Chapter 26
Oxidative Stress Responses in Aquatic and Marine Fishes

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Oxidative stress can be defined as the deleterious cellular effects arising from the production of reactive oxygen species (ROS) beyond the capacity of antioxidant defense systems to detoxify them. ROS are reactive O_2-based molecules including the superoxide anion radical ($O_2^{-\cdot}$), the hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), ozone (O_3) and singlet oxygen ($^1O_2$). The importance of oxidative stress and antioxidant defenses in human health and disease has been a major topic of research and clinical application for decades (see review by [1]). More recently, there has been an increasing appreciation for these phenomena in fishes, particularly in the context of pollution of freshwater and marine systems. This has spurred substantial research into a mechanistic understanding of oxidative stress in fishes. The mechanistic study of oxidative stress in fishes serves many purposes: (1) increase our understanding of the basic biochemical and molecular mechanisms related to oxidative stress in fish; (2) explore evolutionary adaptations to oxidative stress to inform our understanding in other vertebrate species, including humans; and (3) understand the impact of prooxidant environmental stressors on fish population health. Many xenobiotics induce the production of ROS by several biochemical mechanisms (Fig. 26.1) such as the impairment of membrane-bound electron transport (e.g., mitochondrial, microsomal electron transport), redox cycling, inactivation of antioxidant enzymes, depletion of free radical scavengers, photosensitization, and facilitation of Fenton reactions [2]. A number of reviews have been published that discuss the nature of ROS, mechanisms by which they are produced naturally and

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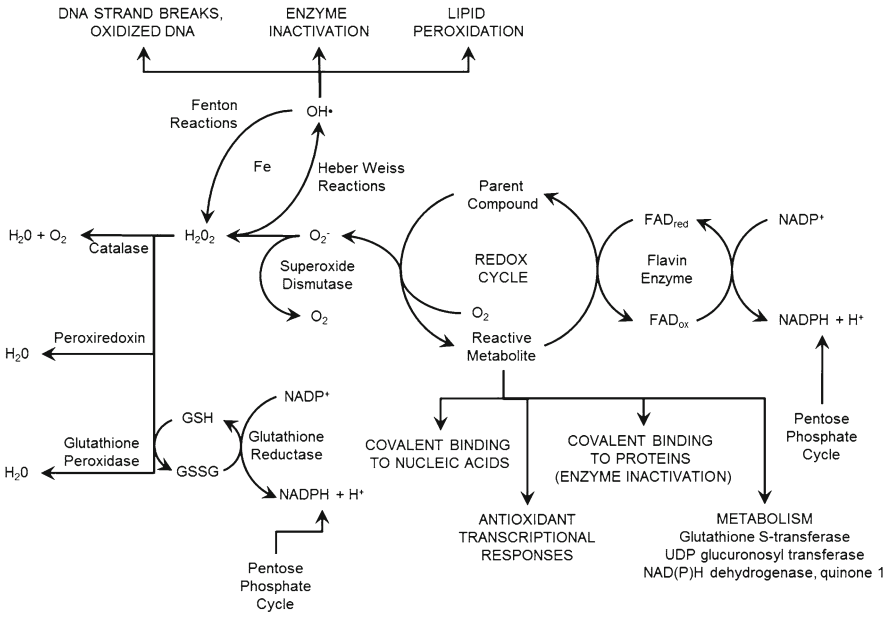

Fig. 26.1 An overview of ROS generation by redox cycling, key enzymatic antioxidant defenses, and cellular targets

via xenobiotics, antioxidant defense systems, cellular targets and organismal consequences [2, 3].

