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Oxidative Stress in Invertebrate Systems

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Abstract

Invertebrates have been valuable research models in the discovery of many scientific principles owing to the numerous advantages they provide. Throughout the life cycle, many of them thrive in pathogen-rich environments, manage harsh weathers, exposed to a number of allochemicals, and adapt well to both terrestrial and marine ecosystems. Their remarkable ability to cope up with the enormous oxidative stress generated in all these circumstances, make them attractive models in this field of research. Endocrine control of oxidative stress in insects is recently emerging. Adipokinetic hormone, glucagon, ecdysteroids and juvenile hormone have been implicated in antioxidative protective role in insects. *Drosophila* and *Caenorhabditis elegans* have provided the largest body of evidence addressing the free radical theory of ageing. Oxidative stress is also induced by pesticides/insecticides. In mollusks, pesticides exert their biological effects via generation of ROS. Oxidative stress has been shown to be associated with exposure to several organophosphorous compounds and different classes of pyrethroids. Malathion is a potential hazard to the environment. Adverse effects induced by malathion in earthworms and insects have been reported. Information is now available in great detail on the role of ROS in modulating insect immunity during parasite invasion and bacterial infection. In *Drosophila melanogaster* ROS are actively produced in the midgut at a basal level in the presence of commensal microbiota and highly generated upon bacterial challenge. The involvement of reactive oxygen species (ROS) in mosquito immunity against bacteria and *Plasmodium* was investigated in the malaria vector *Anopheles gambiae*. The concentration of ROS increased in sand fly midguts after they fed on the insect pathogen *Serratia marcescens*. Elevated oxidative stress was previously reported for a mosquito line experimentally infected with *Wolbachia*, indicating that oxidative stress may be important for *Wolbachia*-mediated antiviral protection. In a nutshell, this chapter highlights the current advances of oxidative stress in invertebrate model systems and its implications.

Keywords: oxidative stress, invertebrates, reactive oxygen species, antioxidative system

1. Oxidative stress: an introduction

Oxidative stress can be defined as a disturbance in the balance between the production of reactive oxygen species and antioxidant defenses [1]. Reactive oxygen species (ROS) are free radicals, which are important for the cellular functions generated in different biological processes. A free radical is unstable and highly reactive and contains one or more unpaired electrons. These free radicals are involved in the regulation of various mechanisms and intercellular signaling and act as bactericidal agents [2]. ROS can also induce cellular senescence, apoptosis, and cell growth regulatory pathways [3]. ROS are generated as a by-product of the aerobic respiration where the superoxide anion (O_2^-) and H_2O_2 are formed when molecular oxygen chemically oxidizes electron carriers. Cellular sources of ROS are produced by the action of different oxidative enzymes, which include plasma membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, intracellular cytosolic xanthine oxidase, peroxisomal oxidases, endoplasmic reticular oxidases, and mitochondrial electron transport components [4]. Apart from this, auto-oxidation of catecholamines, ubihydroquinone, hemoproteins, and flavin enzymes also produces ROS [5]. In response to nutrient stress, cells enter autophagy that can lead to adaptation or death. Starvation-induced autophagy results in reactive oxygen species (ROS) production, DNA damage, and PARP-1 activation, leading to the inhibition of mTOR.

In all these cases, the generation of free radicals is implied.

2. Biological factors of oxidative stress in invertebrates

Reactive oxygen species are naturally produced in all cells and organisms. Modifications of normal conditions alter reactive species generation and may result in oxidative stress. In this chapter, we have discussed the role of the most important causes of the ROS generation in the invertebrate systems.

2.1. Blood feeding

Insects like mosquitoes which feed on human blood face severe oxidative stress due to the release of iron from hemoglobin which in turn can potentially induce oxidative damage and eventually death [6]. To face such odd situation, blood-feeding insects evolved with strong defense mechanisms against the stress. Mosquitoes protect themselves by secretion of the peritrophic matrix (PM) [7] in the midgut which is made up of chitin and a protein-containing layer. It also has a role in protecting the insect from invasive pathogens [8]. The direct contact of the gut epithelium with intermediates of hemoglobin digestion induces oxidative stress in sand fly and *Aedes aegypti* [9].

2.2. Xenobiotic degradation

A source of ROS in the honeybee is the microsomal oxidation of xenobiotics. Microsomes contain enzymes of cytochrome P450 (CYP) system, which catalyzes polyvalent oxidation of

xenobiotics with simultaneous generation of O_2^- and other ROS. The CYP hydroxylase system includes flavoproteins and a family of hemoproteins which are localized on the membranes of the endoplasmic reticulum. Different isoforms of CYP are involved in metabolism of various xenobiotics [10, 11]. CYP groups are distinguished based on the metabolism of endogenous and exogenous substances. Microsomal glutathione S-transferase (GST) is closely linked with the CYP system, which contributes to rapid inactivation of active metabolites produced during the metabolism of xenobiotics.

2.3. Dopamine synthesis

In the insect nervous system, ROS-mediated decline of neuron survival can be observed. The dopamine-producing neurons show the highest sensitivity to oxidative stress as the dopamine production machinery is linked to an endogenous production of high amounts of ROS, thus making these cells prone to damages caused by ROS. These high levels of ROS lead to the development of Parkinson's disease-like phenotypes [12]. It is a suitable mechanism to induce neurodegenerative processes using ROS as typically seen in Parkinson's disease. One of the most reproducible ways to do this is through hyperoxia, which consequently is able to induce these phenotypes enabled *Drosophila* as a model for Parkinson's disease [13]. In an Alzheimer's disease model, based on tau activation in the nervous system, ROS has been shown to modulate the sensitivity, thus demonstrating that oxidative stress plays a major role in the development of Alzheimer's disease.

