The Role of Antioxidant Gene Regulation During Bacterial Oxidative Stress Response

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Lily Vu

Dr. Laura Laranjo
Faculty Advisor
Department of Biology

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Abstract

Reactive oxygen species (ROS) are produced naturally in a cell and when the cell is in homeostasis, there is an equilibrium of ROS and antioxidants (enzymes that combat ROS). Stresses can occur such as “Oxidative Stress” which results in an influx of ROS compared to antioxidants in the cell and could lead to damage of DNA, lipids and proteins and also cell death. To return the cell’s homeostatic state, bacteria have evolved to contain defense mechanisms to eradicate ROS. This paper will focus on the regulation of antioxidants that are produced to eliminate high concentrations of ROS when the cell is under oxidative stress. These antioxidants are encompassed in a mechanism called a “Regulon,” which controls the production of these enzymes. The regulons within this paper include the OxyR, Rpos, SoxRS, PerR, and OhrR regulons. Each regulon plays a critical role in the survival of bacterial species when experiencing oxidative stress. There are many mechanisms such as regulons that help defend the bacterial cell from damage or death from oxidative stress.

Table of Contents

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List of Figures

Introduction

Bacteria have been able to survive for millions and millions of years because of their ability to adapt and evolve to different stresses such as oxidative stress. Oxidative stress is the disruption of homeostasis of ROS (reactive oxygen species) populations in a cell [1]. The ideal state of homeostasis would have an equal balance of ROS and antioxidants. When bacteria experience an imbalance of ROS and antioxidants, their cells stop functioning properly and sometimes it can lead to apoptosis (cell death). Oxidative stress is harmful to bacterial cells and to resist damage and death, the cells must combat ROS.

ROS are found naturally in cell environments. A low-to-moderate amount of ROS is beneficial because ROS have a key role in cell signaling and regulating expression of antioxidant genes [2]. Having a higher ROS population than antioxidant population leads to lipid, protein, and DNA damage. When ROS levels increase, it creates a cytotoxic environment, damaging organs and tissues [3]. High ROS populations are harmful to DNA because it can alter enzymatic functions and disrupt gene expression. DNA strands can be broken during oxidative stress. Lipid membranes and peptide chains (of proteins) breakage is also a result of oxidative stress. Bacterial cells contain mechanisms to reduce damage and eliminate excess ROS when experiencing oxidative stress.

Bacteria have developed ways to defend itself and to repair the damage resulting from oxidative stress through regulation of antioxidant proteins. Oxidative stress in bacteria causes a cascade of events to occur including activation of regulons which induce the production of antioxidants to combat ROS. The ROS will activate sensor

proteins that will initiate transcription of antioxidant genes to produce proteins to reduce the amount of ROS in the cell. The cascade involves gene regulation of the antioxidants.

As published in sources by Seshayee [4], Watson [5], and Ralston [6] bacterial gene regulation comprises of elements evolved in the production od proteins that are necessary for bacterial survival. Regulation of antioxidants is a key component to eliminating excess ROS.

Results

The defense mechanisms for combatting ROS consist of antioxidants in two categories: non-enzymatic and enzymatic antioxidants [7]. Non-enzymatic antioxidants function to block free radical chain reactions. The non-enzymatic defenses forage for free radicals and include Vitamins C and E and glutathione [8]. Vitamin C (ascorbic acid) scavenges for free radicals while Vitamin E (alpha tocopherol) defends the membrane from damage caused by oxidative stress. Glutathione detoxifies hydrogen peroxide into water and oxygen and converts Vitamins C and E into their active forms. The non-enzymatic antioxidants are crucial as the first line of defense in combating ROS when the cell is under oxidative stress.

Enzymatic defenses have helped bacteria survive for as long as they have and they continue to evolve with new mechanisms for survival. The enzymatic antioxidants work to convert ROS into water and oxygen. The enzymatic defenses found in bacteria can be found in regulons and include dismutases, catalases, peroxidases, reductases, etc. as seen in Figure 1. [9]. These enzymatic antioxidants and more are regulated by systems called regulons. Regulons control the production of antioxidant enzymes that are critical

for survival from oxidative stress. Enzymatic defenses such as antioxidants under regulons are necessary in helping bacteria combat oxidative stress.

