Minireview

Oxidative stress responses – what have genome-scale studies taught us?
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Abstract

Oxidative stress arises from an imbalance between generation and elimination of reactive oxygen species, often leading to cell death. Genomic tools are expanding our understanding of the antioxidant defenses aerobes have evolved and the recently discovered role(s) of reactive oxygen species in signaling.

Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^\bullet$) are produced in all aerobic organisms within the cell and are normally in balance with antioxidant molecules. Oxidative stress occurs when this critical balance is disrupted because of depletion of antioxidants or excess accumulation of ROS (Figure 1).

ROS were originally considered to be exclusively detrimental to cells, but it is now recognized that redox regulation involving ROS is key to the modulation of critical cellular functions (Figure 2) [1]. Regardless of how or where they are generated, an increase in intracellular oxidants results in two very important effects: damage to various cell components and activation of specific signaling pathways, both of which influence numerous cellular processes leading to proper cell functions or to cell death [1]. Mounting evidence links oxidants and oxidative stress to a variety of human diseases and aging [2], as well as to senescence, impaired photosynthesis, and necrosis in plants [1]. On the other hand, there are clear examples of how ROS are put to constructive uses, the most powerful being the crucial role that $O_2^{\bullet-}$ plays against invading microbes, by serving as a broad-spectrum antibiotic [3]. Plants also mount a broad-range response to invading pathogens by a rapid and transient production of ROS via the 'oxidative burst' [4]. This article considers a number of recent genome-wide analyses of the response to ROS in bacteria, yeasts and *Arabidopsis* in the light of previous knowledge about oxidative stress responses.

Oxidative stress and gene expression

When oxidative stress occurs, cells function to counteract the resulting oxidant effects and to restore the redox balance. All organisms have adaptive responses to oxidative stress, with antioxidant defense enzymes being induced by changes in the levels of H_2O_2 or $O_2^{\bullet-}$, leading to the activation or silencing of genes encoding defensive enzymes, transcription factors and structural proteins [5]. ROS have also been proposed to function as second messengers independent of oxidative stress and to signal such cellular fates as cell proliferation, necrosis, and apoptosis. Although various observations have led to the suggestion that cells have the means to sense ROS and to induce specific responses [1], the underlying mechanisms are still not fully understood. Regulators of oxidative stress responses are currently best characterized in bacteria [6], but progress is now also being made towards understanding such regulators in higher eukaryotes [5,7].

The transcriptional network that responds to ROS in eukaryotes is only now being unraveled but the prokaryotic system is quite well understood. Seventeen years ago, it was shown by two-dimensional gel electrophoresis that the expression of approximately 30 proteins was induced in bacteria by H_2O_2. Of these 30 proteins, 12 were maximally induced within 10 minutes and the other 18 between 10 and 30 minutes [8]. Subsequent work led to the discovery of the OxyR regulatory protein, which was shown to regulate the expression of 9 of the 12 rapidly induced proteins. The tetrameric OxyR protein is a member of the LysR family of
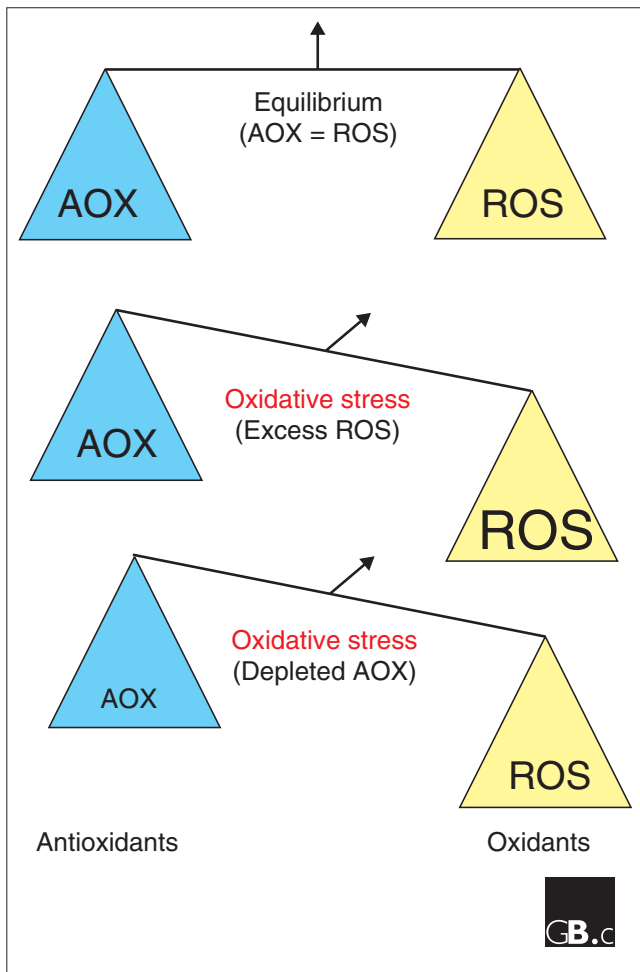
Figure 1
Oxidative stress results from imbalance between the levels of reactive oxygen species (ROS) and antioxidants (AOX). Under normal circumstances, cells are able to balance the production of oxidants and antioxidants (such as catalase and superoxide dismutase), resulting in redox equilibrium. Oxidative stress occurs when cells are subjected to excess levels of ROS, or as a result of depletion in antioxidant defenses.

