Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 9374–9378, August 1999
Microbiology

A highly conserved sequence is a novel gene involved in *de novo* vitamin B6 biosynthesis

(*SOR1*/*PDX1*/photosensitizer/cercosporin)

MARILYN EHRENSHAFT*†, PIOTR BILSKI‡, MING Y. LI‡§, COLIN F. CHIGNELL‡, AND MARGARET E. DAUB*

Departments of *Plant Pathology and §Chemistry, North Carolina State University, Raleigh, NC 27695; and ‡Laboratory of Pharmacology and Chemistry, National Institute for Environmental Health Sciences, Research Triangle Park, NC 27709

Communicated by Arthur Kelman, North Carolina State University, Raleigh, NC, June 14, 1999 (received for review March 10, 1999)

ABSTRACT **The *Cercospora nicotianae SOR1* (singlet oxygen resistance) gene was identified previously as a gene involved in resistance of this fungus to singlet-oxygen-generating phototoxins. Although homologues to *SOR1* occur in organisms in four kingdoms and encode one of the most highly conserved proteins yet identified, the precise function of this protein has, until now, remained unknown. We show that *SOR1* is essential in pyridoxine (vitamin B6) synthesis in *C. nicotianae* and *Aspergillus flavus*, although it shows no homology to previously identified pyridoxine synthesis genes identified in *Escherichia coli*. Sequence database analysis demonstrated that organisms encode either *SOR1* or *E. coli* pyridoxine biosynthesis genes, but not both, suggesting that there are two divergent pathways for *de novo* pyridoxine biosynthesis in nature. Pathway divergence appears to have occurred during the evolution of the eubacteria. We also present data showing that pyridoxine quenches singlet oxygen at a rate comparable to that of vitamins C and E, two of the most highly efficient biological antioxidants, suggesting a previously unknown role for pyridoxine in active oxygen resistance.**

The filamentous, phytopathogenic fungus *Cercospora nicotianae* exhibits a uniquely effective, broad-spectrum resistance to potent photosensitizers of diverse chemical structure and solubility (1, 2). *C. nicotianae* is resistant to cercosporin, a light-activated, singlet oxygen ($^1O_2$)-generating toxin it produces in culture and during plant parasitism, and also to other potent photosensitizers including porphyrins and xanthine and thiazine dyes. Photosensitizers are highly toxic compounds that produce their deleterious effects only after activation by light. Absorbed light energy converts the photosensitizer to an excited (triplet) state molecule that may transfer an electron to oxygen to generate superoxide and/or transfer energy directly to oxygen, yielding $^1O_2$ (3). Exposure of cells to photosensitizers plus light leads to the destruction of critical cellular components including proteins, membranes, and DNA and often results in cell death.

Studies on the mechanisms by which organisms protect themselves against reactive oxygen species have focused primarily on reduced and radical forms of oxygen, including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), and the hydroxyl radical ($OH\cdot$). These active oxygen species are byproducts of normal cellular metabolism, and cells contain numerous and conserved defenses against them. By contrast, the highly reactive, but nonradical $^1O_2$ is produced primarily via light activation of photosensitizing compounds. Most organisms do not tolerate $^1O_2$, and few biological defenses have been identified (2). The broad-spectrum resistance expressed by *Cercospora* species against cercosporin and other photosensitizers of diverse structure make these organisms an excellent model for understanding the cellular basis of $^1O_2$ resistance.

To study specific genes and proteins involved in photosensitizer and $^1O_2$ resistance, we isolated mutants of *C. nicotianae* sensitive to cercosporin (1, 4) and used functional complementation to identify genes required for resistance. Our recent work has focused on the *C. nicotianae SOR1* (singlet oxygen resistance) gene (5, 6). Mutant complementation studies and the production of *SOR1* null mutants by targeted gene replacement suggested that *SOR1* plays a role in *C. nicotianae* resistance both to cercosporin and other photosensitizers. *SOR1* was the first cloned gene with apparent genetic and phenotypic links to resistance against compounds that generate $^1O_2$.