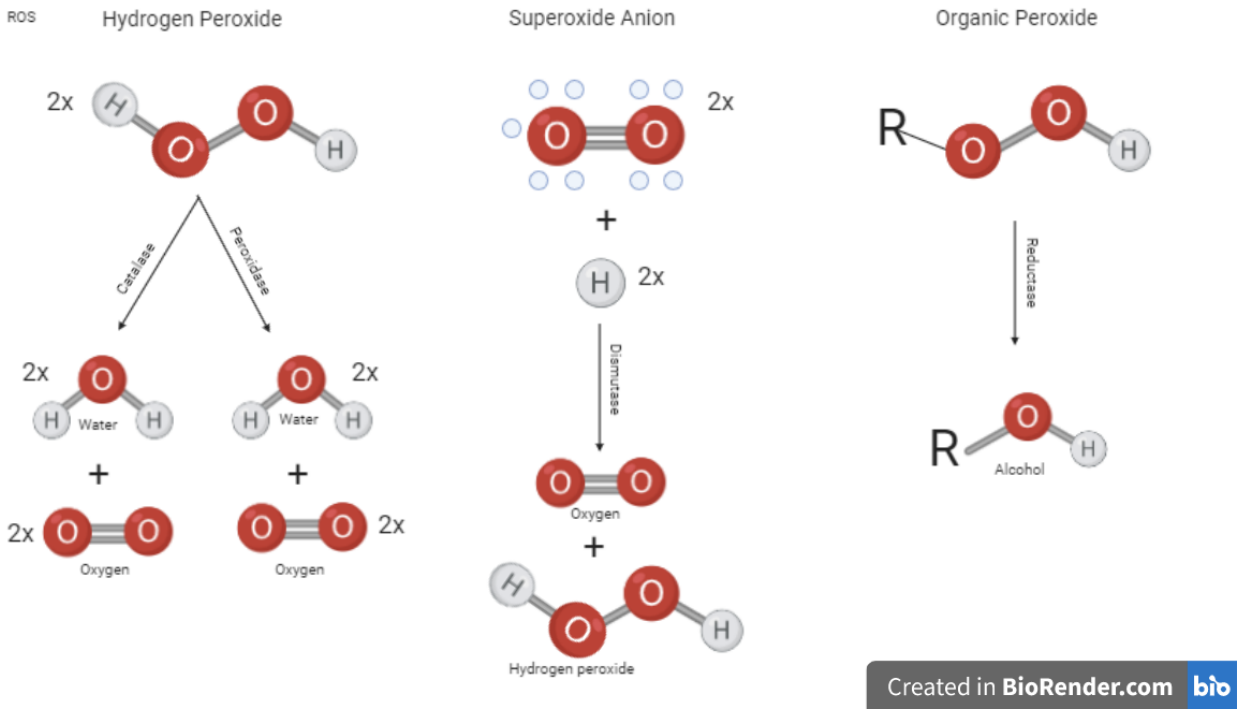

Figure 1. Enzymatic Defenses Against ROS. Catalases function to convert hydrogen peroxides into their corresponding components water and oxygen. Peroxidases will also work to break down hydrogen peroxide into water and oxygen. Dismutases will work to reduce the amount of superoxide anions, in a cell under oxidative stress, by converting it into oxygen and hydrogen peroxide. Reductases are important as they sense ROS imbalance and reduce molecules into their corresponding non-harmful forms under oxidative stress [10].

Regulons regulate the expression of antioxidants, in bacterial cells, that can reduce the amount of ROS when under oxidative stress. Regulons can differ between gram-positive

bacteria and gram-negative bacteria. Gram-negative bacteria contain thin cell walls compared to the thick cell walls seen in gram-positive [11]. Gram- negative bacteria comprise of a rigid, impenetrable outer membrane which make it difficult for any molecules to exit or enter the cell. As ROS levels increase and they cannot escape from within, Gram-negative bacteria must obtain more mechanisms to covert these larger molecules into their smaller cohorts to exit through the membrane [12]. The toxins produced by both differ as well, where gram-positive produce exotoxins and gram-negative produce endotoxins. There are varied levels of sensitivity to ROS seen in gram-positive than in gram-negative bacteria [13]. Although every regulon isn't present in all types of bacteria, a common one seen in many gram-negative and some gram positive is the OxyR regulon.

An influx of hydrogen peroxide (H_20_2) in the cell will initiate a response from the OxyR regulon. The OxyR regulon, a member of the LysR family, is the most prominently known regulon and is mostly found in gram-negative bacteria as well as some gram-positive bacteria. The OxyR regulon is highly conserved and can be seen in a wide range of bacteria from gammaproteobacterial to actinobacteria [14]. It controls over 30 genes including antioxidants to reduce the concentration of hydrogen peroxide as seen in *E.coli and S. enterica*. An increase of H_20_2 will convert OxyR to its oxidized form initiating transcription of its genes as seen in Figure 2. When activated, OxyR will produce antioxidants that function to reduce levels of hydrogen peroxide in the cell due to oxidative stress.

