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OXIDATIVE STRESS IN THE AIRWAY SYSTEM OF THE FRUIT FLY *DROSOPHILA MELANOGASTER*

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19.1 INTRODUCTION

Oxidative stress is a major threat for all organisms living in a high-oxygen atmosphere. Reactive oxygen species (ROS) can be produced either exogenously or endogenously. The major exogenous source of ROS is the atmosphere, which implies that especially those organs or parts of the body that come into close contact with large amounts of air are at risk. This would mean that the airway epithelium is prone to be damaged by ROS and thus needs specialized mechanisms to cope with this situation. Endogenous production of ROS is highest in those tissues with the greatest energy consumption, as it is an inevitable by-product of mitochondrial activity. To cope with this ROS confrontation, animals employ different sets of enzymatic as well as nonenzymatic antioxidants. ROS-mediated damage of tissues and cells is believed to be one major reason for changes associated with aging. The oxidative stress theory of aging that was introduced more than 50 years ago [1] predicts that effective scavenging of ROS may delay aging and thus should increase life span. Free radicals and other ROS induce molecular damage at different levels, leading to increased incidences of mortality as a function of time. These ROS-mediated damages that can occur in all major groups of macromolecules accumulate over time, and, at a certain time-point, the organism is unable to repair all of them. Accumulation of these damages interferes with the normal physiological function of the cells, which may end up in reduced overall performance of the organism and finally in mortality. A great number of studies have supported this theory, especially those performed with model organisms such as the fruit fly *Drosophila melanogaster*. In general, increasing the antioxidant repertoire of these model organisms tends to increase life span. In particular, two enzymatic antioxidants, superoxide dismutase (SOD) and catalase, tend to mediate life-prolonging effects in *Drosophila* or *Caenorhabditis elegans*, an observation that is still matter of debate.

19.2 OXIDATIVE STRESS SYSTEMS IN *DROSOPHILA*

A number of different tissues show a higher sensitivity toward oxidative stress than others. Among these are the nervous system, but also the airway epithelium, as this structure is constantly exposed to high oxygen flow rates and therefore to ROS. Regarding nonenzymatic antioxidants that are employed by the fly, our knowledge is limited. Interestingly, chronic food supplementation with compounds having antioxidant properties has the potential to increase life span, indicating that nonenzymatic antioxidants are of relevance for various aspects of the fly's biology [2]. In addition to these nonenzymatic antioxidants, a very comprehensive armamentarium of enzymatic antioxidants can scavenge these compounds, thus protecting the organism. Among these compounds are superoxide dismutases, catalase, peroxiredoxins,

Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling, First Edition.
Edited by Tahira Farooqui and Akhlaq A. Farooqui.

thioredoxins, and the plethora of enzymes involved in glutathione metabolism. These enzymes are either constitutively present in different organs of the fly or the expression of the corresponding genes is induced after exposure to various stressors.

A large number of signaling pathways are involved in the response to oxidative stress and are therefore necessary to mount an adequate antioxidative response. In most of these cases, these cellular responses comprise not only oxidative stress resistance but also a general stress resistance. Among these signaling pathways that are of central importance for a proper response to oxidative stress is the PI3-kinase pathway, which converges onto activation of the transcription factor FoxO [3]. FoxO activation leads to expression of for example, 4E-BP, which is required for a proper and efficient expression of other target genes, mainly those associated with an increase in oxidative stress resistance. An alternative way of signaling is represented by the Nrf2/Keap1-pathway (Fig. 19.1). This is a system that appears to be tailored to sense oxidative stress and to induce an adequate cellular reaction. In unstressed conditions, Nrf2 is kept in the cytoplasm by its inhibitor Keap1. Keap1 has also the potential to act as a sensor for oxidants, by reacting with these compounds via redox-sensitive cysteine residues [4].