2.4. Microbiome

The intestinal microbiome is one of the densest populations on earth. Thus, attaining a homeostatic balance between fight against potential pathogens and maintenance of the microbial community within the intestinal tract is a major challenge for the gut immune system [14]. Fighting pathogens is achieved via enzymes producing ROS. Dual oxidase (DUOX) is a member of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) family and the most important enzyme secreted from enterocytes in response to pathogen contact [11, 15]. It is independent of classical immune response pathways such as the Toll [16] or the IMD pathway [17]. The killing mechanism of DUOX comprises the production of H_2O_2 , which is a typical feature of DUOX enzymes in general [18]. The fly phenol oxidases (POs) are another enzyme set that produces a melanin coat surrounding invaders of different nature. These enzymes are able to produce a locally high concentration of ROS, which may be one of the major effectors of the prophenoloxidase (PPO) system. This melatonin production is triggered by the recognition of bacterial patterns including peptidoglycans [19].

Airway infection is one of the well-studied areas in fruit fly [20]. In addition to classical aspects of an antibacterial response including antimicrobial peptide genes, some of these enzymatic antioxidants are strongly upregulated. The antioxidant system of the airway epithelium contains both producers of ROS and protectors against them.

2.5. Oxidative burst in macrophages

Motile cells of the innate immune system use production of highly effective ROS which is a versatile method to fight pathogens. The oxidative burst produced by macrophages is the most impressive example highlighting this strategy. Reactive oxygen species (ROS) is generated by many phagocytic cells in response to membrane perturbations such as receptor-ligand interactions and phagocytosis [16, 21], which defend these cells against infectious diseases by virtue of their antimicrobial properties. Initially, superoxide anion (O_2^-) is produced which is spontaneously or enzymatically converted to hydrogen peroxide (H_2O_2), subsequently giving rise to even more toxic products, such as hydroxyl radical ($\cdot OH$), hypochlorous acid ($HOCl$), and singlet oxygen (IO_2). ROS are either directly toxic or exert increased antimicrobial activity in synergism with lysosomal hydrolases and/or reactive nitrogen species (RNS), plasma membrane-bound enzyme complexes, and the NADPH oxidase (NOX) system [22].

Leishmania parasite manages both to survive and proliferate within the mature phagolysosomal compartment of the macrophages [21]. Trypanosomatids do not express essential antioxidant enzymes like catalase and selenium-containing glutathione (GSH) peroxidases [23]. Defense system of the *Leishmania* parasite contains several mechanisms, which include trypanothione (N1, N8-bis(glutathionyl)spermidine adduct) [24, 25]. It is the primary thiol in these parasites and adopts the metabolic role of glutathione. Along with this, ovothiol A acts as a nonenzymatic scavenger of H_2O_2 though it is much less efficient than trypanothione [26]. Trypanothione reductase (TR) is a member of the disulfide reductase family, which is the enzyme responsible for maintaining trypanothione in its reduced form [27]. Tryparedoxin peroxidases or peroxiredoxins are a family of peroxidases that reduce H_2O_2 and alkyl hydroperoxides to water and alcohol, respectively, with the use of reducing equivalents provided by thiol-containing proteins [28]. The enzyme arginase is a part of the host as well as parasite defense. Phagocytosis of promastigotes leads toward the two opposing forms of classical and alternative activation of host macrophages which results in differential L-arginine metabolism [29, 30]. Being effective in modulating macrophage signaling and antimicrobial function, *Leishmania* parasites possess surface protein kinases which phosphorylate members of complement system, thus inactivating cellular cascades. This helps the parasite to evade the innate immune responses and ensure a safe environment for its proliferation [31].

Parasites can inhibit the activation of several inflammatory cytokines like interleukin-12 (involved in T-cell activation), interferon gamma, interleukin-1, and tumor necrosis factor-alpha that strengthens parasite survival. Few species induce heterologous population of host inflammatory cells, such as neutrophils and monocytes/macrophages, which are effective in controlling/clearing infections. The T helper cell type 1 (Th1) response as a result decreased expression of induced nitric oxide synthase and reduced activity of NK cells [32].

Selenocysteine is analogous to a cysteine residue but has sulfur substituted by selenium. Selenoprotein families, some with antioxidant properties, such as glutathione peroxidase

(GPx) and thioredoxin glutathione reductase (TGR) appear to be essential in flatworms and in *Plasmodium falciparum* and other *Plasmodium* species [33, 34].

Ascorbate peroxidase is another antioxidant, which is a heme peroxidase identified in the inner mitochondrial membrane of the *Leishmania* parasite. Overexpression of this enzyme confers tolerance to oxidative stress-mediated cardiolipin oxidation and thus protects the parasites from extensive protein damage. *Leishmania* promastigotes inhibit phagolysosome biogenesis via lipophosphoglycan (LPG), which causes periphagosomal accumulation of F-actin and impaired assembly of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and exclusion of vesicular proton ATPase from phagosomes [35].

2.6. Respiration

The major task of airway epithelial cells is enabling an effective oxygen transport and exchange. These cells react to different stimuli with appropriate responses including massive responses if pathogens are experienced. In principal, all oxygen transport systems are prone to damage caused by high oxidative stress because these cells have direct contact with the air and large volumes of air pass over their surface during the process of gas exchange.

3. Nonbiological factors of oxidative stress in invertebrates

3.1. Effect of pollutants

Environment has a major role in the production of stress because of different pollutants like air, soil, and water. The compounds used in industry and agriculture include metals, metalloids, and numerous other organic compounds. Majority of these compounds have been shown to induce oxidative stress by generating ROS in nontarget species including invertebrates [36].

3.2. Effect of phytochemicals, herbicides, and insecticides

Plant phenolic compounds, such as flavonoids and tannins, which are involved in plant defense mechanism, can produce free radical in herbivorous insects [37]. The midgut of insect is a highly oxidizing environment. Hence in lepidopteran larvae, *Helicoverpa zea* and *Spodoptera littoralis* with phenolic acids were found to increase various indicators of oxidative stress in gut tissues [38, 39]. Additionally, furanocoumarins in some plants and herbicides like paraquat are well known to generate oxidative stress in insect species [36]. Insect growth regulator (IGR) is a substance (chemical) that inhibits the life cycle of an insect and has been used to control the insect pests. Hormonal IGRs typically work by mimicking or inhibiting the juvenile hormone (JH) or ecdysone. Studies on the IGRs like Applaud (buprofezin) as a chitin synthesis inhibitor and Admiral (pyriproxyfen) as juvenile hormone analog (JHA) in the larval body of the cotton leaf worm, *S. littoralis*, resulted in the occurrence of lipid peroxidation in the larval tissues which enhanced different anti-

oxidant defensive systems to overcome its effect like malondialdehyde (MDA) and glutathione reduction [40].

