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New perspectives: insights into oxidative stress from bacterial studies

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Abstract

Studies of the response to hydrogen peroxide as well as the characterization of small, noncoding RNAs and small proteins of less than 50 amino acids in *Escherichia coli* have given new perspectives on redox sensing, the nature of regulators and gene organization that are relevant to all organisms.

Keywords

OxyR; OxyS; MntS

1. Introduction

I first met the Professor Helmut Sies when he was a visiting scientist in the laboratory of Professor Bruce Ames at the University of California, Berkeley and I was a beginning graduate student starting my studies of the OxyR regulator of the *Salmonella enterica* and *Escherichia coli* responses to oxidative stress. Helmut was a terrific influence on the lab; cultured, enthusiastic and informed about so many topics. He not only expanded my scientific knowledge but also extended my perspective in a literal way. As many colleagues know, in addition to being a distinguished scientist, Helmut was a pilot. One memorable day he took me for a flight in a small plane giving me a unique view of the University of California, Berkeley campus (Figure 1A) as well as San Francisco and the Golden Gate Bridge (Figure 1B).

I hope the time in Berkeley also provided Helmut with a new perspective on the value of studying oxidative stress in bacteria. Around the time of Helmut's tenure in the Ames lab, Michael Christman isolated hydrogen peroxide resistant mutants of *S. enterica* and *E. coli* [1]. The mutations were found to affect the gene encoding the OxyR transcription factor. Following up these initial findings, I collaborated with Helmut and Mike on a project to examine the contribution of OxyR and its target genes to protection against DNA damage

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[2]. Using “Ames test” strains, we showed that derivatives lacking OxyR had 10–55 fold higher frequencies of spontaneous mutagenesis. The number of mutations decreased when cells were grown anaerobically and that the largest increase was observed for T:A to A:T transversions, the base substitution most frequently caused by chemical oxidants. In addition, constitutively active OxyR mutants and overexpression of some of the OxyR-regulated targets genes such as *katG* encoding catalase led to reduced mutagenesis. These observations supported the conclusion that OxyR was protecting cells against oxidative DNA damage.

After Helmut left the Ames laboratory, I continued the characterization of the OxyR regulator and its target genes even into my first years as an independent investigator at the National Institutes of Health. I am fortunate that these studies have provided new insights into both the mechanism by which hydrogen peroxide is sensed as well as the functions of genes regulated by OxyR; findings that have had implications beyond *E. coli* and bacteria. After a scientific detour to study small regulatory RNAs and small proteins, my group recently found its way back to oxidative stress through the characterization of a small protein that affects manganese homeostasis and, in turn, enzyme metallation. Below I summarize these findings and the perspectives they provide for all organisms.

2. OxyR: activation by disulfide bond formation

The transcription of the OxyR target genes is induced extremely rapidly, within 1–2 minutes after *E. coli* cells are exposed to hydrogen peroxide. Thus a major question raised by the discovery of OxyR was how the transcription factor was so quickly activated by oxidative stress. Hydrogen peroxide treatment did not result in changes to the levels or stability of OxyR suggesting the protein was directly sensing hydrogen peroxide or an intermediary metabolite [3]. Purification of OxyR allowed a detailed biochemical characterization of its properties. These assays showed that OxyR was directly sensitive to oxidation; the purified protein could be interconverted from an inactive to an active form in vitro solely by changing the concentration of the reducing agent [3]. Subsequent genetic and biochemical studies revealed this activation by oxidation was through the formation of a disulfide bond between cysteine residues Cys199 and Cys208 [4], a surprising finding given the reducing environment of the bacterial cytoplasm. Structures of the regulatory domain encompassing Cys199 and Cys208 [5] and recently full length OxyR [6] determined by X-ray crystallography have given insights into the amino acid environment that potentiates Cys199 reactivity. Oxidation of Cys199 results in a significant conformation that facilitates disulfide bond formation with Cys208 and consequently OxyR binding to its target promoters.

Since the characterization of OxyR, other regulators and enzymes with roles in protecting cells against oxidative stress have been found to be regulated by disulfide bond formation (reviewed in [7]). For example, the subcellular localization of the Yap1p transcription regulator, which, in the yeast *Saccharomyces cerevisiae*, actives expression of many of the same activities as OxyR, is controlled by disulfide bond formation [8]. In the absence of stress, Yap1p is exported from the nucleus. Upon oxidation, the formation of two disulfide bonds masks a nuclear export signal leading to nuclear retention. As more and more thiol-disulfide regulated proteins are discovered in all organisms, it is likely that OxyR will

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continue to serve as paradigm with which to compare the mechanisms and effects of reversible oxidation.

3. OxyS: regulation by small RNAs

Studies of the OxyR target genes have also led to discoveries of general relevance. Among the first enzymes shown to be elevated in the constitutively active *oxyR* mutant strains were the catalase hydroperoxidase I, which detoxifies hydrogen peroxide, and a novel enzyme, initially named an alkyl hydroperoxide reductase, capable of reducing a range of peroxides [1, 9]. This latter enzyme was subsequently found in organisms from all kingdoms with many species having multiple paralogs. Extensive characterization of this family of enzymes, renamed NADH peroxidases or peroxiredoxins, has revealed a broad range of biological roles from protection against many different oxidants and host defenses against infection to suppression of tumor formation and redox signaling (reviewed in [10]). Other identified target genes link the OxyR-regulated response in *E. coli* to the thiol-disulfide status of the cell [4, 11] as well as to iron homeostasis [12], iron sulfur cluster assembly [13] and most recently heme synthesis [14]. In general, these studies illustrate how information about the genes induced by an environmental stress can give insights into what activities are critical to protect against the insult.