Initial identification of *SOR1* concomitantly uncovered an intriguing mystery. Although $^1O_2$ and photosensitizer resistance is uncommon, *SOR1* homologues are widespread, occurring in numerous organisms within four kingdoms, archaebacteria, eubacteria, plants, and fungi (5). In addition to enjoying widespread distribution, SOR1 also is one of the most highly conserved proteins yet identified (7, 8). These results suggested that the SOR1 protein is involved in an unknown but conserved metabolic function. In this paper we describe a second phenotype for *SOR1* that explains its strong conservation in diverse organisms. Our data show that *SOR1* is necessary for synthesis of pyridoxine (vitamin B6) in *C. nicotianae* and in a second filamentous fungus, *Aspergillus flavus*. We propose that *SOR1* is part of a novel pathway for *de novo* biosynthesis of pyridoxine distinct from the previously described *Escherichia coli de novo* pathway (9–14) and that pathway divergence occurred during the evolution of the eubacteria. Finally, we also provide data demonstrating that pyridoxine quenches $^1O_2$ and argue for a heretofore undiscovered role for pyridoxine in antioxidant defense.

MATERIALS AND METHODS

Fungal Strains and Culture Conditions. All *C. nicotianae* mutants used in this study were derived from the wild-type strain ATCC 18366. Mutants used included three UV-generated *sor1* mutant strains (cercosporin-sensitive CS6, CS8, and CS9) (1, 4) and three *sor1* null strains generated by targeted gene replacement (5). The *C. nicotianae SOR1* transformants screened for prototrophy were derived from previous studies. *A. flavus* strain ATCC 60045 is a pyridoxine auxotroph, kindly provided by G. A. Payne, North Carolina State University. Stock cultures of *C. nicotianae* were maintained in the dark, on malt medium (15) at 28°C, conditions nonconducive to cercosporin biosynthesis by *Cercospora* fungi. For assessment of pyridoxine auxotrophy and prototrophy, transformants and the original CS strains were grown on minimal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

†To whom reprint requests should be addressed. E-mail: me1@unity.ncsu.edu.

medium (15) with and without the addition of 1 μg/ml pyridoxine-HCl.

Transformation. Protocols for genetic transformation of *A. flavus* have been described (16). Two plasmids, both expressing only the *SOR1* ORF, were used to transform *A. flavus.* The first expressed *SOR1* under the control of the constitutive *A. nidulans gpdA* (glyceraldehyde-3-phosphate) promoter (17). The second was a previously described (5, 6) clone containing *SOR1* under the control of its own promoter.

Computer Analysis of Data. Database homology searches were performed by using the BLAST server at the National Center for Biotechnology Information (18). Preliminary sequence data were obtained from The Institute for Genomic Research web site at http://www.tigr.org.

Quenching of $^1O_2$ Phosphorescence by Pyridoxine. Singlet oxygen was detected from irradiated solutions of cercosporin or rose bengal in aerobic D_2O via phosphorescence at 1,270 nm (diagnostic for $^1O_2$). The experimental set-up used an NdYag laser (Continuum, Santa Clara, CA) for pulse excitation (532 nm), and $^1O_2$ phosphorescence was detected by a germanium diode as described by Bilski and Chignell (19). The kinetics of decay in the presence and absence of pyridoxine, pyridoxal, pyridoxamine, pyridoxal 5-phosphate, and L-methionine were measured as described (19). Rate constants for $^1O_2$ quenching were determined with increasing concentrations of each quencher in 50 μM rose bengal-sensitized solutions.

Chemicals. Cercosporin was extracted and purified from mycelial cultures of *Cercospora kikuchii* as described (20). Pyridoxine, pyridoxal, pyridoxamine, pyridoxal 5-phosphate, and L-methionine all were purchased from Sigma.

RESULTS

***SOR1* Is Required for Pyridoxine (Vitamin B6) Synthesis.** During experiments to determine whether expression of *SOR1* was sufficient to confer $^1O_2$ and photosensitizer resistance on a sensitive organism, a second phenotype for *SOR1* was discovered. A construct containing the *SOR1* ORF under the control of the constitutive *A. nidulans gpdA* promoter was transformed into an *A. flavus* strain auxotrophic for pyridoxine. Analysis of the transformants indicated that this isolate could be restored to pyridoxine prototrophy by *SOR1* transformation, either under the control of the *gpdA* promoter or its own promoter (data not shown). Transformation with plasmids lacking *SOR1* failed to restore prototrophy, confirming that restoration to pyridoxine prototrophy was a *SOR1*-specific effect. Furthermore, we also found that pyridoxine prototrophy could be used as a selective marker in *A. flavus* transformations with *SOR1.*

Our fungal strains are maintained on complex medium. The above results led us to test our cercosporin-sensitive strains, used in the identification of *SOR1*, for pyridoxine auxotrophy. Six *sor1* mutant *C. nicotianae* strains were tested, three of which were generated by UV mutagenesis and three null strains that were generated by targeted gene replacement (1, 4–6). When tested for growth on minimal medium, all six strains required pyridoxine for growth (Fig. 1). When transformed with *SOR1*, all of the mutant strains were restored to pyridoxine prototrophy (data not shown). We also have learned recently that an *A. nidulans* gene that complements an *A. nidulans* pyridoxine auxotroph encodes a *SOR1* homologue (A. H. Osmani, G. S. May, and S. A. Osmani, personal communication). Thus, *SOR1* homologues are required for pyridoxine synthesis in both *Cercospora* and *Aspergillus*.