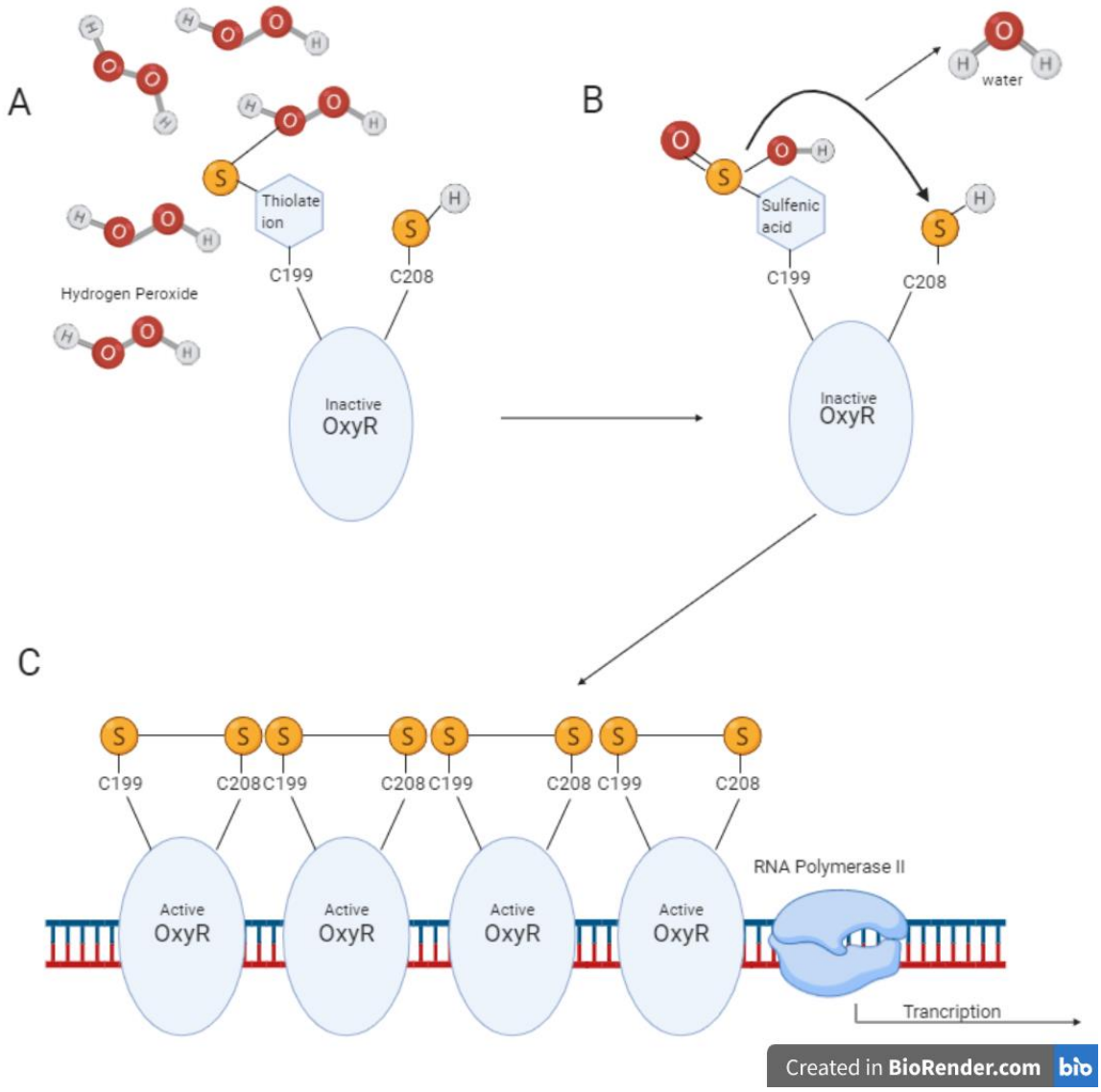

Figure 2. Activation of OxyR Regulon. To activate OxyR, a disulfide bond forms between cysteine residues C199 and C208 in the C-terminal domain [15]. The thiolate ion on C199 will react with H_20_2 to form sulfenic acid which then reacts with C208 to form the disulfide bond which in turn alters the binding of OxyR to DNA and allows for RNA polymerase to bind and start transcription [16].