3.3. Effect of metal ions

Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces symptoms for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders (Alzheimer's disease, Parkinson's disease), chronic inflammation, and others [41].

Metals, such as iron (Fe), copper (Cu), chromium (Cr), and cobalt (Co), induce the formation of the superoxide and hydroxyl radicals (mainly through Fenton reaction) and other ROS, which ultimately leads to the production of malondialdehyde (MDA), 4-hydroxynonenal (HNE), and other exocyclic DNA adducts that are carcinogenic and mutagenic. On the other hand, the redox-inactive metals, such as cadmium (Cd), arsenic (As), and lead (Pb), induce toxicity through bonding to sulphhydryl groups of proteins and depletion of glutathione. Zinc (Zn) is a redox-inert metal, which is an essential component of numerous proteins involved in the defense against oxidative stress. In addition, Zn possesses neuroprotective properties. Depletion of Zn may enhance DNA damage through impairments of DNA repair mechanisms. Cellular antioxidants (ascorbic acid (vitamin C), α -tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants) and chelate metal ions reduce their catalytic activity to form ROS. A novel therapeutic approach to suppress oxidative stress is based on the development of dual function antioxidants which comprise of chelating and scavenging agents [39, 40].

4. ROS production in marine invertebrates

Oxidative stress is highly prevalent in marine invertebrates, particularly bivalve mollusks, due to the degradation of marine ecosystems, massive aquaculture productions, climate change, and pathogenic infections. Mollusk hemocytes produce ROS and RNS as part of their basal metabolism as well as in response to both endogenous and exogenous stimuli. However, sources, pathways, and mechanistic aspects of these reactive species production are currently poorly deciphered. Unique pathways seem to exist in marine bivalves [42].

High oxygen solubility in cold water is responsible for an elevated level of antioxidant protection in marine ectotherms from polar environments. However, tissue oxidative stress is a function of elevated or variable pO_2 , rather than of an elevated tissue oxygen level [43].

To counteract or to regulate the free radical production, several antioxidant mechanisms were evolved (**Figure 1**) [44].

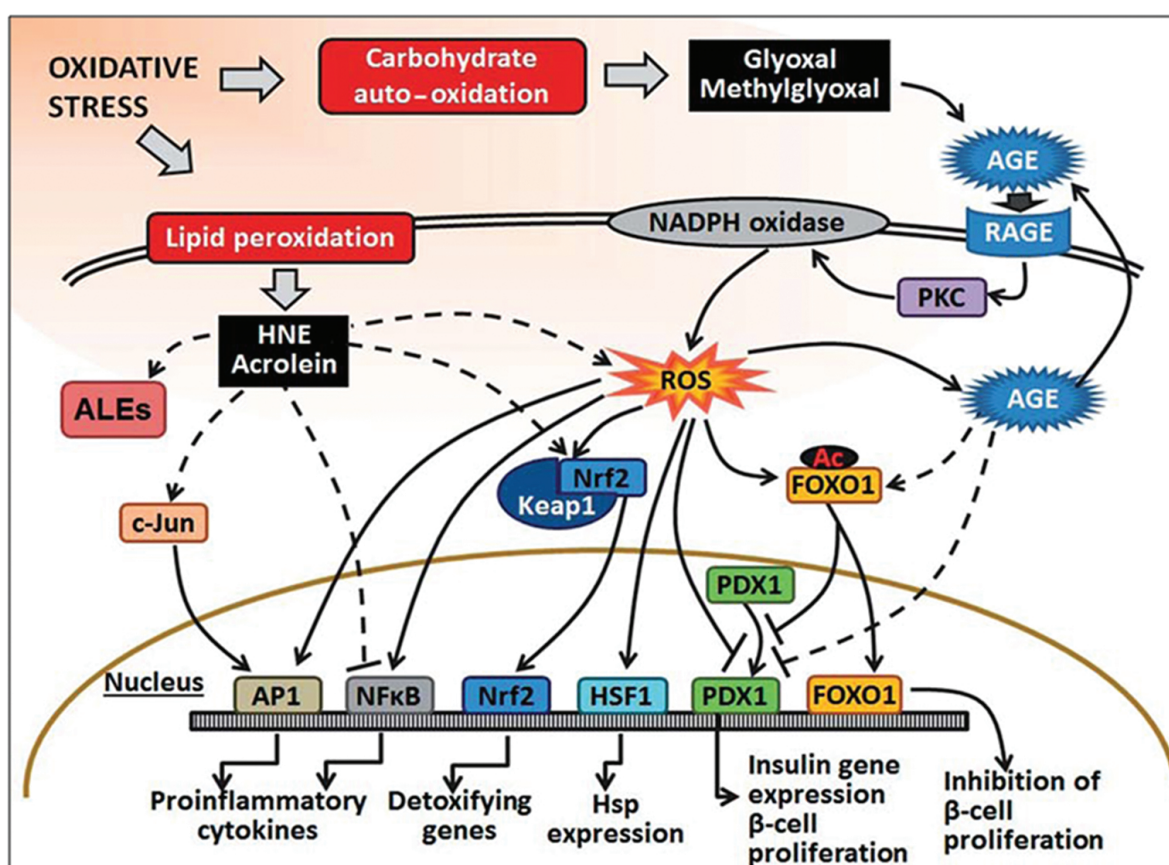


Figure 1. Schematic representation of the pathways activated by the oxidative stress on macromolecules like lipids and carbohydrates leads to the signal transduction through ROS-dependent and ROS-independent mechanisms. Glyoxal and methylglyoxal formed by the oxidation of the carbohydrates were detected by the AGE and activate cell surface molecule RAGE. It passes signal to NADPH oxidase through PKC, which activates the ROS. ROS activates several transcription factors such as AP-1; NF- κ B: pro-inflammatory cytokines; Nrf2: detoxifying genes; HSF1: Hsp expression; and FOXO1: inhibition of B-cell proliferation. It also inhibits the PDX1 transcription factor which activates insulin gene expression and B-cell proliferation. In ROS-independent pathway, AGE can directly activate FOXO1 and inhibits PDX1. In lipid peroxidation, 4-hydroxynonenal (HNE) and acrolein-mediated signaling activate AP-1 through c-Jun and Nrf2. It blocks NF- κ B-mediated transcription activation apart from the ROS-activated signaling.