***SOR1* Encodes a Novel Pyridoxine Pathway Gene.** Studies of pyridoxine biosynthesis have centered on *E. coli* in which pathway genes for *de novo* pyridoxine biosynthesis have been identified via complementation of auxotrophic mutants (9–14, 21–23). Two of the genes (*serC* and *gapB*) encode enzymes also required in other biosynthetic pathways, but three (*pdxA*, *pdxB*, and *pdxJ*) are unique to pyridoxine synthesis. None of these five genes shows homology to *SOR1*. We performed BLAST searches by using *SOR1* and the unique *E. coli* pyridoxine biosynthesis genes (*pdxA, pdxB*, and *pdxJ*) against the GenBank database as a whole as well as the genomes with separate databases. At the time of this writing, the entire genomes of 18 microorganisms and one animal were available for analysis. Without exception, organisms with *SOR1* homologues lack homologues to the *E. coli* pyridoxine biosynthesis genes *pdxA* and *pdxJ*, whereas those with homologues to the *E. coli* genes lack *SOR1* (Table 1). Seven genomes possess neither *SOR1* nor *pdxA*/*pdxJ*. Six of these belong to obligate microbial parasites with greatly reduced coding capacity, presumably because they obtain many nutrients, such as pyridoxine, from their hosts. The seventh is the single animal representative, *Caenorhabditis elegans*.

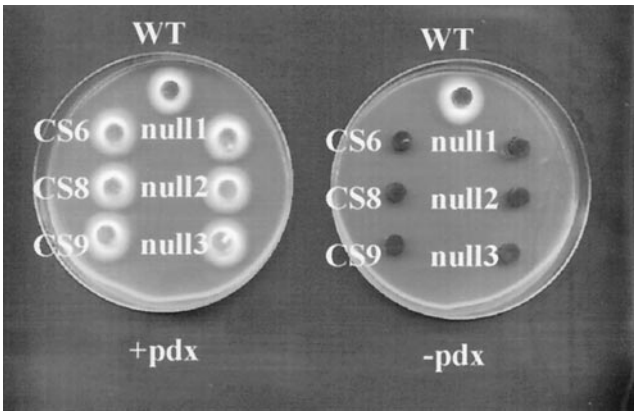

FIG. 1. Growth of *C. nicotianae* wild type (WT) and *sor1* null (null 1, 2, and 3) and cercosporin-sensitive UV-mutant (CS6, CS8, and CS9) strains on minimal medium with (+pdx) and without (−pdx) 1 μg/ml pyridoxine. Six-millimeter fungal plugs were placed mycelium-side down and incubated for 4 days at 28°C.

The data from completely sequenced genomes are corroborated by data from other, incompletely sequenced ones (Table 2). Here, *SOR1* homologues were identified in three

Table 1. Occurrence of *SOR1* and the *E. coli* pyridoxine biosynthetic genes *pdxA* and *pdxJ* in organisms with completely sequenced genomes

Organism	*SOR1*	*pdxA*/*J*	Genome
Fungi			
Saccharomyces cerevisiae	+	−	16 Mb
Archaebacteria			
Methanococcus jannaschii	+	−	1.8 Mb
Pyrococcus horikoshii	+	−	2.0 Mb
Methanobacterium thermoautotrophicum	+	−	1.8 Mb
Archaeoglobus fulgidus	+	−	2.2 Mb
Eubacteria			
Haemophilus influenzae	+	−	1.8 Mb
Bacillus subtilis	+	−	4.2 Mb
Mycobacterium tuberculosis	+	−	4.4 Mb
E. coli	−	+	4.6 Mb
Synechocystis	−	+	3.5 Mb
Helicobacter pylori	−	+	1.7 Mb
Aquifex aeolicus	−	+	1.5 Mb
Mycoplasma pneumoniae	−	−	0.8 Mb
Mycoplasma genitalium	−	−	0.6 Mb
Borrelia burgdorferi	−	−	1.0 Mb
Treponema pallidum	−	−	1.1 Mb
Chlamydia trachomatis	−	−	1.0 Mb
Rickettsia prowazekii	−	−	1.1 Mb
Animal			
Caenorhabditis elegans	−	−	100 Mb

