatory cytokines			
TNF- $\alpha$	$\leftrightarrow$ Rat lung [146] $\leftrightarrow$ Human vascular smooth muscle [147]	$\uparrow$ Rat smooth muscle [148] $\uparrow$ Human pulmonary adenocarcinoma cells [149] $\uparrow$ Human vascular smooth muscle [147]	$\downarrow$ Human fibroblast [122] $\downarrow$ Human vascular smooth muscle [147]
IL-1 $\beta$	$\leftrightarrow$ Rat lung [150]	$\uparrow$ Rat smooth muscle [148] $\uparrow$ Rat glial and neuronal cells [151]	
IL-1 $\alpha$	$\leftrightarrow$ Human vascular smooth muscle [147]	$\uparrow$ Human vascular smooth muscle [147]	$\uparrow$ Human fibroblast [122] $\uparrow$ Rat sertoli cells [123]
IFN- $\gamma$	$\leftrightarrow$ Human vascular smooth muscle [147]	$\uparrow$ Human vascular smooth muscle [147] $\uparrow$ Human lung adenocarcinoma [107] $\uparrow$ Rat glial and neuronal cells [151]	$\uparrow$ Human fibroblasts [122] $\uparrow$ Human vascular smooth muscle [147] $\leftrightarrow$ Rat sertoli cells [123]
TNF- $\alpha$ + IFN- $\gamma$		$\uparrow$ Murine fibrosarcoma [152] $\uparrow$ Human lung adenocarcinoma [107]	$\uparrow$ Rat pneumocytes [124]
Growth factors			
TGF- $\beta$ Fibroblast and epidermal growth factors	$\uparrow$ Rat fibroblasts [153]	$\downarrow$ Murine fibrosarcoma [152]	$\leftrightarrow$ Human fibroblast [122] $\downarrow$ Human vascular smooth muscle [125] $\leftrightarrow$ Rat sertoli cells [123]
Platelet-derived growth factor GM-CSF and GH		$\uparrow$ NIH 3T3 [113]	$\downarrow$ Human vascular smooth muscle [125] $\leftrightarrow$ Human fibroblast [122]
Nitric oxide	$\uparrow$ Human keratinocytes [87]	$\uparrow$ Rat vascular smooth muscle [154]	$\uparrow$ Human aortic smooth muscle [126]
Ozone	$\uparrow$ Rat lung [86]	$\uparrow$ Rat lung [86]	
Lipopolysaccharide	$\leftrightarrow$ Pulmonary epithelial cells [106] $\downarrow$ Rat astrocytes [155]	$\uparrow$ Rat smooth muscle [148] $\uparrow$ Pulmonary epithelial cells [106] $\uparrow$ Rat glial and neuronal cells [151]	$\leftrightarrow$ Human fibroblasts [122]
cAMP			$\uparrow$ Rat glioma [128]
TPA	$\uparrow$ HeLa cells [23]	$\uparrow$ Human lung adenocarcinoma [110] $\uparrow$ HeLa cells [156]	
Angiotensin II	$\leftrightarrow$ Mouse aorta [127]		$\uparrow$ Human vascular smooth muscle [125] $\uparrow$ Mouse aorta [127]

GM-CSF = granulocyte-macrophage colony-stimulating factor; GH = growth hormone.

also at the level of translation by a RNA-binding protein. The 41 bp region, located in the 3'-untranslated part of SOD2 mRNA binds the specific protein that increases its translation efficiency [116]. When this cis-element was positioned after the coding region of chloramphenicol acetyltransferase, it considerably increased the translation efficiency and enzymatic activity of the reporter gene [117]. While the identity of RNA-binding protein has not been determined, recent work shows that SOD2 binding protein is phosphorylated by tyrosine kinase and dephosphorylation is required for its binding activity [118].

### SOD3

In contrast to intracellular SOD1 and SOD2, the expression of SOD3 appears restricted to only a few cell types in several tissues. High levels of SOD3 expression have been documented for alveolar type II cells [37], proximal renal tubular cells (Folz, R.J., unpublished observation), vascular smooth muscular cells [119], lung macrophages [120] and few cultured fibroblast cell lines [121]. The features regulating such highly specific expression are not yet known, but analysis of the 5'-flanking region of human SOD3 reveals several potential regulatory sequences such as a glucocorticoid response element, xenobiotic response element, and an antioxidant response element [32]. Computer analysis of murine SOD3 proximal promoter reveals multiple putative binding sites for the Ets family of transcription factors. The importance of these proteins in regulating cell-specific expression has yet to be elucidated. The promoter region of SOD3 lacks typical TATA or CAAT boxes but possesses purine-rich sequences.