5. Antioxidants

Oxidative stress arises when there is a marked imbalance between the production and removal of reactive oxygen and nitrogen species. Free radical production is normally controlled by the antioxidant defense mechanisms [2]. Antioxidative defenses could essentially be divided broadly into two main mechanisms: one is enzymatic and the other is nonenzymatic.

5.1. Enzymatic antioxidative mechanisms

Insects, like other animals, possess a suite of enzymes that are directed toward the removal of various radicals [45–48]. These include superoxide dismutase present in cytosol and mito-

chondria, catalase present in peroxisomes, ascorbate peroxidase [49], glutathione S-transferase peroxidase, thioredoxin/thioreductase system [50], and so on.

5.2. Nonenzymatic antioxidative mechanisms

In addition to the classical antioxidant enzyme systems, a number of small molecules also play a significant role in scavenging ROS, and some of these small molecules are plant derived, whereas others such as carotenoids, α -tocopherol, ascorbic acid, and glutathione (GSH) can be synthesized by insects [45, 50–52].

In addition to the antioxidant mechanisms and systems described above, insects also possess several water-soluble molecules (uric acid, carbohydrates, and polyols) and iron-binding proteins (ferritin and transferrin) that serve crucial antioxidant functions [45]. Antioxidant properties of vitellogenin are specified by its Zn-binding capacity [53] and preferential oxidative carboxylation under oxidative stress in honeybees [54]. With respect to these properties, vitellogenin is compared to Cu/ZnSOD, a key metal-binding antioxidant enzyme that undergoes preferential carbonylation [55], and serum albumin, a metal-binding protein, that can function as free radical acceptors and reduce oxidative marker levels such as protein carbonylation [56].

Responses to oxidative stress induced by a blood meal were investigated in *A. aegypti* [7]. In female mosquitoes, higher levels of ferritin were observed after repeated blood meals. Ferritin is a sensitive defense mechanism against oxidative stress induced by blood meal [57]. An increase of secreted ferritin due to the induced synthesis of ferritin heavy chain homologue (HCH) was observed [58].

6. Regulation of defenses against oxidative stress

Oxidative stress triggers a range of responses in insect cells which could be physiological, pathological, and adaptive. A large number of signaling pathways are involved in response to oxidative stress. *Drosophila melanogaster* is a well-studied organism in the field of oxidative stress. Among these signaling pathways, in fruit fly, PI3-kinase pathway is one of the important responses to oxidative stress by activating the transcription factor FOXO [13], and an alternative signaling is represented by the Nrf2/Keap1 pathway which is required for a proper and efficient expression of genes associated with an increase in oxidative stress resistance [41]. Other signaling systems include activator protein-1 (AP-1), nuclear factor-kB (NF-kB), protein kinase C (PKC), protein 53 (p53), and redox regulation by redox factor-1 and the nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant response. Nrf2 belongs to a group of specialized transcription factors termed xenobiotic-activated receptors (XARs). These recognize specific xenobiotics and coordinate the transcription of batteries of genes.

Honeybees possess only about half the genes of GST, CYP, and carboxyl/cholinesterases as in other insects [17–19]. This deficit can contribute to insufficient resistance to oxidative stress caused by incomplete microsomal oxidation of xenobiotics [59]. These insects have more genes

of class sigma GST [60], which has a high affinity to the products of lipid peroxidation localized in the thorax muscle of flies to protect these tissues against oxidative stress by-products [61].

The idea behind one of the first theories of aging was oxidative stress-induced damage [42]. Enzymes which regulate the oxidative stress are responsible for the longevity that was supported by shortened life span in flies lacking a functional thioredoxin-2 [60]. Peroxiredoxin of type 2, named Jafrac1 [61], and other peroxiredoxins, especially those present in mitochondria, are also relevant for life span, as their downregulation has a significant impact on this aspect of life [62] along with other enzymes like glutathione reductase [63], SODs, and catalase [64–66].

Intriguingly, genes belonging to the family of antimicrobial peptide genes are obviously relevant for this adaptation to extreme oxygen concentrations and therewith to higher levels of oxidative stress [67, 68]. In insects, compared to vertebrates, genes encoding glutathione reductase (GR) and glutathione peroxidase (GPX) are absent. Their functions are performed by homologous genes that encode thioredoxin reductase (TrxR) [52] and thioredoxin peroxidase (TPx) [67]. In addition, insects' genes encode enzymes of antioxidant defense that act as peroxidases: phospholipid-hydroperoxide GPx homologues with TPx activity (GTPx) [69] and GST [70, 71]. Thus, the secondary antioxidant enzymes in insects that act on ROS indirectly include TrxR, which converts both Trx and GSH, and methionine sulfoxide reductases (MsrA and MsrB), which are involved in protein repair by catalyzing the Trx-dependent conversion of methionine sulfoxide to methionine [72, 73].

In the nervous system, the dopamine metabolism produces high ROS concentration leading to the SOD overexpression. Increase in the glutathione S-transferase activity in the nervous system protects these dopaminergic neurons [74]. However, this ROS-based mechanism of neurodegenerative diseases is not applicable for Huntington's disease. Neither overexpression of enzymatic antioxidants nor supplementation with nonenzymatic antioxidants decreased the lethality in this model [61].