When experiencing high levels of hydrogen peroxide during oxidative stress, OxyR will activate or repress its antioxidant genes. OxyR is both a repressor and activator of its genes. In its repressive form, OxyR will inactivate the generation of disulfide bonds. In its activator state, it will recruit RNA polymerase and stimulate transcription of genes. Genes such as OxyS (regulatory RNA), ahpCF (alkyl hydroperoxide reductase), katG (HPI catalase), dps, fur, and yaaA are antioxidants that are upregulated by the OxyR regulon [17]. OxyR represses its expression of itself (peroxide sensing protein), in its reduced and oxidized forms, in bacteria such as *E.coli*. KatG and ahpCF will function as scavengers of hydrogen peroxide free radicals. Dps, fur, and yaaA will minimize the level of free iron present in the cell to reduce Fenton reactions when under oxidative stress. The genes controlled under the OxyR regulon can be seen as homologs in different bacteria. The gene for catalase, katE (hydroperoxidase II), is seen as katA and katY in *Yersinia sp.*, and hktE in *Haemophilus sp*. OxyR will regulate the production of its antioxidants in response to the disruption of hydrogen peroxide levels in the cell due to oxidative stress.

Another regulon that responds to an increase of hydrogen peroxide is the Rpos regulon. Rpos stands for RNA polymerase of stationary phase and is an alternative sigma factor that regulates antioxidants, in bacteria such as *E.coli*, when under oxidative stress [18]. It belongs to the sigma 70 factor family. When induced, Rpos will recruit RNA polymerase to its promoters to initiate transcription of its genes. Genes such as osmY, katE, dps, xthA (exonuclease III), sodC (CuZnSOD), and otsA fall within the control of

the Rpos regulon. Gammobacteria such as *Vibrio vulnificus* contain the conserved catalase gene, katE in E.coli, as KatG and and P. putida contain the catalase gene as catB. Rpos will work to eliminate excess hydrogen peroxide and other reactive oxygen species to return the cell to its homeostatic condition. The Rpos regulon will regulate production of antioxidants to respond to high levels of hydrogen peroxide.

Thiols are critical organic compounds that make up the majority of antioxidants and maintenance of them are key for bacterial survival from oxidative stress [19]. Thiol maintenance consists of glutathione reductase, thioredoxin reductase, and glutaredoxin [20]. *S. pneumoniae* contain a thiol peroxidase, TpxD, which is induced by oxidative stress and is crucial in bacterial survival as it detoxifies hydrogen peroxide during oxidative stress. Glutathione reductase helps to keep Glutathione in its reduced form to respond to an increase of ROS when the cell is under oxidative stress [21]. Thioredoxin reductase is a crucial enzyme present in *Lactobacillus plantarum* to respond to oxidative stress [22]. Glutaredoxin is important in that it will deactivate OxyR when H_20_2 levels are reduced to a normal range. Thiol maintenance is vital for the survival of bacteria in oxidative stress environments.

The response to an increase of superoxide radical anions due to oxidative stress is induced by the SoxRS regulon [23]. The SoxRS regulon functions to reduce superoxide overaccumulation in any gammaproteobacteria such as *E.coli, Salmonella* and *Klebsiella*. This regulon positively regulates over 30 proteins [24]. Within the SoxRS regulon is the SoxR, sensor protein related to the AraC family, and SoxS, transcriptional activator (similar to MerR protein). Nonenteric bacteria do not contain the SoxS gene, therefore the SoxR gene must regulate and detoxify ROS independently [25]. Activation of

SoxRS is seen in a two-step process. SoxR detects an imbalance of superoxide radical anions and will activate when the iron-sulfur binding domain of a superoxide anion is oxidized [26]. SoxR will magnify transcription of SoxS and together they activate the SoxRS regulon. The SoxRS regulon will be induced by the activation of SoxR which activates SoxS and the antioxidants will be produced to scavenge for superoxide radical anions. An activated SoxRS regulon will result in the decrease of superoxide radical anions as they are eradicated by antioxidants.

When the SoxRS regulon is activated it will regulate the production of its genes. Antioxidants within the SoxRS regulon are superoxide dismutases, catalases, glucose-6-phosphate dehydrogenase and NADH dehydrogenase. Manganese superoxide dismutase (SOD) works with iron SOD to reduce the concentration of free metal cations and lessen the damage from hydrogen peroxides. Hydroperoxidase (HPI and HPII) are catalases that convert hydrogen peroxide into water and oxygen. Glucose-6-phosphate dehydrogenase prevents the formation of hydroxyl radicals and NADH dehydrogenase helps to decrease the concentration of organic hydroperoxide. Activated SoxRS will additionally elevate expression of the fur repressor to decrease iron uptake and reduce the formation of hydroxyl radicals. The antioxidants under the SoxRS regulon function to reduce the amount of superoxides present in a cell when experiencing oxidative stress.