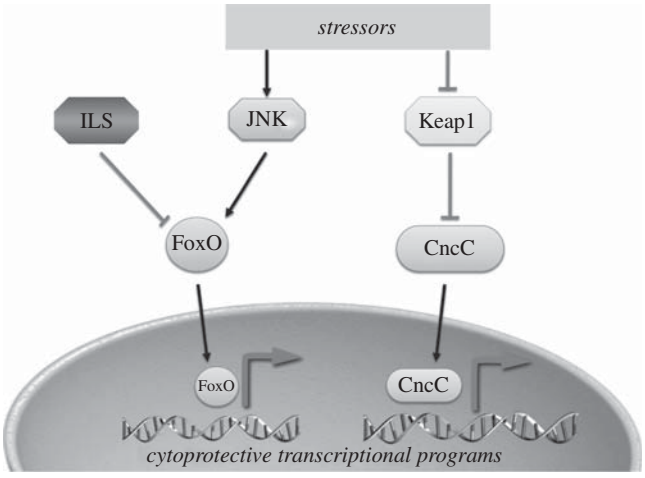

Fig. 19.1 The two transcription factors FoxO and Nrf2 (CncC) are activated by stressors and induce a cytoprotective transcriptional program operative in the airway epithelial cell. FoxO can be activated by a variety of stressors via the JNK pathway, whereas it is inhibited by the insulin-like signaling pathway (ILS). After activation, FoxO translocates into the nucleus, where it initiates transcription of various genes, e.g., those coding for enzymatic antioxidants. On the other hand, Nrf2 (CncC in *Drosophila*) is usually inhibited by Keap1 and held back in the cytoplasm. After stress application, Keap1 is inhibited, thus releasing CncC and allowing it to translocate into the nucleus, where it also starts a cytoprotective transcriptional program (e.g., transcription of glutathione-*S*-transferases).

Thus oxidants lead to dissociation of the complex between Nrf2 (CncC in *Drosophila*) and Keap1, allowing Nrf2 to translocate into the nucleus, where it binds to the antioxidant response element (ARE), an enhancer sequence present in the target genes of Nrf2 activation. Expression of these target genes represents the so-called electrophile counterattack comprising a whole battery of enzymatic antioxidants of all flavors. Among these are thioredoxins, enzymes of the glutathione metabolism, but also chaperones and parts of the proteasome [5]. In the fly, the Nrf2/Keap1 system fulfils all the tasks described above, and persistent activation of Nrf2 signaling via depletion of Keap1 increases resistance against oxidative stress and life span.

Other signaling systems known to be relevant for the resistance against stressors in general are also known to have an impact on the resistance against oxidative stress. The *Drosophila* p38 ortholog, which is an essential part of the p38 mitogen-activated protein kinase (MAPK) cascade, is essential for a reaction and the resistance against oxidative stress, but also for the resistances against heat stress and starvation [6]. p38, as one of the major parts of the MAPK pathway, has a high potential as a target for intervention in chronic inflammatory diseases including rheumatoid arthritis or asthma, but the side effects caused by chronic interference with its normal function are still not well understood.

19.3 REACTIVE OXYGEN SPECIES AND AGING

A direct connection between aging and oxidative stress induced damages was the idea behind one of the first theories of aging [1]. Accumulation of these damages over time leads to impairment of cellular function and finally to death of the organism. Studies from different fields show a connection between aging and ROS-mediated impairments of various different features. Aging and the reaction to oxidative stress share a number of identical transcript signatures. In particular, genes coding for enzymatic antioxidants and immune relevant genes are upregulated under these two different conditions [7]. Nevertheless, it must be kept in mind that the correlation between oxidative stress-mediated impairments and the physiological decline of various abilities during aging is not necessarily direct [8]. Decline in sensory perception occurs at the same rate in control flies and those showing a high oxidative stress resistance. Although the contribution of single parts of the antioxidant armamentarium to life span is hard to quantify, it is common sense that a highly effective antioxidant response is beneficial. A relatively large number of enzymatic antioxidants appear to have a positive effect on longevity if present in the organism. Among these

are, for example, the thioredoxins; corresponding knockout flies lacking a functional Thioredoxin-2 have a shortened life span [9]. A comparable effect on life span has been attributed to a peroxiredoxin of type II, named Jafrac1, whose overexpression in the nervous system appears to increase life span whereas its downregulation has the opposite effect [10]. Interestingly, Jafrac1 is a target gene of the JNK pathway that is activated after stress and cellular damage to restore the cellular homeostasis. 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 [11].