Table 2. Occurrence of *SOR1* and the *E. coli* pyridoxine biosynthetic genes *pdxA* and *pdxJ* in organisms with partially sequenced genomes

Organism	SOR1	pdxA/J
Plant		
Arabidopsis thaliana	+	−
Oryza sativa	+	−
Hevea brasiliensis	+	−
Stellaria longipes	+	−
Brassica napus	+	−
Physcomitrella patens	+	−
Fungi		
Schizosaccharomyces pombe	+	−
A. nidulans	+	−
Candida albicans	+	−
Dictyostelid		
Dictyostelium discoidum	+	−
Archaebacteria		
Methanococcus vannielli	+	−
Pyrococcus furiosis	+	−
Eubacteria		
Mycobacterium leprae	+	−
Francisella tularensis	+	−
Clostridium acetobutylicum	+	−
Streptococcus pneumoniae	+	−
Thermotoga maritima	+	−
Deinococcus radiodurans	+	−
Shewanella putrefaciens	−	+
Caulobacter crescentus	−	+
Porphyromonas gingivalis	−	+
Chlorobium tepidum	−	+
Erwinia herbicola	−	+
Yersinia pestis	−	+
Pseudomonas aeruginosa	−	+
Neisseria meningitidis	−	+
Neisseria gonorrhoeae	−	+
Campylobacter jejuni	−	+
Vibrio cholerae	−	+
Salmonella typhi	−	+
Bordetella pertussis	−	+

fungi, six plants, two archaebacteria, six eubacteria, and the Dictyostelid *Dictyostelium discoidum*. Thirteen additional eubacteria contain homologues to both *pdxA* and *pdxJ* (Table 2), whereas four others (*Aquifex pyrophilus*, *Bradyrhizobium japonicum*, *Sphingomonas aromaticivorans*, and *Burkholderia cepacia*) contain either *pdxA* or *pdxJ*. None of these 35 organisms encode both *SOR1* and *pdxA* and/or *pdxJ*.

In contrast to *pdxA* and *pdxJ*, results with *pdxB* were inconclusive. Because the *pdxB* gene product is a dehydrogenase, there is widespread, but low, homology to the *E. coli* PDXB protein. Potential *pdxB* homologues were found in organisms with *SOR1* homologues as well as in *pdxA*/*pdxJ*-containing organisms (data not shown). Further, *pdxB* homologues could be identified in only two (*Synechocystis* and *Aquifex aeolicus*) of the three completely sequenced *pdxA*/*pdxJ*-containing organisms. However, the data with *pdxA* and *pdxJ* strongly suggest that there are two different sets of genes involved in *de novo* pyridoxine synthesis. One set, of which *SOR1* is a part, is found in some eubacteria, in all archaebacteria so far examined, and in eukaryotes such as fungi and plants. The second set, the *E. coli* version, is found so far only in some, but not all, eubacteria.

Pyridoxine and Biologically Active Pyridoxine Vitamers Quench $^1O_2$ *in Vitro*. The connection in *C. nicotianae* between photosensitizer resistance and pyridoxine synthesis was completely unanticipated. Extensive surveys of $^1O_2$ quenchers (24, 25) do not include pyridoxine, nor has this vitamin been implicated previously in cellular antioxidant defense. To determine whether pyridoxine could directly deactivate $^1O_2$, we examined the interaction of these molecules *in vitro*. Singlet oxygen can be identified by its characteristic IR phosphorescence at 1,270 nm after irradiation of a photosensitizer-containing sample (Fig. 2*A*). Photosensitizer-containing samples with and without 0.1 mM pyridoxine were subjected to a laser pulse, and phosphorescence was measured at 1,270 nm. $^1O_2$ phosphorescence decayed faster in the presence of pyridoxine (Fig. 2*B*), indicating that pyridoxine decreases $^1O_2$ lifetime. Dose-response experiments confirmed that increasing concentrations of pyridoxine and pyridoxal 5-phosphate increase the rate of $^1O_2$ decay (Fig. 2*C*). These data were used to calculate rate constants for pyridoxine and other biologically active forms of vitamin B6 (Table 3). All four vitamers tested exhibited quenching constants at or approaching 1×10^8 $M^{-1}\cdot sec^{-1}$. They are thus more efficient quenchers than sulfur-containing antioxidants and quench $^1O_2$ at a rate comparable to that of vitamins E and C, two of the most efficient biological antioxidants identified to date.