The intestinal microbiome regulation DUOX plays a crucial role with killing mechanism by production of H_2O_2 [31]. To protect the gut tissue from the action of ROS, an enzymatic antioxidant, immune-regulated catalase (IRC), is produced [29].

Previous microarray studies in *Drosophila* suggested that IRC is the only catalase to be induced upon infection and may be regulated by the Toll pathway. The IMD pathway could be another candidate for regulation of antioxidants including IRC, since it is induced systemically by Gram-negative bacteria and in turn activates the JNK pathway.

Malpighian tubules also perform crucial roles in stress sensing and response [75]. At molecular level, multiple cell-specific signaling pathways including cAMP, cGMP, and calcium modulate the tissue and, hence, organism responds to various stress, such as detoxification and xenobiotic handling, and stress sensing of oxidative, osmotic (ionic/salt) and immune challenges [76–78].

7. ROS-dependent biological processes in insects

7.1. Autophagy

In the regulation of intestinal microbiome, apart from the ROS-induced OS regulation of pathogens, ROS production induces a JNK-triggered autophagy in the enterocytes in *D. melanogaster* [59].

7.2. Differentiation of hemocytes

Motile cells of the innate immune system use oxidative burst to control invading pathogens. Apart from that, ROS play an important role in the differentiation of hemocytes. In *Drosophila*, multipotent hematopoietic progenitors react to different ROS levels with a speeding up or arresting of their differentiation [79]. Both the JNK and the FOXO pathways are involved in the transduction of ROS levels into developmental signals [80].

8. Hormonal regulation of oxidative stress

8.1. Adipokinetic hormones (AKHs): stress hormones of insects

The insect endocrine system produces three main groups of hormones [81] including (a) ecdysteroids, (b) juvenile hormones (JHs), and (c) neurohormones. Out of these, a group of neurohormones belonging to the AKH/RPCH family (adipokinetic hormone/red pigment-concentrating hormone family) is associated with stress responses [82, 83]. Recently, it has been found that AKHs are also involved in the control of oxidative stress (OS) in insects [79]. The balance between the OS and its control by AKHs was attained by feedback regulation between an oxidative stressor and AKH actions. The effect is reported for paraquat [84, 85], *Galanthus nivalis* agglutinin and *Bacillus thuringiensis* toxin [84] and also for endosulfan and malathion [86] insecticides with an OS effect. Reports are available for the elevation of AKH in stress other than OS [53–56]. It was suggested that the activation of protective anti-oxidative mechanisms is derived from the effect of oxidative stressors on AKH level in the insect body.

Not only have AKHs been implicated to be involved in hormonal control of antioxidative protective reactions in insects, but other hormones such as glucagon, ecdysteroids, and JHs have also been suggested to be involved in antioxidative protective mechanisms. Analogous to vertebrates [87–89], it was suggested for insects that glucagon could play a role in defense against OS. Ecdysteroids were shown to be involved in the control of OS by inhibiting the oxidation of cholesterol and the peroxidation of polyunsaturated fatty acids in the lipoproteins, microsomes, and other components of biological systems [90].

8.2. 20-Hydroxyecdysone (20E)

In *D. melanogaster*, ecdysone-induced methionine sulfoxide reductase A enhances resistance to hydrogen peroxide [91]. It also regulates the methionine sulfoxide reductase [92, 93] enzyme

expression via the ecdysone receptor (EcR-UPS) complex [54]. Treatment with insect 20E protected mammals against cerebral ischemia injury by inhibiting production of ROS/RNS and modulating OS-induced signal transduction pathways. Treatment of B35 rat neuroblastoma cells with hydrogen peroxide led to OS-induced apoptosis, mitochondrial membrane potential dissipation, neuronal injury, generation of intracellular ROS/RNS, decrease of cellular antioxidant potential, and increase of lipid peroxidation, all of which were significantly eliminated by 20E [54, 94–97].

8.3. Juvenile hormone

JHs are synthesized and secreted in/from the corpora allata in the insect brain. Recent studies have implicated that lack of JH may confer resistance to OS. The elimination of JH synthesis extended the survival of flies exposed to hydrogen peroxide. The survival times returned to those in control when the knockout flies were treated with JH analog methoprene. Moreover, another JH analog, pyriproxyfen, induced OS in the wax moth *Galleria mellonella*, as the antioxidative enzyme activities, CAT and SOD, increased after the pyriproxyfen application. Similarly, pyriproxyfen increased the activity of enzymes CAT and GST, as well as the accumulation of MDA and GSH, in larvae of *S. littoralis*. JHs are also involved in the control of antioxidative reactions albeit indirectly via the regulation of vitellogenin and transferrin synthesis [54, 94–97].

9. Summary

This chapter summarizes current knowledge on pro- and antioxidant processes in invertebrates particularly insects. Reactive oxygen species (ROS) formation and their adaptations in different organisms were discussed in the context of the usage of invertebrates as a model organism in the field of OS to study the OS-mediated diseases in humans.