One other surprising target of *E. coli* OxyR was a gene encoding a 109 nucleotide RNA [15]. Characterization of this noncoding RNA, named OxyS, whose expression was very strongly induced by hydrogen peroxide, demonstrated that a small RNA (sRNA) can act as a post-transcriptional regulator. OxyS has become a model for a large family of bacterial sRNAs of 50–300 nucleotides, which act similarly to eukaryotic microRNAs to modulate the translation and stability of mRNAs through base pairing. Studies of *E. coli* OxyS showed that sRNAs can repress translation by base pairing at or near the ribosome binding site [16]. This work also revealed that many base pairing sRNAs require the RNA chaperone Hfq to facilitate the sRNA-mRNA interaction [17]. In the years since the discovery of OxyS, regulatory sRNAs have been found to be associated with many different stress responses in bacteria including other environmental conditions that impact oxidative stress such as limited iron availability and transitions between aerobic and anaerobic growth (reviewed in [18]).

4. MntS: roles of manganese and small proteins

Although my group eventually put aside our investigations of OxyR to focus on studies of regulatory sRNAs as well as small proteins, we recently re-encountered the oxidative stress response through our characterization of a small *E. coli* protein that impacts manganese homeostasis. We initiated studies of small proteins, which we have arbitrarily defined as proteins of 50 amino acids or fewer, when we realized that a subset of transcripts annotated as sRNAs encode small proteins. Small proteins are ignored by most standard genome annotation and are missed by many biochemical approaches. This was the case for an sRNA initially designated RybA. Alignment of closely related homologs from enteric bacteria revealed conservation indicative of protein coding, and tagging of the open reading frame confirmed that the 42-amino acid protein is indeed synthesized [19]. Clues to the

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physiological role for this small protein, now denoted MntS, came from the realization that MntS was only expressed under conditions of low manganese. Follow up biochemical and physiological assays showed that overexpression of MntS causes the same sensitivity to high concentrations of manganese as a strain lacking the manganese exporter MntP suggesting that MntS inhibits the activity of this exporter [20]. The studies also showed that MntS is required for the activation of manganese-dependent enzymes such as superoxide dismutase under conditions when manganese is limiting. These observations and other findings [21] support the conclusion that the expression of manganese-dependent isozymes or the substitution of manganese for iron in other enzymes is advantageous when iron levels are low or cells are exposed to oxidative stress. This protective role for manganese was presaged by the report that OxyR activates the expression of the manganese importer MntH [22] and is bolstered by findings of antioxidant activity associated with manganese in a wide range of organisms (reviewed in [23, 24]).

The characterization of MntS as well as other small bacterial proteins, the majority of which are hydrophobic, has led to the hypothesis that a major role for this ignored class of proteins is modulating the activities of larger membrane proteins (reviewed in [25]). As increasing numbers of “noncoding” RNAs in eukaryotic cells are also being shown to encode small proteins, some of which also have been found to interact with membrane proteins [26], it will be interesting to see how broad this hypothesis extends.

5. Perspectives

When I began my thesis work in the Ames lab with the limited vision of a graduate student, I wondered how much could still be learned from studies of the *E. coli* response to oxidative stress. My perspective has expanded through interactions with Bruce Ames, Helmut Sies and other visionary scientists. Nevertheless, I remain amazed, thirty years later, by the many new concepts and insights relevant to all organisms that continue to be derived from bacterial models.

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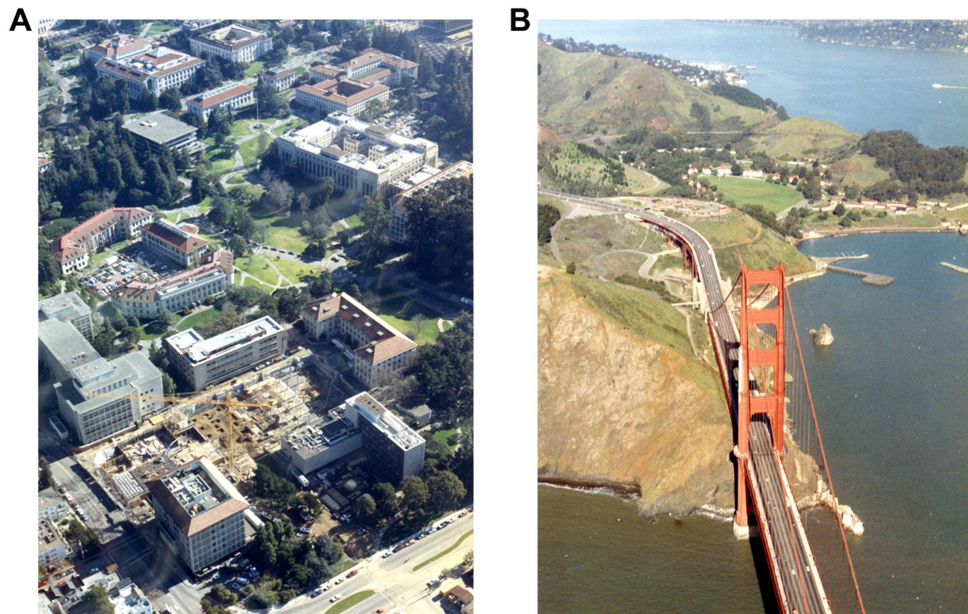

Fig. 1.
Photographs taken above the University of California, Berkeley campus (A) and the Golden Gate Bridge leading in to San Francisco on a flight piloted by Professor Sies. The Biochemistry building in which we carried out collaborative studies on the role of the OxyR transcription factor in protecting against oxidative DNA damage is in the bottom left corner of (A).