Regarding the role of enzymes involved in glutathione metabolism, the picture is relatively complex because of the large number of gene products being part of this machinery. Glutathione reductase increases resistance under hyperoxic conditions, whereas no effects on life span could be observed at normoxia [12]. The hypothesis that glutathione metabolism is involved in life span regulation was further supported by the observation that overexpression of the glutamate-cysteine ligase, which is the rate-limiting enzyme in glutathione de novo synthesis, increases life span by more than 20% [13].

Two other sets of enzymatic antioxidants have been implicated in life span extension, the SODs and catalase. In particular, the role of SODs in this context is still a matter of debate. Overexpression of a human SOD1 in motoneurons only was shown to be sufficient for a 40% life span extension [14]. As mentioned above, overexpression of SODs was shown to increase life span significantly, but this effect appeared to be restricted to short-living strains of *Drosophila melanogaster* [15]. A combined overexpression of SOD and catalase appears to represent a more robust way of life span extension compared with overexpression of either of the two components [15, 16]. This observation is in line with the enzymatic activities of both enzymes that act in common.

Hyperoxia is a relatively simple way to induce oxidative stress. Although very high oxygen concentrations are lethal to naive flies, it was possible to select for survivors under these hostile conditions. These flies show phenotypical differences compared with control flies, and their gene expression profile was altered accordingly. Very interestingly, 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 [17, 18].

The plethora of signaling and effector molecules involved in the response to oxidative stress indicates that this response is of vital importance for the organism. Apparently, this response is tightly controlled and obviously of vital importance, especially regarding life span-associated aspects.

19.4 OXIDATIVE STRESS IN THE NERVOUS SYSTEM

Most important among these is obviously the nervous system, where ROS-mediated decline of neuron survival can be observed. As could have been expected, one class of neurons shows highest sensitivity to oxidative stress, namely, the dopamine-producing cells. Obviously, the dopamine production machinery is inevitably linked to an endogenous production of relatively high amounts of ROS, thus making these cells prone to damages caused by "extra doses" of ROS. This high sensitivity is the major reason why dopamine-producing neurons die whereas other neurons are almost unaltered [19]. This highly interesting observation enables us to link high ROS concentration with the development of Parkinson disease-like phenotypes. One major reason for the development of this disease is believed to be chronic confrontation with very high ROS concentration in conjunction with the sensitivity of dopamine-producing cells in general [19]. Consequently, confrontation with ROS doses is a suitable mechanism to induce neurodegenerative processes as typically seen in Parkinson disease. One of the most reproducible ways to do this is hyperoxia, which consequently is able to induce these phenotypes [20]. Especially based on these approaches, it was possible to verify hypotheses that ROS-scavenging enzymes should reduce the sensitivity to these high ROS levels in corresponding *Drosophila* models. SOD overexpression was sufficient to protect the dopaminergic cells, whereas catalase was not [20]. In addition, increasing the glutathione-*S*-transferase activity in the nervous system protects these dopaminergic neurons in another *Drosophila* model of Parkinson disease [21].

In an Alzheimer disease model based on tau-activation in the nervous system, ROS have been shown to modulate the sensitivity of the animals, thus demonstrating that oxidative stress plays a major role in the development of Alzheimer disease [22]. The positive effects of enzymatic and nonenzymatic antioxidants on disease progression appear not to hold true for all neurodegenerative diseases. In a well-established model for Huntington disease, neither overexpression of enzymatic antioxidants nor supplementation with nonenzymatic antioxidants decreased the lethality in this model [23].