DISCUSSION

SOR1 originally was identified as a gene involved in resistance to cercosporin and other $^1O_2$-generating photosensitizers in the fungus *C. nicotianae*. Although $^1O_2$ resistance is rare in nature, sequence analysis indicated *SOR1* homologues were present in widely divergent organisms not reported to be photosensitizer resistant, including fungi, plants, eubacteria, and archaebacteria (5). In addition to the gene's widespread distribution, the predicted SOR1 protein is one of the most highly conserved proteins yet identified (7, 8). However, the precise metabolic function of the SOR1 protein remained undefined. In this study, we demonstrate that *SOR1* is required for synthesis of pyridoxine, a vitamin that is a direct precursor for pyridoxal 5-phosphate, a required cofactor in enzymatic

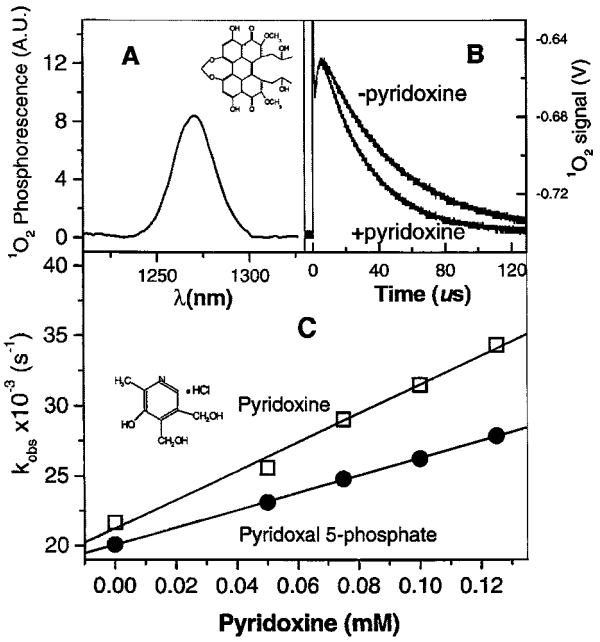

FIG. 2. Quenching of $^1O_2$ phosphorescence by pyridoxine. (*A*) Spectrum of $^1O_2$ phosphorescence after irradiation of a solution of cercosporin (*Inset*) in aerobic D_2O. (*B*) $^1O_2$ phosphorescence decay observed at the maximum of $^1O_2$ emission after one-shot laser $^1O_2$ production in the absence (−pyridoxine) and presence (+pyridoxine) of 0.1 mM pyridoxine. (*C*) Observed rate constant for $^1O_2$ quenching as a function of the increasing concentration of pyridoxine (*Inset*) and pyridoxal 5-phosphate at pD 7.4 in D_2O aerobic phosphate buffer (20 mM). The line slopes yield the quenching-rate constants (Table 3). Rose bengal (50 μM) was used to photosensitize $^1O_2$.

Table 3. Total quenching* of $^1O_2$ by pyridoxine derivatives and other physiological antioxidants

Quencher/antioxidant	k_q, $M^{-1}\cdot s^{-1}$	Conditions, solvent	Ref.
Pyridoxine	$10.3\ (\pm 0.5) \times 10^7$	pD = 7.4, D_2O	This work
Pyridoxal	$8.7\ (\pm 0.1) \times 10^7$	pD = 7.4, D_2O	This work
Pyridoxamine	$9.9\ (\pm 0.2) \times 10^7$	pD = 7.4, D_2O	This work
Pyridoxal 5-phosphate	$6.2\ (\pm 0.2) \times 10^7$	pD = 7.4, d_2O	This work
L-Methionine	$1.1\ (\pm 0.1) \times 10^7$	D_2O	This work
Vitamin C	0.83×10^7	H_2O, pH 6.8	(26)
	15×10^7	CD_3OD	(27)
Vitamin E	10×10^7	CCl_4	(28)
	3×10^7	$ClCF_2CCl_2F$	(29)
Glycyl-methionine	1.5×10^7	50% CH_3CN, 50% H_2O	(30)
Glutathione	0.087×10^7	D_2O	(31)
Cysteine	5×10^7	75% D_2O, 25% EtOH	(32)

*The quenching-rate constants, k_q, reported in this work were calculated from time-resolved decays of $^1O_2$ phosphorescence recorded after a single-pulse laser irradiation (19) of rose bengal in aerobic D_2O solution containing the pyridoxine quencher.

reactions such as transaminations, which are involved primarily in amino acid metabolism. The linkage of *SOR1* with pyridoxine synthesis provides a singularly clear solution to the initial mystery of why *SOR1* is so conserved and so much more prevalent than photosensitizer resistance. Nearly all microbes, prokaryotic and eukaryotic, as well as plants synthesize pyridoxine. Organisms that do not synthesize pyridoxine would be expected to lack genes associated with its production, clarifying why *SOR1* is not present in any of the animal genomes being sequenced. Although our original analysis detected a *SOR1* homologue in *C. elegans* (5), completion of this animal genome indicated that the supposed homologue was actually a yeast sequence.