Many environmental chemicals of varying chemical classes have been shown to cause oxidative stress in marine and freshwater fishes. The severity of the oxidative stress can be influenced by temperature [4–6], salinity [7–9], and hypoxia [10–12]. It is unclear in many cases whether these stressors are acting synergistically or additively with xenobiotic chemicals since antioxidants and oxidative stress responses can be influenced by many environmental factors, including temperature [13–15], dissolved oxygen [16–19], salinity [20], and acidification [21]. A very interesting case is the notothenoid ice fish that have adapted to the cold, oxygen-rich waters of Antarctica through genomic loss or gene amplification [22, 23] and have a pronounced loss of heat shock response and a non-traditional battery of oxidative stress-responsive genes [24]. This suggests that they might be highly sensitive to the effects of oxidative stress or any other stressor that might promote oxidative stress [25]; therefore, questions arise regarding their ability to adapt to warming in the Antarctic and the increased environmental pollution via global transport mechanisms and expanding ecotourism.

The association of pesticide exposure and oxidative stress is well established for many environmentally-relevant agents, and their structural diversity leads to a

spectrum of effects. For example, a number of pesticides have been shown to impact catalase activity [26–28], lipid peroxidation [29–31], and mitochondrial function [32–34]. A relatively recent and thorough review of pesticide-induced oxidative stress in fish was produced by Slaninova and coworkers [35].

Metals are known inducers of oxidative stress in many species of fish, promoting the formation of ROS through either redox cycling [36–38] or interaction with antioxidant defenses, especially with thiol-containing antioxidants and enzymes [39–41]. Sevcikova et al. have published a thorough review of metals and oxidative stress in fish [42].

Aromatic hydrocarbons are found in nearly all aquatic environments around the globe. They are structurally diverse, but all are composed of one or more benzene rings. In general, aromatic hydrocarbons are metabolized in fish by Phase I enzymes (e.g., cytochrome P450s or epoxide hydrolase) to reactive intermediates that are substrates for conjugation by Phase II enzymes to create hydrophilic metabolites for excretion. As part of this metabolic process, reactive oxygen species are produced that lead to the induction of antioxidant enzymes and protective molecules [43–45]. Also, the metabolic products of PAHs include quinones that can generate ROS via redox cycling [46]. The toxicity of aromatic hydrocarbons can also be increased by exposure to UV light in the environment, which has been demonstrated in a number of fish species and life stages [47–49].

Microcystins are potent cyanobacterial toxins produced by *Microcystis spp.* during bloom events, and their effects on fish and other aquatic animals have been reviewed recently [50, 51]. In cultured fish cells, purified microcystin causes increases in lipid peroxidation and the expression of several antioxidant enzymes [52]. In many whole organism studies using fish, the exposures occur via the addition of cultured *Microcystis spp.* to the water in which the fish are being held. Under these conditions, there is also an increase in lipid peroxidation and the expression of several antioxidant enzymes with the liver being the most affected organ followed by kidney and gills [53]. A few studies have observed that *Microcystis spp.* exposure also inhibits protein serine/threonine phosphatase in liver that may initiate a metabolic response to the toxin (Olivares [54]). Interestingly, *Amado* et al. suggest that hyperphosphorylation linked to ROS is responsible for inducing and maintaining the antioxidant response to *Microcystis spp.* exposure [50]. The exact mechanistic linkages between oxidative stress, phosphorylation state and oxidative stress responses induced by *Microcystis spp.* remain to be determined.

26.1 Small Molecule Antioxidant Defenses

A number of small molecules have been shown to have a protective effect in fish under environmentally-induced oxidative stress. We will briefly discuss what are often considered the most important small molecules in the prevention of

ROS-induced damage—glutathione, ascorbic acid (vitamin C), and tocopherol (vitamin E). Glutathione is considered the most important small molecule for cellular defense against ROS-induced damage. As observed in mammals, tissue glutathione levels are often depleted after short-term oxidant exposures but elevated after long-term exposures [3, 55, 56]. Glutathione-associated antioxidant enzymes—glutathione peroxidase, glutathione reductase, and glutathione S-transferase—(discussed below) are also critical for maintenance of normal cellular redox status and protection against ROS [2]. Non-glutathione small molecules are increasingly under scrutiny and evidence suggests they also play a critical role in protection against ROS. Ascorbic acid (vitamin C) levels are modulated by environmental chemicals [57, 58] and other environmental stressors including osmotic stress [20] and redox stress associated with air breathing [59]. Tissue ascorbic acid levels have been shown to protect against lipid peroxidation [60]. The majority of studies using fish to investigate the protective effect of tocopherol (vitamin E) are based on dietary supplementation, which can reduce oxidative stress-related biomarkers [61–63]. Environmental toxicants can modulate the levels of tocopheral which may influence ROS-induced damage [64, 65].