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References

- [1] Betteridge, D.J., What is oxidative stress? *Metabolism*, 2000. 49(2 Suppl 1): p. 3–8.
- [2] Evans, P. and B. Halliwell, Free radicals and hearing. Cause, consequence, and criteria. *Ann N Y Acad Sci*, 1999. 884: p. 19–40.
- [3] Valko, M., et al., Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*, 2006. 160(1): p. 1–40.
- [4] Uday B. et al., Reactive oxygen species: oxidative damage and pathogenesis. *Curr Sci*, 1999. 77(5–10).
- [5] Ahmad, S. and R.S. Pardini, Mechanisms for regulating oxygen toxicity in phytophagous insects. *Free Radic Biol Med*, 1990. 8(4): p. 401–13.
- [6] Kirkinezos, I.G. and C.T. Moraes, Reactive oxygen species and mitochondrial diseases. *Semin Cell Dev Biol*, 2001. 12(6): p. 449–57.
- [7] Saeaeue, L., et al., Antioxidative systems defense against oxidative stress induced by blood meal in *Aedes aegypti*. *Southeast Asian J Trop Med Public Health*, 2011. 42(3): p. 542–9.
- [8] Bhatnagar, R.K., et al., Synthetic propeptide inhibits mosquito midgut chitinase and blocks sporogonic development of malaria parasite. *Biochem Biophys Res Commun*, 2003. 304(4): p. 783–7.
- [9] Araújo A.P., et al., Disruption of the peritrophic matrix by exogenous chitinase feeding reduces fecundity in *Lutzomyia longipalpis* females. *Mem Inst Oswaldo Cruz* 2012 107(4): 543–45.
- [10] Tellam, R.L., G. Wijffels, and P. Willadsen, Peritrophic matrix proteins. *Insect Biochem Mol Biol*, 1999. 29(2): p. 87–101.
- [11] Zhao, H.W., et al., Experimental selection for *Drosophila* survival in extremely high O₂ environments. *PLoS One*, 2010. 5(7): p. e11701.
- [12] Ha, E.M., et al., A direct role for dual oxidase in *Drosophila* gut immunity. *Science*, 2005. 310(5749): p. 847–50.
- [13] Chaudhuri, A., et al., Interaction of genetic and environmental factors in a *Drosophila* parkinsonism model. *J Neurosci*, 2007. 27(10): p. 2457–67.
- [14] Gruenewald, C., et al., Hyperoxia-induced neurodegeneration as a tool to identify neuroprotective genes in *Drosophila melanogaster*. *Free Radic Biol Med*, 2009. 46(12): p. 1668–76.
- [15] Kounatidis, I. and P. Ligoxygakis, *Drosophila* as a model system to unravel the layers of innate immunity to infection. *Open Biol*, 2012. 2(5): p. 120075.

- [16] Wagner, C., et al., Molecular architecture of the fruit fly's airway epithelial immune system. *BMC Genomics*, 2008. 9: p. 446.
- [17] Ha, E.M., et al., An antioxidant system required for host protection against gut infection in *Drosophila*. *Dev Cell*, 2005. 8(1): p. 125–32.
- [18] Ha, E.M., et al., Regulation of DUOX by the Galphaq-phospholipase C β -Ca²⁺ pathway in *Drosophila* gut immunity. *Dev Cell*, 2009. 16(3): p. 386–97.
- [19] Ryu, J.H., E.M. Ha, and W.J. Lee, Innate immunity and gut-microbe mutualism in *Drosophila*. *Dev Comp Immunol*, 2010. 34(4): p. 369–76.
- [20] Bidla, G., M.S. Dushay, and U. Theopold, Crystal cell rupture after injury in *Drosophila* requires the JNK pathway, small GTPases and the TNF homolog Eiger. *J Cell Sci*, 2007. 120(Pt 7): p. 1209–15.
- [21] McConville, M.J., et al., Living in a phagolysosome; metabolism of *Leishmania* amastigotes. *Trends Parasitol*, 2007. 23(8): p. 368–75.
- [22] Koppenol, W.H., et al., Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res Toxicol*, 1992. 5(6): p. 834–42.
- [23] Castro, H., et al., Complementary antioxidant defense by cytoplasmic and mitochondrial peroxiredoxins in *Leishmania infantum*. *Free Radic Biol Med*, 2002. 33(11): p. 1552–62.
- [24] Henderson, G.B., et al., Biosynthesis of the trypanosomatid metabolite trypanothione: purification and characterization of trypanothione synthetase from *Crithidia fasciculata*. *Biochemistry*, 1990. 29(16): p. 3924–9.
- [25] Krauth-Siegel, R.L. and M.A. Comini, Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochim Biophys Acta*, 2008. 1780(11): p. 1236–48.
- [26] Ariyanayagam, M.R. and A.H. Fairlamb, Ovothiol and trypanothione as antioxidants in trypanosomatids. *Mol Biochem Parasitol*, 2001. 115(2): p. 189–98.
- [27] Dumas, C., et al., Disruption of the trypanothione reductase gene of *Leishmania* decreases its ability to survive oxidative stress in macrophages. *EMBO J*, 1997. 16(10): p. 2590–8.
- [28] Rhee, S.G., et al., Peroxiredoxin, a novel family of peroxidases. *IUBMB Life*, 2001. 52(1–2): p. 35–41.
- [29] Kropf, P., et al., Signaling through the T1/ST2 molecule is not necessary for Th2 differentiation but is important for the regulation of type 1 responses in nonhealing *Leishmania* major infection. *Infect Immun*, 2003. 71(4): p. 1961–71.
- [30] Iniesta, V., L.C. Gomez-Nieto, and I. Corraliza, The inhibition of arginase by N(omega)-hydroxy-L-arginine controls the growth of *Leishmania* inside macrophages. *J Exp Med*, 2001. 193(6): p. 777–84.