been found to play a role in regulating eukaryotic antioxidant genes. In yeast, transcriptional regulators of antioxidant genes include ACE1, MAC1, YAP1, YAP2, HAP1, and HAP2/3/4 [11]. In higher eukaryotes, oxidative stress responses are more complex and are modulated by several different regulators. In mammalian systems, nuclear factor κB (NFκB) and activator protein-1 (AP-1) are involved in regulating the oxidative stress response. The antioxidant responsive element (ARE) is present in the promoter region of genes encoding mammalian glutathione *S*-transferase (GST), metallothionein-I and manganese superoxide dismutase (MnSOD), and is responsible for the induction of these genes in response to oxidants [12]. The promoters of antioxidant genes in higher plants also contain the ARE and the NFκB and AP-1 binding sites [1]. The role of these factors is not unique to activation of antioxidant genes, however: NFκB, in particular, is known to play central roles in regulating cellular responses to stresses other than oxidants, as well as regulating normal growth and metabolism.

There is substantial evidence to suggest that a variety of biotic and abiotic stresses induce H_2O_2, which serves as a common factor in regulating various signaling pathways [1,13]. Similar stresses also activate mitogen-activated protein (MAP) kinases, with kinetics that either precede or parallel H_2O_2 production, suggesting that MAP kinases may be among the many converging points in the defense-signaling network [14]. In addition, exogenous application of several plant hormones and toxins has been shown to induce synthesis of $O_2^{\bullet-}$ and H_2O_2, leading to differential induction of some antioxidant genes and isozymes [1,7,12,15]. Thus, from a utilitarian viewpoint, the identification of all genes and proteins regulated by H_2O_2 is an important step toward treatments that might confer tolerance to multiple, but interrelated, stresses. In addition to induction or repression of antioxidant defense genes, ROS are known to similarly affect expression of a variety of other genes involved in different signaling pathways in microbes [6], yeast [16], plants [17], and animals [2].

Gene expression on a genomic scale
Efforts to identify ROS-responsive genes on a global scale were limited until the advent of microarray-based gene-expression analysis [18]: DNA microarrays are now being used to comprehensively examine gene expression networks during oxidative stress. Reports on the stress responses of *Escherichia coli* [19], yeast [16,20], and higher plants [21] have now provided significant progress in surveying gene expression in response to H_2O_2.