19.5 OXIDATIVE STRESS IN THE DIGESTIVE SYSTEM

The intestinal immune system is characterized by a very special situation. It faces one of the most dense

populations on earth, the intestinal microbiome. Thus the major regulatory challenge for this organ is to hold a homeostatic balance between fight against potential pathogens and maintenance of the microbial community within the intestinal tract. Intestinal immune reactions are therefore different from those of other epithelia such as the airway epithelium. Whereas the latter reacts quickly and strongly with the expression of antimicrobial compounds to confrontation with bacteria, the intestinal epithelium is refractory to this type of stimuli and reacts only relatively weakly. To complement this type of response and to fight potential pathogens, the intestinal immune system acts in a completely different way. Fighting pathogens is achieved via enzymes producing ROS. Most important among them is obviously the dual oxidase (DUOX) that is secreted from enterocytes in response to pathogen contact [24, 25]. DUOX is a member of the nicotinamide adenine dinucleotide oxidase (NADPH oxidase) family. Surprisingly, the intracellular signaling pathway transducing the recognition of pathogens into the secretion of DUOX has nothing to do with classical immune relevant pathways such as the Toll or the IMD pathway. It comprises signaling pathways well known from neurotransmitter or hormonal signaling systems, namely, G-protein coupled receptor systems involved with the Gαq G-protein in a central position [26]. Downstream is the phospholipase C-β, also known as norpA. Animals defective in PLC-β have a shortened life span due to massive proliferation of an otherwise harmless microbe, the yeast *Saccharomyces cerevisiae*. This yeast is the major nutritional microbe in most *Drosophila* diets and has usually no pathogenic potential. The observation that PLC-β mutants, which are consequently unable to release DUOX, lost the ability to control this bug indicates that DUOX plays a very important role in maintenance and shaping of the microbiome [26]. The receptor transmitting these effects is yet not known, but activation of this pathway leads to IP_3 production and subsequent Ca^{2+} release from internal stores, which in turn is the trigger for DUOX release into the intestinal lumen. DUOX-defective mutants have a phenotype very similar to that of PLC-β-defective mutants. They are hypersensitive to proliferation of bacteria, even of nutritional bacteria that are otherwise harmless. Yet-unknown bacterial products, also acting through the unidentified G protein-coupled receptors mentioned above, trigger regulation of DUOX expression and release into the gut. The killing mechanism of DUOX comprises the production of H_2O_2, which is a typical feature of DUOX enzymes in general [27]. Although ROS production by DUOX is an important way to fight pathogens, some of these are resistant to oxidative stress; for this type of pathogens, NF-κB-mediated expression of antimicrobial peptide genes is a complementary type of reaction.

Oxidative stress produced by the epithelial cell is well suited to kill most of the invading bacteria but has the disadvantage that the organism's own tissue can be heavily injured. Thus the fly must have a two-sided type of response: While export of ROS-producing enzymes is important; protecting the fly's own epithelium from ROS-mediated damage is also. To ensure this type of concerted action, an enzymatic antioxidant, immune-regulated catalase (IRC), is produced to protect the cells from ROS-mediated damage. Consequently, IRC is required to survive contact with bacteria in the intestine, even if they are heat killed. These bacteria induce a massive ROS reaction that, if IRC is not there, can cause fatal damage to the cells of the intestine [25]. In addition, other mechanisms operative in the intestinal epithelium protect the organism's own cells from ROS present in the intestinal lumen. Sensing of this activates the JNK pathway, which in turn leads to activation of cellular adaptations allowing the organism to cope with this situation. ROS confrontation induces a JNK-triggered autophagy reaction of the enterocytes that helps to keep them alive under these otherwise very hostile conditions [28].

19.6 OXIDATIVE STRESS IN THE IMMUNE SYSTEM

Production of highly effective ROS is a versatile method to fight pathogens that is used by a great variety of cells of the innate immune system. The oxidative burst produced by, for example, macrophages is the most impressive example highlighting this strategy. Thus this way to combat pathogens is primarily used by motile cells of the innate immune system. In insect immunity, especially in the fruit fly's immune system, this type of response, namely an oxidative burst, has not been shown to be operative. Only the hemocytes, the motile cells of the immune system that have the ability to phagocytose invaders, come into consideration for this task, but they appear not to employ this strategy.