26.2 Antioxidant Transcriptional Response

Cellular homeostatic mechanisms have evolved to deal with low levels of oxidative stress through modulation of the basal cellular concentrations of glutathione and the transcription factor commonly known as NRF2 (HGNC approved name: nuclear factor, erythroid 2-like 2, symbol: NFE2L2). With elevated levels of oxidative stress, cells can adapt by up-regulation of networks of responsive proteins regulated by NRF2 or NFkB (Fig. 26.2). Most proteins whose expression is regulated by NRF2 activity function as cryoptotectants [66]. The cellular pool of NRF2 is regulated through binding to KEAP1 (kelch-like ECH-associated protein 1), which promotes NRF2 ubiquitination and limits protein half-life. Under oxidative stress conditions, ubiquitination of NRF2 is dramatically reduced and KEAP1 binding sites are rapidly saturated. This leads to an increase in free cytosolic NRF2, which acts as a redox probe and translocates to the nucleus under oxidative conditions [66]. In the nucleus, NRF2 dimerizes with small MAF proteins to up-regulate the transcription of numerous target genes via binding to antioxidant response elements (also known as electrophile response elements) [67].

NFkB is known to regulate proteins that are cryoprotectants and some that are pro-oxidant. While these two functions seem contradictory, the expression of NFkB target genes typically promotes cellular survival in response to numerous cellular stressors [68]. The canonical NFkB pathway is activated mostly under proinflammatory conditions. The NFKB1-RELA dimer is held inactive via interaction with IkB inhibitory proteins. Under oxidative stress conditions, IkB is phosphorylated and subsequently ubiquinated, which allows the NFKB1-RELA dimer to translocate to the nucleus and bind to NFkB binding sites [68].