The transcriptional profile of *E. coli* cells exposed to H_2O_2 was examined with a DNA microarray composed of 4,169 *E. coli* open reading frames [19]. Gene expression was measured in isogenic wild-type and *oxyR*-deletion mutants (Δ*oxyR*) to confirm that the H_2O_2-response regulator OxyR

transcription activators and exists in two forms, reduced and oxidized; the latter is the only form able to activate transcription. Further studies led to the identification of a number of OxyR-activated genes [9]. Similarly, the superoxide response or SoxRS regulatory proteins were found to regulate expression of $O_2^{\bullet-}$-responsive proteins in bacteria [10]. Regulation of the *soxRS* regulon occurs by conversion of SoxR protein to an active form, which enhances *soxS* transcription. The enhanced levels of SoxS, in turn, activate expression of the regulon [10]. In addition to SoxR and OxyR, several other transcriptional regulators modulate the expression of antioxidant genes in bacteria, indicative of the complexity and connectivity of overlapping regulatory networks [6].

There are no apparent homologs of OxyR, SoxR, or SoxS in eukaryotes, but a number of other transcription factors have
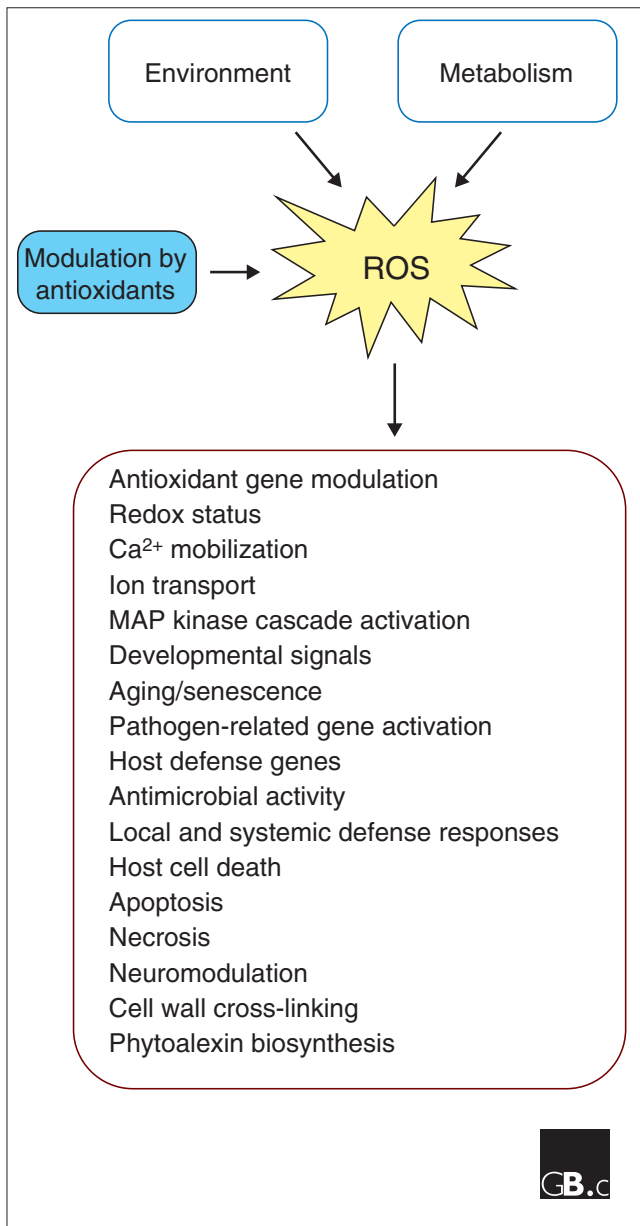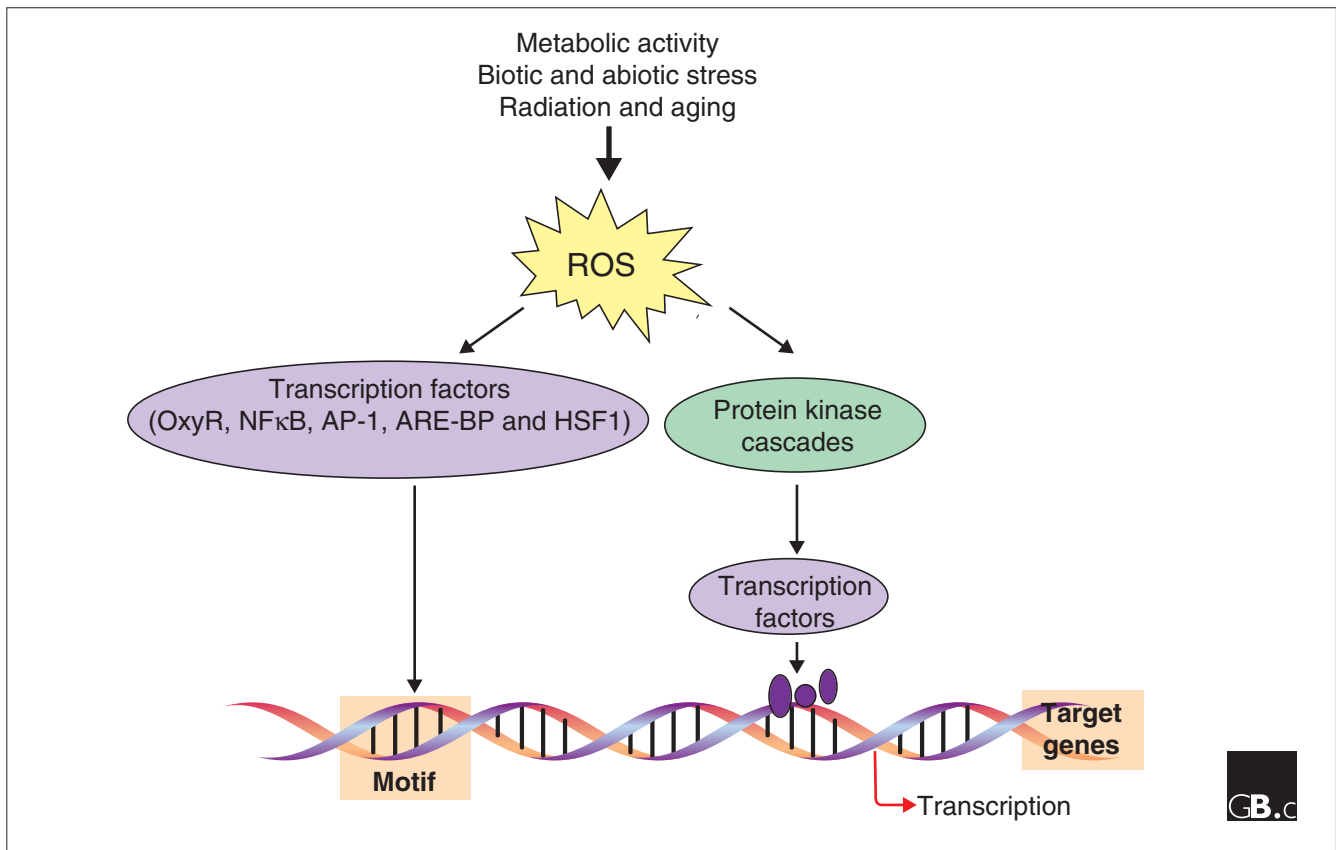
Figure 2
Some of the pleiotropic effects of ROS. They have many roles as signaling molecules in addition to their scavenging protective functions in biological systems.