To date, all genes involved in *de novo* pyridoxine synthesis are from *E. coli.* Biochemical and functional complementation analyses of *E. coli* pyridoxine auxotrophs led to the identification of the intermediate products and the genes necessary for *de novo* synthesis, respectively (9, 11–14). Our evidence, however, indicates that the *E. coli* pathway is not universal. With the exception of animals and obligate parasites, homology searches using *SOR1* and the *E. coli* genes *pdxA* and *pdxJ* (Tables 1 and 2) clearly divide organisms into those with *SOR1* homologues and those with *pdxA*/*pdxJ* homologues. The *SOR1* group includes archaebacteria, eukaryotes such as fungi and plants, and some eubacteria, whereas the *pdxA*/*pdxJ* group includes only eubacteria. The evolutionary shift appears to have occurred in the gamma subdivision of the proteobacteria taxon of the eubacteria group. Two bacterial species in this subdivision encode *SOR1* homologues, whereas numerous others, including *E. coli*, do not.

In addition to our evidence, ^{15}N-labeling studies in yeast, which has three unlinked *SOR1* homologues (7) and no homologues to either *pdxA* or *pdxJ*, determined that the nitrogen atom of yeast pyridoxine is derived from the amide moiety of glutamine (33), whereas in *E. coli*, glutamic acid provides the nitrogen. The ^{15}N-labeling data, in conjunction with our database analysis, suggest that the yeast pyridoxine pathway, like the yeast thiamin synthetic pathway (34), is distinct from that of *E. coli*. Interestingly, there is a second gene (*SNO* or *SNZB*) found in organisms with *SOR1* homologues, generally in physical proximity to *SOR1*. The protein encoded by this gene has been predicted to possess glutamine amidotransferase activity (8, 35), a hypothesis consistent with the ^{15}N-labeling data.

It is unclear at this time whether the pathways in *E. coli* and in *SOR1*-containing organisms are completely disparate, or if they only partially diverge. *pdxA* and *pdxJ* encode enzymes catalyzing the final step in *E. coli* pyridoxine synthesis, that of ring closure (9, 21, 23). The dehydrogenation catalyzed by the *pdxB* gene product and the addition of the nitrogen moiety, which in *E. coli* is catalyzed by an aminotransferase, occur in that order and before ring closure. However, homology searches with *pdxb* produced far less clear results than the *pdxA*/*pdxJ* homology searches. Although *SOR1*-containing organisms contain putative *pdxB* homologues, not all *pdxA*/*pdxJ*-containing organisms do so. Furthermore, the homology between putative *pdxB* homologues is less pronounced than the homologies between either *pdxA* or *pdxJ* homologues. It seems feasible that SOR1 substitutes for PDXA/PDXJ and is involved in ring closure whereas SNO/SNZB are required for addition of the nitrogen moiety via an amidotransferase reaction. However, further studies need to be performed to determine the extent of conservation and/or divergence between the *E. coli de novo* pyridoxine synthesis pathway and the "*SOR1*" pathway. Interestingly, although there is divergence in the *de novo* synthesis of pyridoxine, the salvage pathway that interconverts and recycles the various vitamers of pyridoxine into the active coenzyme (pyridoxal 5-phosphate), appears to be conserved (12).

In this study, we also suggest a previously unrecognized role for pyridoxine in active oxygen resistance. Cellular oxidative damage has become the focus of intense interest in both the scientific and popular literature. Medical studies seek to quantify both the curative and protective effects of nutritive substances with antioxidant properties. In advertising and the popular press, numerous compounds with antioxidant properties have been touted as prophylactic or restorative agents. Although the importance of many compounds, such as vitamins C and E and β-carotene, are well known, even the highly enthusiastic lay press rarely mentions vitamin B6 in this capacity. Surveys quantifying the $^1O_2$-quenching ability of both natural and synthesized compounds also fail to include pyridoxine or its vitamers (24, 25). Thus, the linkage of pyridoxine synthesis with photosensitizer resistance was completely unanticipated. Direct measurements of the ability of pyridoxine and its vitamers to quench $^1O_2$ (Fig. 2 and Table 3), however, provides evidence that this vitamin may play a heretofore unforeseen biological function in addition to its known role as a cofactor in enzymatic reactions.