- [31] Forget, G., et al., Role of host phosphotyrosine phosphatase SHP-1 in the development of murine leishmaniasis. *Eur J Immunol*, 2001. 31(11): p. 3185–96.
- [32] Campos-Neto, A., What about Th1/Th2 in cutaneous leishmaniasis vaccine discovery? *Braz J Med Biol Res*, 2005. 38(7): p. 979–84.
- [33] Maiorino, M., et al., A selenium-containing phospholipid-hydroperoxide glutathione peroxidase in *Schistosoma mansoni*. *Eur J Biochem*, 1996. 238(3): p. 838–44.
- [34] Williams, D.L., et al., Molecular cloning and sequencing of glutathione peroxidase from *Schistosoma mansoni*. *Mol Biochem Parasitol*, 1992. 52(1): p. 127–30.
- [35] Das, S., et al., The complex world of cellular defense in the *Leishmania* parasite. *Proc Ind Natl Sci Acad*, 2015. 81(3): p. 629–41.
- [36] Ahmad, S., Oxidative stress from environmental pollutants. *Arch Insect Biochem Physiol*, 1995. 29(2): p. 135–57.
- [37] Sakihama, Y., et al., Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, 2002. 177(1): p. 67–80.
- [38] Krishnan, N. and F. Sehnal, Compartmentalization of oxidative stress and antioxidant defense in the larval gut of *Spodoptera littoralis*. *Arch Insect Biochem Physiol*, 2006. 63(1): p. 1–10.
- [39] Jomova, K. and M. Valko, Advances in metal-induced oxidative stress and human disease. *Toxicology*, 2011. 283(2–3): p. 65–87.
- [40] Nedal M.F., Impact of two insect growth regulators on the enhancement of oxidative stress and antioxidant efficiency of the cotton leaf worm, *Spodoptera littoralis* (Biosd.). *Egypt Acad J Biolog Sci*, 2012. 5(1): p. 137–49.
- [41] Hybertson, B.M., et al., Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol Aspects Med*, 2011. 32(4–6): p. 234–46.
- [42] Donaghy, L., et al., The known and unknown sources of reactive oxygen and nitrogen species in haemocytes of marine bivalve molluscs. *Fish Shellfish Immunol*, 2015. 42(1): p. 91–7.
- [43] Abele, D. and S. Puntarulo, Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comp Biochem Physiol A Mol Integr Physiol*, 2004. 138(4): p. 405–15.
- [44] Jaganjac, M., et al., Reactive aldehydes—second messengers of free radicals in diabetes mellitus. *Free Radic Res*, 2013. 47 Suppl 1: p. 39–48.
- [45] Felton, G.W. and C.B. Summers, Antioxidant systems in insects. *Arch Insect Biochem Physiol*, 1995. 29(2): p. 187–97.

- [46] Krishnan, N. and D. Kodrik, Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): are they enhanced to protect gut tissues during oxidative stress? *J Insect Physiol*, 2006. 52(1): p. 11–20.
- [47] Krishnan, N., et al., Stage-specific distribution of oxidative radicals and antioxidant enzymes in the midgut of *Leptinotarsa decemlineata*. *J Insect Physiol*, 2007. 53(1): p. 67–74.
- [48] Krishnan, N., et al., 20-Hydroxyecdysone prevents oxidative stress damage in adult *Pyrrhocoris apterus*. *Arch Insect Biochem Physiol*, 2007. 65(3): p. 114–24.
- [49] Mathews, M.C., C.B. Summers, and G.W. Felton, Ascorbate peroxidase: a novel antioxidant enzyme in insects. *Arch Insect Biochem Physiol*, 1997. 34(1): p. 57–68.
- [50] Krishnan, N., et al., Glutathione-ascorbic acid redox cycle and thioredoxin reductase activity in the digestive tract of *Leptinotarsa decemlineata* (Say). *Insect Biochem Mol Biol*, 2009. 39(3): p. 180–8.
- [51] Yu, B.P., Cellular defenses against damage from reactive oxygen species. *Physiol Rev*, 1994. 74(1): p. 139–62.
- [52] Kanzok, S.M., et al., Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster*. *Science*, 2001. 291(5504): p. 643–6.
- [53] Amdam, G.V., et al., Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. *Exp Gerontol*, 2004. 39(5): p. 767–73.
- [54] Seehuus, S.C., et al., Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proc Natl Acad Sci U S A*, 2006. 103(4): p. 962–967.
- [55] Choi, J., et al., Oxidative modifications and aggregation of Cu,Zn-superoxide dismutase associated with Alzheimer and Parkinson diseases. *J Biol Chem*, 2005. 280(12): p. 11648–55.
- [56] Quinlan, G.J., G.S. Martin, and T.W. Evans, Albumin: biochemical properties and therapeutic potential. *Hepatology*, 2005. 41(6): p. 1211–9.
- [57] Sanders, H.R., et al., Blood meal induces global changes in midgut gene expression in the disease vector, *Aedes aegypti*. *Insect Biochem Mol Biol*, 2003. 33(11): p. 1105–22.
- [58] Geiser, D.L., D. Zhang, and J.J. Winzerling, Secreted ferritin: mosquito defense against iron overload? *Insect Biochem Mol Biol*, 2006. 36(3): p. 177–87.
- [59] Wu, H., M.C. Wang, and D. Bohmann, JNK protects *Drosophila* from oxidative stress by transcriptionally activating autophagy. *Mech Dev*, 2009. 126(8–9): p. 624–37.
- [60] Dias-Santagata, D., et al., Oxidative stress mediates tau-induced neurodegeneration in *Drosophila*. *J Clin Invest*, 2007. 117(1): p. 236–45.