activates most of the H_2O_2-inducible genes. There was a very rapid and strong induction of a set of OxyR-regulated genes in the wild-type but not in the $\Delta oxyR$ strain, providing an internal validation of the experiment and confirmation of the induction of the oxidative stress genes identified 17 years ago by other means [8]. In addition, several new H_2O_2-inducible genes were identified: some are members of the OxyR regulon and some are induced by an OxyR-independent mechanism. These findings indicate that other H_2O_2 sensors and regulators are present in *E. coli* [19]. Several

genes that are known to be repressed by OxyR were found to be significantly expressed in the $\Delta oxyR$ mutant. Overall, the mRNA of 140 genes in the wild-type and 167 genes in the $\Delta oxyR$ strain were significantly induced after H_2O_2 treatment. It was also found that the superoxide response transcription factor gene *soxS* was induced by H_2O_2, indicating an overlap with other regulatory pathways. Also highly induced by H_2O_2 in both wild-type and $\Delta oxyR$ cells were two genes known to be members of the SoxRS regulon, *Fpr* and *sodA* (encoding NADH-ferredoxin oxidoreductase and manganese-superoxide dismutase, respectively). The microarray data revealed an overlap between the oxidative stress and heat-shock and 'SOS' DNA-damage responses [19]. Thus, the results from *E. coli* microarrays clearly indicate that the activities of transcription factors in addition to OxyR and SoxRS are likely to be modulated by oxidative stress.