Corroborative evidence that pyridoxine, via expression of *SOR1* and its homologues, is involved in active oxygen resistance can be found in expression studies in plants, yeast (*Saccharomyces cerevisiae*), and fission yeast (*Schizosaccharomyces pombe*). In rubbertree (*Hevea brasiliensis*), *SOR1* homologue transcripts increase to higher amounts in response to treatment with ethylene and salicylic acid (36), two inducers of plant-defense reactions. Plant-defense reactions, including those induced by ethylene and salicylic acid, are associated with increases in production of active oxygen species that serve multiple roles as signal molecules, as substrates in the production of structural defense compounds, and as defense molecules against pathogens (37). In *S. cerevisiae*, one of the three

SOR1 homologues (*SNZ1*) encodes a protein that accumulates to high levels in stationary-phase culture (7). Stationary-phase yeast cultures are subjected to increased oxidative stress, and entry into this growth stage is associated with a dramatic increase in resistance to a diversity of oxidants (38). Finally, in *S. pombe*, overexpression of the PAP1 transcription factor increases expression of the *S. pombe SOR1* homologue (W. M. Toone, Laboratory of Gene Regulation, Imperial Cancer Research Fund, London, personal communication). PAP1 is an AP-1-like transcription factor that is a member of the basic leucine zipper superfamily of DNA-binding transcription factors (39). PAP1 is an essential part of the *S. pombe* cellular response to oxidative stress (40), as is the comparable *S. cerevisiae* YAP1 transcription factor (41, 42). We have found an AP-1 consensus region upstream of the *C. nicotianae SOR1* promoter, suggesting that *SOR1* may be regulated by this family of transcription factors and as a general response to cellular oxidative stress.

Ironically, our observation that *sor1* mutants lack resistance to $^1O_2$-generating photosensitizers now is complicated by our data on pyridoxine quenching. We have determined recently that pyridoxine and its vitamers quench $^1O_2$ primarily via a chemical quenching mechanism during which the vitamin is consumed (data not shown). Measurements conducted during growth experiments indicate that pyridoxine concentrations drop rapidly in the presence of photosensitizers and light. Thus, in the process of quenching $^1O_2$, a nutrient required by our *sor1* mutants is consumed. We currently are concentrating our efforts on dissecting the relationship between photosensitizer sensitivity and auxotrophy in our mutant strains. Because the *sor1* phenotype is clearly that of pyridoxine auxotrophy, we have changed the name of this gene to *PDX1* (GenBank accession no. AF035619).

We thank J. D. Williamson for critical review of this manuscript, G. A. Payne for providing the A. flavus culture, and A. H. Osmani, G. S. May, S. A. Osmani, and W. M. Toone for sharing unpublished results. Preliminary sequence data were obtained from The Institute for Genomic Research web site at http://www.tigr.org. This work was supported by National Science Foundation Grant MCB-9631375 and U.S. Department of Agriculture National Research Initiative Competitive Grants Program Grant 96-35303-3204, both to M.E.D. and M.E.