*Stimuli upregulating SOD3.* In human fibroblasts, the level of SOD3 was elevated by IFN- $\gamma$  and IL-1 $\alpha$ , while other cytokines such as IL-2, IL3, IL-4, IL-6, and IL-8 demonstrated no effect on its expression [122]. Similar results were reported for induction of SOD3 in rat sertoli cells, except IFN- $\gamma$  has no effect on the enzyme expression [123]. TNF- $\alpha$  and IFN- $\gamma$  appear to be a potent combination for the induction of SOD3 expression in rat alveolar type II pneumocytes through NF- $\kappa$ B activation [124]. Because SOD3 exerts an important protective role in the vascular wall, the vasoactive factors such as histamine, vasopressin, oxytocin, endothelin-1, serotonin, and heparin markedly increased enzyme level in the cultured arterial smooth muscle cells [125]. Further, exercise training increases production of nitric oxide in mouse vessel endothelial cells, which in turn upregulates expression of SOD3 in adjacent smooth muscle cells [126]. Thus, increased concentration of SOD3 prevents

the degradation of NO by oxygen radicals. Angiotensin II strongly induces SOD3 activity in mouse aortas [127] and in cultured human smooth muscle cells [125] through transcriptional activation and stabilization of mRNA. Interestingly, the effect of angiotensin II on SOD3 expression is due to activation of p42/44 MAP kinase pathway, while nitric oxide exerts its effect through MAP kinase p38. There are contradictory data on regulation of SOD3 expression by cyclic nucleotides. The exposure of rat glioma cells to cAMP increases SOD3 production while in mouse aortas it has no effect [126,128]. Interesting data on upregulation of SOD3 mRNA level in HepG2 cells expressing nuclear receptor CAR have been recently published, but the physiological relevance of this regulation is still uncertain [129].

*Stimuli downregulating SOD3.* The expression of SOD3 is repressed by different types of growth factors. Transforming growth factor- $\beta$  in human fibroblasts [122] and platelet-derived growth factors and fibroblast growth factor in vascular smooth muscle cells [125] markedly downregulate expression and excretion of SOD3. These responses are slow and develop over several days.

### ONTOGENY

The developmental regulation of SOD enzymes is crucial for adaptation of fetuses to the relatively high oxygen environment after parturition. The lung is one of the most important organs for protection of newborn organisms against harmful oxygen radicals, but increased SOD activity in the kidney of fetuses may have the same protective effect against extrauterine environment as in the lung. The expression of SOD enzymes in lung and kidney during development differs substantially among the species, but in most cases the level of SOD activity increases considerably just after birth as has been shown for fetal lamb [130] or rabbit [131] lung. It is not clear which particular SOD enzymes were attributed to this increase. Here, we will summarize the data on developmental expression of SOD enzymes in varied organs of different species.

### SOD1

The level of mRNA and enzymatic activity for SOD1 slightly increases from birth to adulthood in lung of the guinea pig [132], rat [133–135], and rabbit [131]. This rise in SOD1 activity is due mostly to an increased rate of mRNA synthesis. SOD1 is also developmentally regulated in the rat kidney where its activity increases 1.7-fold from gestational day 18 to day 22, while in heart its activity remains unchanged [135]. In the mouse, the expres-

sion pattern for SOD1 is highly variable among different strains, but shows an increased level in lung and brain during aging, but no differences in mRNA levels in both heart and kidney [136,137]. The data on expression of human SOD1 during development are quite limited and contradictory. Several groups show no increase in SOD1 activity in lung during late-fetal period [138,139], while others document an increase of mRNA level as well as enzymatic activity toward adulthood [140,141]. The reason for such discrepancies is not clear, but they may be attributed to the use of different assay methods and/or high variation of SOD1 activity among individuals.

### SOD2

The expression profile of SOD2 is somewhat similar to that of SOD1 and appears to be species-specific. SOD2 mRNA increases during developmental stages in guinea pig lungs [132], but shows no such changes in rat lungs [134]. SOD2 protein levels increase in the late gestational stages 2.2- and 2.5-fold in rat lung and kidney, respectively [135]. In the sheep and guinea pig, kidney SOD2 activity and mRNA concentration increase in neonatal and adult animals compared to early and late gestation fetuses [142,143]. In humans the expression profiles of SOD1 and SOD2 almost coincide, increasing toward adulthood in lung and liver, but the activities do not always correlate with mRNA level [141].

### SOD3

The developmental expression of SOD3 has been documented only in rabbit lung at preterm, term, 8 days old, 1 month, and adult stages [144]. While activity of SOD3 increases almost six times from preterm to adult, the SOD3 protein level remains constant during these times. The reason for such discrepancy between enzymatic activity and protein level is not clear but one explanation may be that some of the SOD3 protein, that is localized to intracellular compartments, may be inactive. Indeed, in airway epithelial and endothelial cells, localization of SOD3 clearly changes from intracellular in fetuses to extracellular after birth to adulthood. Our own data shows that levels of SOD3 mRNA increases dramatically just before and after birth while the levels of SOD1 and SOD2 mRNA remains mostly unchanged in mouse lung (Fig. 3). A somewhat similar pattern of SOD3 developmental expression is observed in rat lung, where the level of mRNA spikes just before parturition and then gradually increases toward adulthood (Fig. 4). It is interesting to point out that the level of SOD3 mRNA in mouse and rat has been undetectable until late gestation, indicating that SOD3 is vital for protection of fetal