In addition to bacteria, the transcriptional profile in response to oxidative stress has also been characterized in eukaryotes. In one broad-ranging study, the expression profile was examined in *Saccharomyces cerevisiae* cells exposed to H_2O_2, in addition to other stresses, and the global set of genes induced or repressed by each environmental signal was identified [16,20]. The results indicated that about two-thirds of the genome is involved in the response to environmental changes and the response to oxidative stress involves about one-third of the yeast genome, with the maximal effects on gene expression occurring slightly later relative to other stresses examined during similar time courses. Most of the transcriptome returns to pre-stress levels within 2 hours of exposure to H_2O_2 [20]. Genes that are repressed for approximately 1 hour after exposure to H_2O_2 are only transiently repressed in other stress time courses. Thus, genes encoding the translation apparatus and its regulators are remarkably coordinated in the responses to each environmental change, although the dynamics of each response are different. The expression programs following H_2O_2 or $O_2^{\bullet-}$ treatment were essentially identical, despite the fact that different ROS are involved. There was strong induction of genes known to be involved in detoxification of both H_2O_2 and $O_2^{\bullet-}$, such as catalase, superoxide dismutase, and glutathione peroxidase, as well as genes involved in oxidative and reductive reactions (for example, thioredoxin, glutathione reductase, and glutaredoxin). The genes most strongly induced in response to H_2O_2 and $O_2^{\bullet-}$ were dependent on the transcription factor Yap1 for their induction. Genes that are moderately induced by ROS and other signals are regulated by different transcription factors, depending on the conditions, and their response may be governed by different upstream signaling pathways [20].

Recently, it has also been demonstrated that H_2O_2 activates the Sty1 (stress-activated MAP kinase) pathway in *Schizosaccharomyces pombe* in a dose-dependent manner, via two sensing mechanisms [22]. At low H_2O_2 levels, Sty1 is

Figure 3
The major signaling pathways activated in response to oxidative stress. ROS can originate from metabolic activity or from external environmental signals and are modulated by antioxidants to nontoxic levels at which point they serve as signaling molecules. ROS can activate gene transcription in two ways: either via transcription factors (such as, NFκB, AP-1 and ARE-binding proteins, ARE-BP) that can interact directly with specific DNA motifs, including ARE, on promoters of target genes; or via activation of MAP kinase cascades, which in turn activate transcription factors that trigger target gene transcription. The degree to which a given pathway is activated is dependent on the nature and duration of the stress, as well as on cell type and developmental stage.

regulated by a two-component signaling pathway that feeds into either of the two - Wak1 or Win1 - stress-activated MAP kinase kinase kinases upstream of Sty1. In contrast, at high H_2O_2 levels, Sty1 activation is controlled mainly by an independent two-component mechanism, requiring the function of both Wak1 and Win1. In addition, the individual bZip transcription factors Pap1 and Atf1 were found to function within a limited range of H_2O_2 concentrations: Pap1 activates target genes at low H_2O_2 concentration, whereas Atf1 controls transcriptional responses to high H_2O_2, with some minor overlap. Some apparent cross-talk among Sty1, Atf1, and Pap1 has been detected [23]. Thus, *S. pombe* deploys a combination of stress-responsive regulatory proteins to gauge and trigger the appropriate transcriptional response to increasing H_2O_2 concentrations [22]. This yeast mounts two separate responses to oxidative stress: an adaptive response to low-level H_2O_2 exposure that protects it from subsequent exposures to higher H_2O_2 levels, and an acute response that allows the cell to survive a sudden, potentially lethal dose of H_2O_2.