The PerR regulon is another regulon that responds to increased levels of hydrogen peroxide [27]. It can be compared to the OxyR regulon as it regulates similar genes. It is a substitute or co-regulator with OxyR and found mostly in gram-positive bacteria. Unlike OxyR, PerR acts more as a repressor than an activator. PerR, dimeric zinc protein, will activate the PerR regulon when it loses its iron cofactor to detach itself from DNA

and derepress the transcription of antioxidants [28]. It contains metal binding sites in order to bind to DNA and will repress transcription if bound to Mn (II) or Fe (II). When bound with a metal, PerR is able to bind to a promoter either directly on it, upstream or downstream of it. It has been observed that high levels of Zn (II) will interfere with the binding of Fe (II) and Mn (II) to initiate transcription [29]. The PerR regulon controls similar genes under the OxyR regulon. Within the PerR regulon are the KatA, ahpC, sodB and mrgA genes. The first three genes are repressed by PerR under normal conditions, but under oxidative stress, they will be de-repressed to extinguish hydrogen peroxide [30]. MrgA prevents apoptosis of cells when under oxidative stress. PmtA is a monomeric protein under the PerR regulon and has a key role in peroxide resistance. The PerR regulon has multiple similarities with the OxyR regulon and differs slightly based on its requirement of using metals to co-regulate it. The PerR regulon functions to eliminate hydrogen peroxide when the cell is under oxidative stress.

The OhrR regulon is another regulon of interest in responding to oxidative stress in bacteria and belongs to the MarR family. It can be found in both gram-positive and gram-negative bacteria. OhrR co-resides with OxyR and PerR and its function is to respond to organic peroxides. Ohr is an organic peroxide sensor and is mostly seen as a repressor in transcription. It is similar to the OspR regulon seen in *Pseudomonas aeruginosa* and the MgrA Regulon in *S. aureus*. OhrR is activated when one of the two highly conserved cysteine residues is oxidized to create a sulfenic acid intermediate that attacks the second cysteine to form a disulfide bond. Once an organic peroxide is detected, OhrR de-represses the ohrA gene to produce the ohr protein that scavenges for

organic peroxides. OhrR functions to identify and destroy organic peroxides during oxidative stress.

The OhrR regulon contains two subfamilies that differ in their peroxide sensing mechanism. In bacteria such as *B. subtilis*, OhrR's ability to bind to DNA is controlled by cysteine oxidation. Bacteria such as *Xanthomonas campestris* contain the second subfamily of OhrR which functions using the reversible formation of the disulfide bridge of the two conserved cysteines to act as a repressor. The strength of OhrR can vary between bacterial species. One of the two conserved cysteines within the OhrR regulon, Cys_{11}, is important in determining the strength of the regulon to sensing organic peroxides [31]. Between organisms, OhrR can contain different chosen organic peroxide inducers. In *B.subtilis* and *Xanthomonas campestris*, OhrR is strong as it is more sensitive to complex peroxides. Whereas in *Agrobacterium* tumedaciens, OhrR is weaker because it is less sensitive to peroxides. OhrR can respond to in vitro and intracellular peroxide stress in bacteria such as *Mycobacterium smegmatis*. The OhrR regulon can differ in strength based on bacterial species, but all OhrR will remove surplus organic peroxides when the bacterial cell is under oxidative stress.

Conclusion

Bacteria have evolved to contain mechanisms to respond to and survive under different intracellular and extracellular stress conditions. Oxidative stress is a result of an imbalance of reactive oxygen species (ROS) and antioxidants present in the cell. Defenses against ROS include enzymatic and non-enzymatic molecules/systems. Regulons such as OxyR, SoxRS, OhrR, etc. are examples of enzymatic mechanisms and they regulate production of antioxidants to combat different ROS in a cell when under

oxidative stress. An increase of ROS will initiate responses from the regulons to produce antioxidants to help scavenge and decrease the levels of ROS present in the cell. Along with the enzymatic defenses, there are non-enzymatic defenses (Vitamins C and E and Glutathione) that help defend the cell from damage from excess ROS during oxidative stress. Thiols are important to responding to oxidative stress as they sense and help reduce the levels of ROS. These mechanisms have improved the survival of bacterial species from oxidative stress. Oxidative stress can create a toxic and damaging environment for bacterial cells and to deal with that, bacteria have advanced to comprise of defenses to help their survival from oxidative stress and other stresses.

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