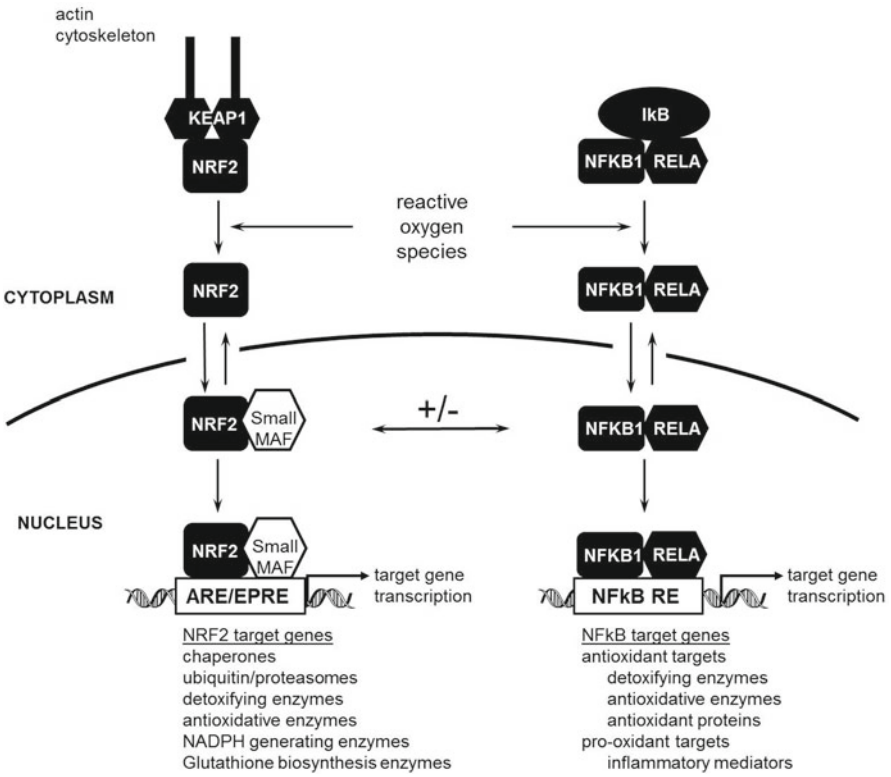

Fig. 26.2 Transcriptional activation in response to oxidative stress via NRF2 and NFkB pathways. Both pathways play a key role in the transcriptional responses to oxidative stress. Interestingly, the two pathways have been described as mutually stimulatory and inhibitory in an apparently cell-types specific manner

26.3 Antioxidant Enzymes and Protective Targets

These two pathways are largely responsible for transcriptional activation of gene products that protect the cell from oxidative stress-associated damage (Table 26.1). These proteins can be broadly described as antioxidant enzymes or protective targets. Antioxidant enzymes catalyze the elimination of reactive metabolic intermediates and ROS, or are important modulators of these processes. Enzymes such as superoxide dismutase and catalase act directly to detoxify the inorganic free radicals superoxide and hydrogen peroxide, respectively [69]. Enzymes such as glutathione S-transferase and UDP glucuronosyltransferases generally catalyze the conjugation of small molecules (glutathione and glucuronic acid, respectively) to reactive intermediates which makes them readily excretable [70, 71]. In contrast, the enzyme glutamate-cysteine ligase, which has a catalytic subunit (GCLC) and a modifier subunit (GCLM), is the rate-limiting enzyme in glutathione synthesis [72].

Table 26.1 Selected antioxidant genes controlled by NRF2 and/or NFkB and identified in fish species

Activity	Gene name	Symbol
Glutathione biosynthesis	Glutamate-cysteine ligase, catalytic subunit	GCLC
	Glutamate-cysteine ligase, modifier subunit	GCLM
	Glutathione reductase	GSR
ROS detoxification	Glutathione peroxidase 1A	GPX1A
	Glutathione peroxidase 1B	GPX1B
	Superoxide dismutase 1, soluble	SOD1
	Superoxide dismutase 2, mitochondrial	SOD2
	Superoxide dismutase 3, extracellular a	SOD3A
	Superoxide dismutase 3, extracellular b	SOD3B
	Catalase	CAT
	Peroxiredoxin 1	PRDX1
	Peroxiredoxin 6	PRDX6
Metal binding	Thioredoxin	TXN
	Metallothionein 2	MT2
Glutathione S-transferase	Glutathione S-transferase M	GSTM
	Glutathione S-transferase M3 (brain)	GSTM3
	Glutathione S-transferase pi 1	GSTP1
	Glutathione S-transferase pi 2	GSTP2
	Microsomal glutathione S-transferase 1.1	MGST1.1
	Microsomal glutathione S-transferase 1.2	MGST1.2
	Microsomal glutathione S-transferase 2	MGST2
	Microsomal glutathione S-transferase 3	MGST3
UDP glucuronosyl transferase	UDP glucuronosyltransferase 1 family, polypeptide A6	UGT1A6
	UDP glucuronosyltransferase 2 family, polypeptide B1	UGT2B1
	UDP glucuronosyltransferase 2 family, polypeptide B5	UGT2B5
Reduction	NAD(P)H dehydrogenase, quinone 1	NQO1
	Aldo-keto reductase family 1, member A1A (aldehyde reductase)	AKR1A1a
	Aldo-keto reductase family 1, member A1b (aldehyde reductase)	AKR1A1B
Heme oxygenase	Heme oxygenase (decycling) 1a	HMOX1A
	Heme oxygenase (decycling) 1b	HMOX1B
Hydrolysis	Epoxide hydrolase 1, microsomal (xenobiotic)	EPHX
Iron transport	Ferritin, heavy polypeptide 1a	FTH1A
	Ferritin, heavy polypeptide 1b	FTH1B