Nevertheless, ROS play an important role in the differentiation of hemocytes. In *Drosophila*, multipotent hematopoietic progenitors, the stem cells of the hemocyte lineage, react to different ROS levels with a speeding up or arresting of their differentiation [29]. In these progenitor cells, ROS levels are slightly enhanced, presumably to sensitize them for further differentiation processes. Further increasing the levels of ROS leads to a precocious differentiation into the final cells of this lineage (all three different types of hemocytes). Involved in the transduction of ROS levels into developmental

signals are obviously both the JNK and the FoxO pathway. In particular, the role of FoxO is interesting, as it usually induces the expression of genes that produce oxidative scavengers such as enzymatic antioxidants.

It has been suggested that ROS defense and immune response do not always act in the same direction. One particular peroxiredoxin, namely, Prd5, appears to have the capacity to modulate the immune response. Interestingly, knockout flies are more resistant to infection, whereas flies overexpressing this gene are more susceptible. Interestingly, these effects seem to be mediated via the JNK pathway, which has the capacity to link "damage" signals with the immune response of the fly [30].

One special aspect of the role of oxidative stress in immunity can be attributed to phenol oxidases (POs). POs are enzymes that produce a melanin coat surrounding invaders of different natures. These enzymes are produced as proenzymes, and are thus called pro-phenol oxidases (PPOs). In *Drosophila* three different PPOs are present (PPO1–3), which are predominantly expressed in different hemocyte subtypes. During melanization, these enzymes are able to produce a locally high concentration of ROS, which may be one of the major effectors of the PPO system. The conversion from PPOs to PO that occurs via limited proteolysis is tightly controlled because the end product, melanin, is highly toxic. This conversion is triggered by the recognition of bacterial patterns including peptidoglycans. Surprisingly, only for PPO3, can the conversion into the enzymatically active form PO be achieved without additional stimuli. Overexpression of this form of PPO was sufficient to induce massive melanization in almost every tissue targeted [31]. PPO1 and 2 are primarily expressed in crystal cells that are constitutively present in the hemolymph and react strongly to injury. These cells can release PPO1 and 2 to induce melanization in the hemolymph. PPO3, in turn, is restricted to lamellocytes that are usually not found constitutively in the hemolymph but are induced in the presence of larger intruders, such as parasitoids that inject their eggs into the body cavity of larvae. These cells surround these intruders and form a melanizing barrier to immobilize and finally kill the intruder [32]. Taken together, it is not known to what extent ROS are involved in the PO-mediated killing of invading microorganisms or larger intruders, or whether ROS production is only an inevitable by-product of melanin synthesis.

19.7 OXIDATIVE STRESS IN THE AIRWAY SYSTEM

The airway system of the fly has a very peculiar structure, made of interconnected tubes in a hierarchical order. First-order tubes, second-order tubes, and final branches make up the entire system. This organization guarantees that almost every cell of the body has direct access to an oxygen supply (Fig. 19.2). Throughout the entire system, a single layer of epithelial cells surrounds a central air-filled lumen. The corresponding cells are seemingly uniform in their response characteristics, while fulfilling the major tasks of airway epithelial cells, enabling an effective oxygen transport and exchange. These cells react to different stimuli with appropriate responses including massive responses if pathogens are experienced (Fig. 19.2). Oxygen is transported through this system to all parts of this body, causing a reasonable oxidative stress. 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. Our knowledge regarding the effects on the airways and mechanisms of the airway epithelium to counteract this stressful situation is almost nonexistent.