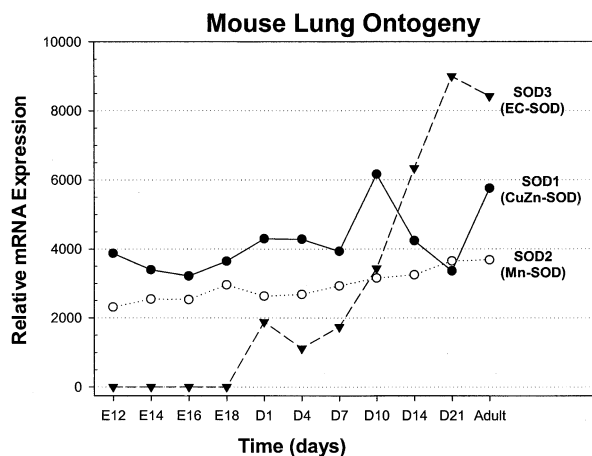


Fig. 3. Developmental expression profile of superoxide dismutase mRNA in mouse lung. Total RNA was isolated from whole mouse lung tissue harvested at the indicated times. RNA was used for target generation and hybridization to the Affymetrix Mu11K GeneChip set according to standard protocols [157]. Relative mRNA expression level represents the Average Difference Value generated for each probe set at each timepoint. SOD1 and SOD2 show moderate, fairly constitutive levels of expression throughout lung development. SOD3 shows low levels of expression during embryonic development and increases substantially during postnatal maturation. Comparing intensity values between genes may not represent accurate relative abundance. E-embryonic day; D-postnatal day.

lung against high-oxygen environment seen after birth. The similar dramatic increase in SOD3 mRNA level has also been observed in rat testicles between 20 and 60 days of age [123]. In humans, plasma levels of SOD3 in children are considerably higher compared with adults and decreases toward adulthood about 2% per year reaching a plateau at age 20 [145].

## Ontogeny Northern Blot

### Rat EC-SOD

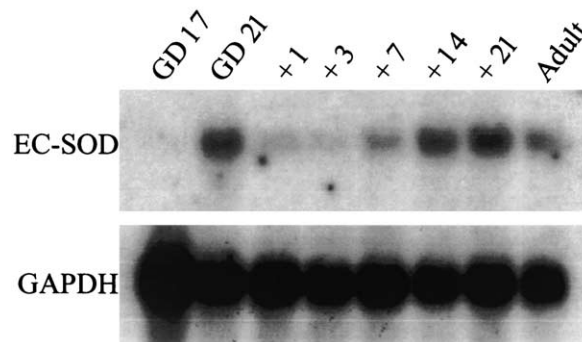


Fig. 4. Developmental expression of SOD3 in the rat lung. One microgram of poly A mRNA from the rat lung samples were prepared from fetuses at day 17 and 21 during gestation, and days 1, 3, 7, 14, 21 after birth as well as from adult rat. The mRNA was electrophoresed, transferred to membrane and hybridized with  $^{32}\text{P}$ -labeled probe specific to SOD3 (upper panel) or GAPDH (lower panel).

## CONCLUSION

The past decade has brought us new evidence of SOD's involvement in a number of diseases and pathologies: ALS, Down's syndrome, and premature aging are probably just some of the pathological conditions that develop due to altered SOD activity and ROS concentration. What other discoveries await us? New, emerging questions such as what role the extracellular form of SOD plays in cardiovascular and pulmonary diseases, and how it affects our ability to learn, still need to be answered. With a wealth of information provided in this field over the last few years we are just beginning to understand the significance of SOD in biology and pathology. The further gain of knowledge about the mechanisms of cell and tissue-specific regulation of SOD gene expression and their signal transduction pathways may also lead to the design of new drugs and strategies directed at regulating levels of these enzymes in particular tissues, cell types, and compartments without affecting other cells.

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#### ABBREVIATIONS

C/EBP—CCAAT/enhancer binding protein  
 CuZn-SOD—SOD1 or copper, zinc SOD  
 EC-SOD—SOD3 or extracellular SOD  
 Egr-1—early growth response-1  
 GRE—glucocorticoid response element  
 HSF—heat shock factor  
 IFN- $\gamma$ —interferon  $\gamma$   
 kDa—kilodaltons  
 Mn-SOD—SOD2 or manganese SOD  
 ROS—reactive oxygen species  
 RT-PCR—reverse transcription-polymerase chain reaction  
 SOD—superoxide dismutase  
 NF- $\kappa$ B—nuclear factor kappa B  
 TNF- $\alpha$ —tumor necrosis factor  $\alpha$   
 TPA—12-O-tetradecanoylphorbol-13-acetate