- [61] Bahadorani, S. and A.J. Hilliker, Antioxidants cannot suppress the lethal phenotype of a *Drosophila melanogaster* model of Huntington's disease. *Genome*, 2008. 51(5): p. 392–5.
- [62] Tettweiler, G., et al., Starvation and oxidative stress resistance in *Drosophila* are mediated through the eIF4E-binding protein, d4E-BP. *Genes Dev*, 2005. 19(16): p. 1840–3.
- [63] Zhang, D.D., Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metab Rev*, 2006. 38(4): p. 769–89.
- [64] Harman, D., Aging: a theory based on free radical and radiation chemistry. *J Gerontol*, 1956. 11(3): p. 298–300.
- [65] Svensson, M.J. and J. Larsson, Thioredoxin-2 affects lifespan and oxidative stress in *Drosophila*. *Hereditas*, 2007. 144(1): p. 25–32.
- [66] Lee, F.K., et al., The role of ubiquitin linkages on alpha-synuclein induced-toxicity in a *Drosophila* model of Parkinson's disease. *J Neurochem*, 2009. 110(1): p. 208–19.
- [67] Radyuk, S.N., et al., Mitochondrial peroxiredoxins are critical for the maintenance of redox state and the survival of adult *Drosophila*. *Free Radic Biol Med*, 2010. 49(12): p. 1892–902.
- [68] Mockett, R.J., R.S. Sohal, and W.C. Orr, Overexpression of glutathione reductase extends survival in transgenic *Drosophila melanogaster* under hyperoxia but not normoxia. *FASEB J*, 1999. 13(13): p. 1733–42.
- [69] Missirlis, F., et al., A putative glutathione peroxidase of *Drosophila* encodes a thioredoxin peroxidase that provides resistance against oxidative stress but fails to complement a lack of catalase activity. *Biol Chem*, 2003. 384(3): p. 463–72.
- [70] Tang, A.H. and C.P.D. Tu, Biochemical-characterization of *Drosophila* glutathione S-transferases D1 and D21. *J Biol Chem*, 1994. 269(45): p. 27876–84.
- [71] Toba, G. and T. Aigaki, Disruption of the microsomal glutathione S-transferase-like gene reduces life span of *Drosophila melanogaster*. *Gene*, 2000. 253(2): p. 179–87.
- [72] Moskovitz, J., H. Weissbach, and N. Brot, Cloning and expression of a mammalian gene involved in the reduction of methionine sulfoxide residues in proteins. *Proc Natl Acad Sci U S A*, 1996. 93(5): p. 2095–9.
- [73] Kumar, R.A., et al., Reaction mechanism, evolutionary analysis, and role of zinc in *Drosophila* Methionine-R-sulfoxide reductase. *J Biol Chem*, 2002. 277(40): p. 37527–35.
- [74] Whitworth, A.J., et al., Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *Proc Natl Acad Sci U S A*, 2005. 102(22): p. 8024–9.
- [75] Davies, S.A., et al., Cell signalling mechanisms for insect stress tolerance. *J Exp Biol*, 2014. 217(Pt 1): p. 119–28.

- [76] Chintapalli, V.R., J. Wang, and J.A. Dow, Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. *Nat Genet*, 2007. 39(6): p. 715–20.
- [77] Dow, J.A., Insights into the Malpighian tubule from functional genomics. *J Exp Biol*, 2009. 212(Pt 3): p. 435–45.
- [78] Wang, J., et al., Function-informed transcriptome analysis of *Drosophila* renal tubule. *Genome Biol*, 2004. 5(9): p. R69.
- [79] Owusu-Ansah, E. and U. Banerjee, Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. *Nature*, 2009. 461(7263): p. 537–41.
- [80] Radyuk, S.N., et al., Peroxiredoxin 5 modulates immune response in *Drosophila*. *Biochim Biophys Acta*, 2010. 1800(11): p. 1153–63.
- [81] Orr, W.C. and R.S. Sohal, Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science*, 1994. 263(5150): p. 1128–30.
- [82] Scarborough, R.M., et al., Isolation and primary structure of two peptides with cardioacceleratory and hyperglycemic activity from the corpora cardiaca of *Periplaneta americana*. *Proc Natl Acad Sci U S A*, 1984. 81(17): p. 5575–9.
- [83] Goldsworthy, G., K. Opoku-Ware, and L. Mullen, Adipokinetic hormone enhances laminarin and bacterial lipopolysaccharide-induced activation of the prophenoloxidase cascade in the African migratory locust, *Locusta migratoria*. *J Insect Physiol*, 2002. 48(6): p. 601–8.
- [84] Kodrik, D., N. Krishnan, and O. Habustova, Is the titer of adipokinetic peptides in *Leptinotarsa decemlineata* fed on genetically modified potatoes increased by oxidative stress? *Peptides*, 2007. 28(5): p. 974–80.
- [85] Vecera, J., et al., Adipokinetic hormone-induced enhancement of antioxidant capacity of *Pyrrhocoris apterus* hemolymph in response to oxidative stress. *Comp Biochem Physiol C Toxicol Pharmacol*, 2007. 146(3): p. 336–42.
- [86] Velki, M., et al., Oxidative stress elicited by insecticides: a role for the adipokinetic hormone. *Gen Comp Endocrinol*, 2011. 172(1): p. 77–84.
- [87] Lu, S.C., et al., Hormonal regulation of glutathione efflux. *J Biol Chem*, 1990. 265(27): p. 16088–95.
- [88] Lu, S.C., et al., Hormone-mediated down-regulation of hepatic glutathione synthesis in the rat. *J Clin Invest*, 1991. 88(1): p. 260–9.
- [89] Alquicer, G., et al., Activation of insect anti-oxidative mechanisms by mammalian glucagon. *Comp Biochem Physiol B Biochem Mol Biol*, 2009. 152(3): p. 226–33.
- [90] Lacort, M., et al., Protective effect of estrogens and catecholestrogens against peroxidative membrane damage in vitro. *Lipids*, 1995. 30(2): p. 141–6.

- [91] Roesijadi, G., et al., Ecdysone induction of MsrA protects against oxidative stress in *Drosophila*. *Biochem Biophys Res Commun*, 2007. 354(2): p. 511–6.
- [92] Weissbach, H., L. Resnick, and N. Brot, Methionine sulfoxide reductases: history and cellular role in protecting against oxidative damage. *Biochim Biophys Acta*, 2005. 1703(2): p. 203–12.
- [93] Hu, X., L. Cherbas, and P. Cherbas, Transcription activation by the ecdysone receptor (EcR/USP): identification of activation functions. *Mol Endocrinol*, 2003. 17(4): p. 716–31.
- [94] Jamroz, R.C., et al., Transferrin in a cockroach: molecular cloning, characterization, and suppression by juvenile hormone. *Proc Natl Acad Sci U S A*, 1993. 90(4): p. 1320–4.
- [95] Harizanova, N., et al., *Aedes aegypti* transferrin. Gene structure, expression pattern, and regulation. *Insect Mol Biol*, 2005. 14(1): p. 79–88.
- [96] Andrus, P.K., et al., Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem*, 1998. 71(5): p. 2041–8.
- [97] Kodrik, D., et al., Hormonal regulation of response to oxidative stress in insects—an update. *Int J Mol Sci* 2015, 16, 25788–816.