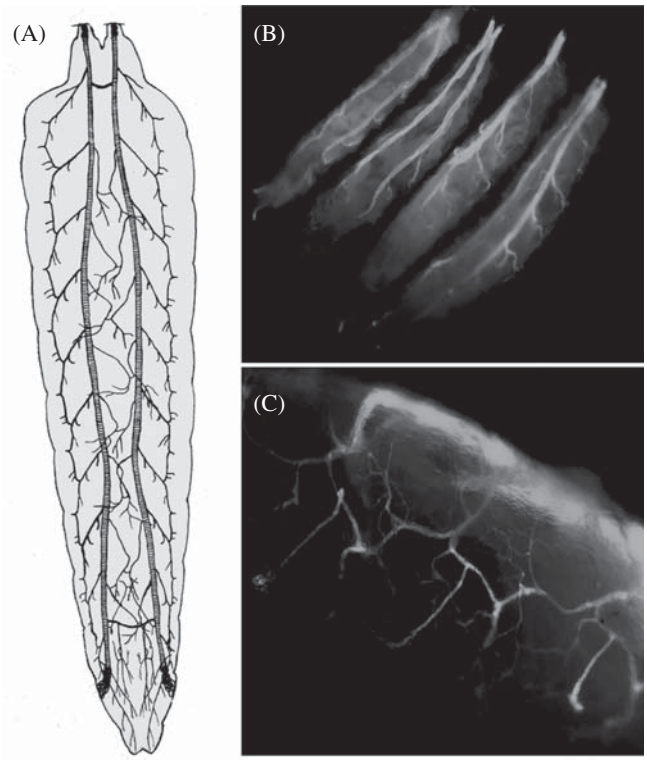

Fig. 19.2 Structure of the airway system of *Drosophila*. The airway system (trachea) of a larval fly is made up of interconnected tubes that deliver oxygen to almost every cell in the body (A). Upon stimulation with different stressors including infection, the airway epithelium launches a very effective response, comprising the expression of antimicrobial peptide genes (B and C, the latter at higher magnification). *(See color insert.)*

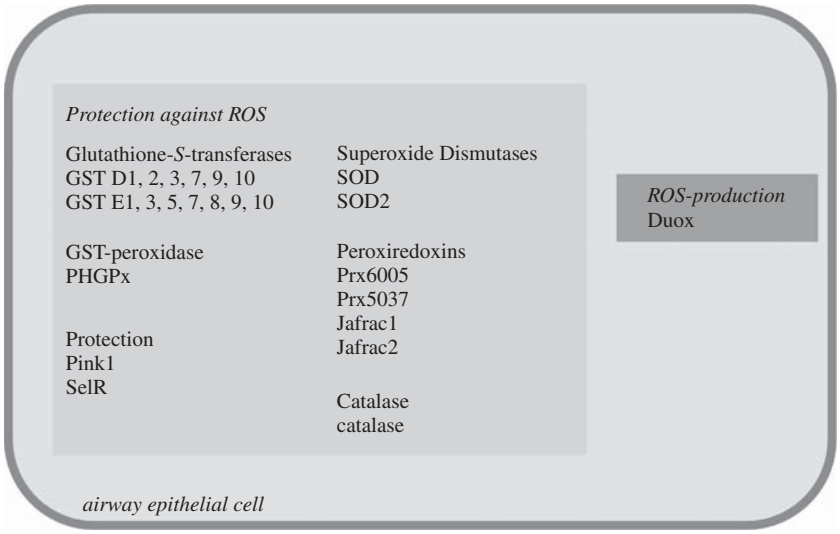

Fig. 19.3 Schematic drawing of reactive oxygen species (ROS)-producing enzymes and those protecting from ROS in the airway epithelial cells of the fly.

A recent study was performed with the aim of determining the relationship between oxygen concentration, oxidative stress, and life span [33]. Counterintuitively, the relation between these parameters is not linear, and reduced life spans were observed at maximal and minimal oxygen concentrations in the atmosphere.

It has been shown that all major enzymatic antioxidants are expressed in the airway epithelium. Among these, superoxide dismutase (SOD), catalase (cat), and all molecules involved in glutathione metabolism are of greatest importance. These enzymatic antioxidants are not only expressed in the airway epithelium but are also part of the reaction to stressful situations. The only well-studied situation is the reaction to airway infection [34]. In addition to classical aspects of an antibacterial response including of antimicrobial peptide genes, some of these enzymatic antioxidants are strongly upregulated.