The oxidative-stress response has also recently been characterized in an organism more complex than yeast and bacteria. A recent large-scale cDNA microarray analysis of the *Arabidopsis* transcriptome during oxidative stress identified 175 non-redundant expressed sequence tags (ESTs) from a sample of 11,000 that are regulated by H_2O_2. Of these, 62 are repressed and 113 are induced; and RNA blots showed that some of the H_2O_2-regulated genes are also modulated by other signals known to involve oxidative stress [21]. Furthermore, a substantial number of these genes have predicted functions in defense responses, cell signaling, transcription, and cell rescue (from environmental insults and developmental arrest), underscoring the pleiotropic effects of H_2O_2 in the response of plants to stress. Overall, the microarray used was estimated to represent only about 30% of the *Arabidopsis* genome, depending on redundancy, and 1% to 2% of the genes represented in the array are affected by H_2O_2-imposed oxidative stress [21], which is comparable to the situation in yeast [20]. Of the 175 genes identified as H_2O_2-responsive, most have no obvious direct role in oxidative stress but may

be linked to oxidative stress indirectly, as a consequence of other biotic and abiotic stresses, explaining their sensitivity to H_2O_2. Among the genes induced by H_2O_2 were genes encoding transcription factors, suggesting that they may mediate downstream H_2O_2 responses consistent with genomic studies in other species. Also, expression of the MAP kinases in *Arabidopsis* is induced by oxidative stress, as in other organisms, which in turn can mediate the induction of oxidative stress-responsive genes [24].

Towards an integrated understanding of stress responses

During the past ten years, the traditional view of ROS as mere indiscriminate reactive byproducts of cellular metabolism has undergone a metamorphosis. This change is primarily as a result of the discovery that ROS, and particularly H_2O_2, may act as signal-transducing molecules, and that activation of intracellular transcription factors such as OxyR, SoxRS, NFκB, and AP-1 occur via interaction with ROS, leading to gene transcription (Figure 3). More recently, genome sequencing and expression profiling using DNA or oligonucleotide microarrays, and related technologies, have been used effectively in the study of global gene-expression patterns in response to different growth and environmental conditions to which organisms are exposed. Subsequent hierarchical clustering methods allow for the allocation of genes, co-regulated temporally or in response to a given signal, into specific expression groups, or regulons [19,25]. The numbers of genes that can be detected by these methods in response to H_2O_2 or any given environmental or developmental signal far exceed the limited number that could have been detected only a few years ago.

Given that transcription of genes into mRNA is governed by transcription factors, which bind to *cis*-regulatory regions of the DNA in the vicinity of their target genes, the question arises as to whether large co-regulated groups of genes share *cis*-regulatory elements that bind to common transcription factors. The data available with respect to oxidative stress seem to suggest this is indeed the case. *Cis*-acting elements within the promoters of ROS-activated genes are being defined as well as their cognate *trans*-acting factors [1]. A comparative analysis of promoter sequences of genes with similar expression profiles should provide a basis for unraveling common regulatory sequences and overlapping gene-expression networks modulating ROS-responsive genes. The antioxidant enzymes catalase and superoxide dismutase play key roles in modulating the levels of endogenous H_2O_2 and $O_2^{\bullet-}$, which at specific concentrations act, in turn, to modulate the expression of other ROS-responsive genes.

Despite some limitations, mentioned in the papers cited [16, 19-21], it has become clear through the use of microarrays that there are far more genes responding to ROS than previously thought. We currently have a large and growing number of sequenced genomes and emerging technologies presenting us with enormous opportunities to advance biological science and our knowledge of how genomes perceive signals to respond to variable environments. Knowing the sequences of tens of thousands of ROS-responsive genes, however, only reminds us that we still do not know the many proteins they encode, nor the biochemical or biological function of the great majority of such proteins. How such proteins interact with ROS to drive the various physiological processes in aerobic organisms remains a great puzzle. For the future, the fundamental challenge will be to integrate the information now being obtained on gene-expression patterns with structural and functional parameters and interactions of the various proteins encoded by ROS-responsive regulons, and to view the cell in which they function holistically. The future indeed seems exciting for studies of oxidative stress responses.

Acknowledgements

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