1. Jenns, A. E., Scott, D. L., Bowden, E. F. & Daub, M. E. (1995) *Photochem. Photobiol.* **61,** 488–493.
2. Daub, M. E., Ehrenshaft, M., Jenns, A. E. & Chung, K. R. (1998) in *Phytochemical Signals and Plant-Microbe Interactions, Recent Advances in Phytochemistry*, eds. Downum, K. R. & Verpoorte, R. (Plenum, New York), Vol. 32, pp. 31–56.
3. Spikes, J. D. (1989) in *The Science of Photobiology*, ed. Smith, K. C. (Plenum, New York), pp. 79–110.
4. Jenns, A. E. & Daub, M. E. (1995) *Phytopathology* **85,** 906–912.
5. Ehrenshaft, M., Jenns, A. E., Chung, K. R. & Daub, M. E. (1998) *Mol. Cell* **1,** 603–609.
6. Ehrenshaft, M., Chung, K. R., Jenns, A. E. & Daub, M. E. (1999) *Curr. Genet.* **34,** 478–485.
7. Braun, E. L., Fuge, E. K., Padilla, P. A. & Werner-Washburne, M. (1996) *J. Bacteriol.* **178,** 6865–6872.
8. Galperin, M. Y. & Koonin, E. V. (1997) *Mol. Microbiol.* **24,** 443–445.
9. Hill, R. E. & Spenser, I. D. (1996) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 1, pp. 695–703.
10. Lam, H. M. & Winkler, M. E. (1990) *J. Bacteriol.* **172,** 6518–6528.
11. Zhao, G. & Winkler, M. E. (1996) *FEMS Microbiol. Lett.* **135,** 275–280.
12. Yang, Y., Zhao, G., Man, T. K. & Winkler, M. E. (1998) *J. Bacteriol.* **180,** 4294–4299.
13. Hockney, R. C. & Scott, T. A. (1979) *J. Gen. Microbiol.* **110,** 275–283.
14. Drewke, C., Klein, M., Clade, D., Arenz, A., Muller, R. & Leistner, E. (1996) *FEBS Lett.* **390,** 179–182.
15. Jenns, A. E., Daub, M. E. & Upchurch, R. G. (1989) *Phytopathology* **79,** 213–219.
16. Woloshuk, C. P., Seip, E. R. & Payne, G. A. (1989) *Appl. Environ. Microbiol.* **55,** 86–90.
17. Punt, P. J., Zegers, N. D., Busscher, M., Pouwels, P. H. & van den Hondel, C. A. M. J. J. (1991) *Biotechnology* **17,** 19–24.
18. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
19. Bilski, P. & Chignell, C. F. (1996) *J. Biochem. Biophys. Methods* **33,** 73–80.
20. Daub, M. E. (1982) *Phytopathology* **72,** 370–374.
21. Roa, B. B., Connolly, D. M. & Winkler, M. E. (1989) *J. Bacteriol.* **171,** 4767–4777.
22. Schoenlein, P. V., Roa, B. B. & Winkler, M. E. (1989) *J. Bacteriol.* **171,** 6084–6092.
23. Lam, H. M., Tancula, E., Dempsy, W. B. & Winkler, M. E. (1992) *J. Bacteriol.* **174,** 1554–1567.
24. Bellus, D. (1979) *Adv. Photochem.* **11,** 105–205.
25. Wilkinson, F., Helman, W. P. & Ross, A. B. (1995) *J. Phys. Chem. Ref. Data* **24,** 663–1021.
26. Chou, P. T. & Khan, A. U. (1983) *Biochem. Biophys. Res. Commun.* **115,** 932–937.
27. Scurlock, R., Rougee, M. & Bensasson, R. V. (1990) *Free Radical Res. Commun.* **8,** 251–258.
28. Krasnovsky, A. A. J. (1979) *Photochem. Photobiol.* **29,** 29–33.
29. Stevens, B. & Marsh, K. L. (1982) *J. Phys. Chem.* **86,** 4473–4476.
30. Miskoski, S. & Garcia, N. A. (1993) *Photochem. Photobiol.* **57,** 447–452.
31. Chacon, J. N. & Truscott, T. G. (1991) *J. Photochem. Photobiol. B.* **11,** 261–267.
32. Michaeli, A. & Feitelson, J. (1994) *Photochem. Photobiol.* **59,** 284–289.
33. Tazuya, K., Adachi, Y., Masuda, K., Yamada, K. & Kumaoka, H. (1995) *Biochim. Biophys. Acta.* **1244,** 113–116.
34. Tazuya, K., Yamada, K. & Kumaoka, H. (1993) *Biochem. Mol. Biol. Int.* **30,** 893–899.
35. Padilla, P. A., Fuge, E. K., Crawford, M. E., Errett, A. & Werner-Washburne, M. (1998) *J. Bacteriol.* **180,** 5718–5726.
36. Sivasubramaniam, S., Vanniashingham, V. M., Tan, C. T. & Chua, N. H. (1995) *Plant Mol. Biol.* **29,** 173–178.
37. Mehdy, M. C., Sharma, Y. K., Sathasivan, K. & Bays, N. W. (1996) *Physiol. Plant.* **98,** 365–374.
38. Jamieson, D. J. (1995) *Redox Rep.* **1,** 89–95.
39. Toda, T., Shimanuki, M. & Yanagida, M. (1991) *Genes Dev.* **5,** 60–73.
40. Toone, M. W., Kuge, S., Samuels, M., Morgan, B. A., Toda, T. & Jones, N. (1998) *Genes Dev.* **12,** 1391–1397.
41. Stephen, D. W., Rivers, S. L. & Jamieson, D. J. (1995) *Mol. Microbiol.* **16,** 415–423.
42. Kuge, S. & Jones, N. (1994) *EMBO J.* **13,** 655–664.