The complex armamentarium of enzymatic antioxidants comprises both SODs (SOD and SOD2), the sole catalase, four different peroxiredoxins (Jafrac 1 and Jafrac 2, Prx 6005 and Prx 5037), a big consortium of glutathione-*S*-transferases (GST D1, 2, 3, 7, 9, 10, GST E1, 3, 5, 7, 8, 9, 10), the glutathione-peroxidase PHGPx, and molecules involved in protection such as *PINK1* and *SelR* (Fig. 19.3). A very potent producer of reactive oxygen species, the dual oxidase Duox, which is known to be very effective against invading bacteria, supplements this armamentarium. Thus the antioxidant system of the airway epithelium contains both producers of ROS and protectors against them. This architecture of oxidant and antioxidant systems allows the organism to cope with the very peculiar system in the airway, namely, that very high ROS concentrations have to be tolerated by these very peculiar cells.

19.8 CONCLUSION

ROS are an omnipresent threat for almost all cells in the body. In particular, those organs with highest metabolic rates and those directly exposed to the atmosphere are in danger. Thus the airway epithelium especially has to cope with this situation. To overcome problems associated with ROS-mediated damage, corresponding cytoprotective systems must be installed. In particular, enzymatic antioxidants including SOD, catalase, peroxiredoxins, thioredoxins, and those involved in glutathione metabolism are relevant. Although the vast majority of the corresponding genes are transcribed constitutively, different stressors can induce their expression further via the JNK-FoxO axis or the Nrf2 system. Our knowledge regarding their physiological role under different conditions is limited, although we know the almost complete armamentarium of antioxidants in the airway epithelium. With the experimental advantages of the *Drosophila* system, it should be possible to obtain an in-depth understanding of this very important regulatory system.

ACKNOWLEDGMENTS

The work in our group has been supported by the Deutsche Forschungsgemeinschaft (DFG SFB TR22).

REFERENCES

1. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956; 11: 298–300.