Proteins that function as protective targets can generally be considered ROS scavengers. Many members of the globin gene family (including myoglobin, neuroglobin, cytoglobin) are thought to play an important role in ROS scavenging and are prominent stress-responsive proteins in fish [73–75]. The globin X member of the gene family is found only in fishes and may either protect the lipids in cell membrane from oxidation or may act as a redox-sensing or signaling protein [76]. As in

other vertebrates, metallothioneins and thioredoxins are also important free radical scavengers [16, 77].

26.4 Adverse Outcome Pathways, Oxidative Stress, and the Health of Wild Fish Populations

The effects of oxidative stress can be measured and interpreted at the level of individual fish as correlations between biomarker activation and measured changes in physiology. However, when considering impacts on wildlife, it is the impacts at the population level that are most relevant. This relationship between molecular responses, individual health, and population effects are rarely straightforward or readily apparent. The Adverse Outcome Pathway (AOP) framework as defined by Ankley et al. [78] is an approach toward understanding the linkages from a molecular initiating event, through a series of biological processes, to an ultimate adverse outcome of relevance to human or ecological risk assessors (Fig. 26.3). This approach is based on the 2007 report by U.S. National Research Council (NRC) Committee on Toxicity Testing and Assessment of Environmental Agents that sought to transform toxicity testing and embrace newly-developed high-throughput and computational approaches focused on pathways to inform risks to humans (NRC 2007). One primary difference between the NRC approach and the AOP framework approach for ecotoxicology or public health is the focus on population-level effects in the AOP.

Oxidative stress and oxidative stress responses are a very important component of many AOPs; however, the nature of oxidative stress responses as homeostatic pathways precludes consideration as a defined AOP [79]. For example, oxidative stress is a key component of proposed AOPs for drug-induced cholestasis and chemical-induced liver fibrosis [80]. At this time, the role of oxidative stress in the initiation of specific disease or dysfunction processes (or AOPs) is rarely well-defined.

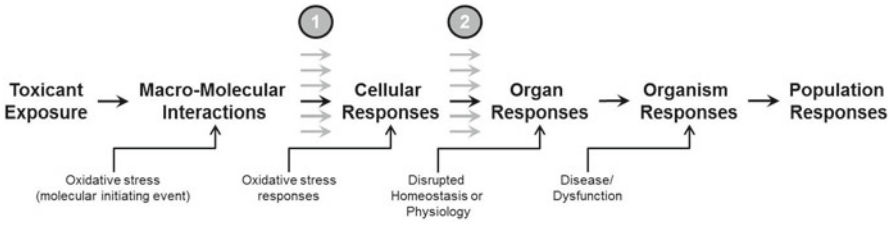

Fig. 26.3 Schematic of a generalized Adverse Outcome Pathway. Points where oxidative stress and subsequent and transcriptional responses play a role are indicated. The gray arrows indicate the multiplicity of cellular- and organ-level responses to oxidative stress. Biomarkers are commonly used to indicate (*1*) oxidative damage that initiates stress responses (e.g. lipid peroxidation, oxidative DNA damage, depleted glutathione levels) to indicate (*2*) processes that are initiated when homeostatic mechanisms are overwhelmed (e.g. apoptosis, necrosis)

There are numerous biomarkers of oxidative stress that include macromolecular damage (e.g., lipid peroxidation, oxidative DNA damage) or changes in expression levels of oxidative stress-responsive genes (Table 26.1). There is a substantial body of scientific literature that clearly connects toxicant exposure with the generation of oxidative stress and the expression or certain biomarkers. Enhancing our understanding of these linkages to disease and dysfunction will increase the certainty with which one can apply oxidative stress biomarkers to predict and assess the potential for adverse effects at the organism and population level.

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