2. Bonilla E, Medina-Leendertz S, Diaz S. Extension of life span and stress resistance of *Drosophila melanogaster* by long-term supplementation with melatonin. *Exp Gerontol* 2002; 37: 629–638.
3. Tettweiler G, Miron M, Jenkins M, Sonenberg N, Lasko PF. Starvation and oxidative stress resistance in *Drosophila* are mediated through the eIF4E-binding protein, d4E-BP. *Genes Dev* 2005; 19: 1840–1843.
4. Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metabol Rev* 2006; 38: 769–789.
5. Sykiotis GP, Bohmann D. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Dev Cell* 2008; 14: 76–85.
6. Craig CR, Fink JL, Yagi Y, Ip YT, Cagan RL. A *Drosophila* p38 orthologue is required for environmental stress responses. *EMBO Rep* 2004; 5: 1058–1063.
7. Landis GN, Abdueva D, Skvortsov D, Yang J, Rabin BE et al. Similar gene expression patterns characterize aging and oxidative stress in Drosophila melanogaster. *Proc Natl Acad Sci USA* 2004; 101: 7663–7668.
8. Cook-Wiens E, Grotewiel MS. Dissociation between functional senescence and oxidative stress resistance in *Drosophila*. *Exp Gerontol* 2002; 37: 1347–1357.
9. Svensson MJ, Larsson J. Thioredoxin-2 affects lifespan and oxidative stress in *Drosophila*. *Hereditas* 2007; 144: 25–32.
10. Lee FK, Wong AK, Lee YW, Wan OW, Chan HY et al. The role of ubiquitin linkages on alpha-synuclein induced-toxicity in a Drosophila model of Parkinson's disease. *J Neurochem* 2009; 110: 208–219.
11. Radyuk SN, Rebrin I, Klichko VI, Sohal BH, Michalak K et al. Mitochondrial peroxiredoxins are critical for the maintenance of redox state and the survival of adult *Drosophila*. *Free Radic Biol Med* 2010; 49: 1892–1902.
12. Mockett RJ, Sohal RS, Orr WC. Overexpression of glutathione reductase extends survival in transgenic *Drosophila melanogaster* under hyperoxia but not normoxia. *FASEB J* 1999; 13: 1733–1742.
13. Orr WC, Radyuk SN, Prabhudesai L, Toroser D, Benes JJ et al. Overexpression of glutamate-cysteine ligase extends life span in *Drosophila melanogaster*. *J Biol Chem* 2005; 280: 37331–37338.
14. Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP et al. Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. *Nat Genet* 1998; 19: 171–174.
15. Orr WC, Sohal RS. Does overexpression of Cu, Zn-SOD extend life span in *Drosophila melanogaster? Exp Gerontol* 2003; 38: 227–230.
16. Orr WC, Sohal RS. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 1994; 263: 1128–1130.
17. Zhao HW, Zhou D, Haddad GG. Antimicrobial peptides increase tolerance to oxidant stress in *Drosophila melanogaster*. *J Biol Chem* 2011; 286: 6211–6218.
18. Zhao HW, Zhou D, Nizet V, Haddad GG. Experimental selection for Drosophila survival in extremely high O_2 environments. *PloS One* 2010; 5: e11701.
19. Chaudhuri A, Bowling K, Funderburk C, Lawal H, Inamdar A et al. Interaction of genetic and environmental factors in a *Drosophila* parkinsonism model. *J Neurosci* 2007; 27: 2457–2467.
20. Gruenewald C, Botella JA, Bayersdorfer F, Navarro JA, Schneuwly S. Hyperoxia-induced neurodegeneration as a tool to identify neuroprotective genes in *Drosophila melanogaster*. *Free Radic Biol Med* 2009; 46: 1668–1676.
21. Whitworth AJ, Theodore DA, Greene JC, Benes H, Wes PD et al. Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *Proc Natl Acad Sci USA* 2005; 102: 8024–8029.
22. Dias-Santagata D, Fulga TA, Duttaroy A, Feany MB. Oxidative stress mediates tau-induced neurodegeneration in *Drosophila*. *J Clin Invest* 2007; 117: 236–245.
23. Bahadorani S, Hilliker AJ. Antioxidants cannot suppress the lethal phenotype of a *Drosophila melanogaster* model of Huntington's disease. *Genome* 2008; 51: 392–395.
24. Ha EM, Oh CT, Bae YS, Lee WJ. A direct role for dual oxidase in *Drosophila* gut immunity. *Science* 2005; 310: 847–850.
25. Ha EM, Oh CT, Ryu JH, Bae YS, Kang SW et al. An antioxidant system required for host protection against gut infection in *Drosophila*. *Dev Cell* 2005; 8: 125–132.
26. Ha EM, Lee KA, Park SH, Kim SH, Nam HJ et al. Regulation of DUOX by the Galphaq-phospholipase Cbeta-Ca^{2+} pathway in *Drosophila* gut immunity. *Dev Cell* 2009; 16: 386–397.
27. Ryu JH, Ha EM, Lee WJ. Innate immunity and gut-microbe mutualism in *Drosophila*. *Dev Comp Immunol* 2010; 34: 369–376.
28. Wu H, Wang MC, Bohmann D. JNK protects Drosophila from oxidative stress by trancriptionally activating autophagy. *Mech Dev*. 2009; 126: 624–637.
29. Owusu-Ansah E, Banerjee U. Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. *Nature* 2009; 461: 537–541.
30. Radyuk SN, Michalak K, Klichko VI, Benes J, Orr WC. Peroxiredoxin 5 modulates immune response in *Drosophila*. *Biochim Biophys Acta* 2010; 1800: 1153–1163.
31. Nam HJ, Jang IH, Asano T, Lee WJ. Involvement of prophenoloxidase 3 in lamellocyte-mediated spontaneous melanization in *Drosophila*. *Mol Cells* 2008; 26: 606–610.
32. Bidla G, Dushay MS, Theopold U. Crystal cell rupture after injury in *Drosophila* requires the JNK pathway, small GTPases and the TNF homolog Eiger. *J Cell Sci* 2007; 120: 1209–1215.
33. Rascon B, Harrison JF. Lifespan and oxidative stress show a non-linear response to atmospheric oxygen in *Drosophila*. *J Exp Biol* 2010; 213: 3441–3448.
34. Wagner C, Isermann K, Fehrenbach H, Roeder T. Molecular architecture of the fruit fly's airway epithelial immune system. *BMC Genomics* 2008; 9: 446.