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The impact of metagenomic interplay on the mosquito redox homeostasis

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Abstract

Mosquitoes are exposed to oxidative challenges throughout their life cycle. The primary challenge comes from a blood meal. The blood digestion turns the midgut into an oxidative environment, which imposes pressure not only on mosquito fecundity and other physiological traits but also on the microbiota in the midgut. During evolution, mosquitoes have developed numerous oxidative defense mechanisms to maintain redox homeostasis in the midgut. In addition to antioxidants, SOD, catalase, and glutathione system, sufficient supply of the reducing agent, NADPH, is vital for a successful defense against oxidative stress. Increasing evidence indicates that in response to oxidative stress, cells reconfigure metabolic pathways to increase the generation of NADPH through NADP-reducing networks including the pentose phosphate pathway and others. The microbial homeostasis is critical for the functional contributions to various host phenotypes. The symbiotic microbiota is regulated largely by the Duox-ROS pathway in *Drosophila*. In mosquitoes, Duox-ROS pathway, heme-mediated signaling, antimicrobial peptide production and C-type lectins work in concert to maintain the dynamic microbial community in the midgut. Microbial mechanisms against oxidative stress in this context are not well understood. Emerging evidence that microbial metabolites trigger host oxidative response warrants further study on the metagenomic interplay in an oxidative environment like mosquito gut ecosystem. Besides the classical *Drosophila* model, hematophagous insects like mosquitoes provide an alternative model system to study redox homeostasis in a symbiotic metagenomic context.

Introduction

Hematophagy is the feeding habit of some animals that involves the ingestion of blood. Hematophagous arthropods include Diptera (mosquitoes, flies, and biting midges), Hemiptera (bed bugs and assassin bugs), Phthiraptera (sucking lice), and Siphonaptera (fleas). Most hematophagous insects possess piercing-and-sucking mouthparts and prey on much larger animals. This blood feeding behavior provides a point of pathogen transmission between host and insect. Among these hematophagous insects, vector mosquitoes are responsible for the transmission of human and animal diseases, such as Zika, Dengue fever,

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West Nile fever, Chikungunya, yellow fever, Eastern equine encephalitis, St. Louis encephalitis, malaria, and filariasis.

Mosquito females require a blood meal for egg production. Blood contains proteins and lipids, which are needed for oogenesis. The hemoglobin digestion releases a significant amount of heme. As a pro-oxidant, heme can induce oxidative stress by generating hydroxyl radicals through the Fenton reaction [1]. Mosquitoes also employ reactive oxygen species (ROS) to fight against various pathogens [2, 3]. For example, in a malaria-refractory mosquito strain, elevated levels of ROS are one of the factors that limit malaria parasite development [4]. As such, there is a selective pressure for adaptive strategies to mitigate the massive pulse of oxidative stress accompanied by the blood feeding and simultaneously retain the ability to combat infection through oxidative bursts [5].

The mosquito midgut harbors a dynamic microbiota. Symbiotic associations are ubiquitous in nature. Cross-kingdom interactions throughout the co-evolution have shaped the structure and functions of the microbiome; this process occurs largely in oxidative environments [6]. The shift of microbial structure after a blood meal [7] may represent an adaptive response to altered oxidative conditions in the blood-fed midgut. In the ecological niche in the midgut, both partners work in concert to maintain a redox homeostasis by launching multiple mechanisms to cope with elevated ROS levels. In this review, we summarize recent advances regarding the complex interplay of host and microbiota in maintaining redox homeostasis.

Heme signaling in transcriptional response in mosquitoes

Blood meal is the principal source of iron for egg production in mosquitoes. In a blood meal, iron is present in two forms, hemoglobin in erythrocytes and ferric-transferrin. According to Zhou *et al.* [8], 98% of iron in the eggs is obtained from hemoglobin, and 2% is from ferric-transferrin. However, only 7% of ingested heme iron is utilized in eggs; the remainder is discharged in excrement [8]. Heme is a pro-oxidant. The Fenton reaction using the iron from heme promotes hydroxyl radical formation, which in essence amplifies the magnitude of internal sources of ROS, and if not controlled, may lead to cell damage [9–11]. Adaptive response to the large quantity of heme in a blood meal is therefore of considerable importance to host physiology.

Heme has been shown to be a signaling molecule that is involved in the activation of steroid hormone 20-hydroxyecdysone (20E)-driven gene expression [12]. In an *Aedes* cell line based transcriptome interrogation, heme itself appears to signal the transcription of a wide range of genes that are involved in redox, energy metabolism and immune responses [13]. This transcriptional profile induced by heme in the cell line is similar to the transcriptional patterns induced in blood-fed mosquitoes [14], suggesting heme *in vivo* is one of the regulators that direct the transcriptional responses to a blood meal. Several key observations from the microarray analysis of the heme exposed cell line include: (i) heme upregulates expression of the genes encoding antioxidants such as ferritin, glutathione S-transferase X2 (GSTX2), cytochrome P450 and heat shock proteins; (ii) heme induces more transcripts than the ROS inducer paraquat (PQ) does, suggesting that heme and PQ may induce distinct signaling pathways, and heme signaling has a broader transcriptional impact; (iii) genes in

several metabolic pathways are induced, including genes in glycolysis and pentose phosphate pathway (PPP), which would favor the regeneration of NADPH, an important reducing equivalent in redox reactions. These transcriptional responses to heme implicate a well-organized systemic coordination to control ROS elevation, which is consistent with the heme mediated decrease of net ROS observed in the midgut epithelial cells post blood meal [15]; (iv) heme down-regulates the transcription of immune genes, such as *peptidoglycan recognition receptor protein LB*, *TEP20*, a set of genes encoding CLIP serine proteases, and antimicrobial peptide genes *defensing* and *cecropins*, after heme supplementation in the cell line. *In vivo*, heme ingestion suppresses the expression of antimicrobial peptide genes *cecropin D*, *cecropin G* and *attacin* upon oral infection with *Serratia marcescens*. Heme supplementation in sugar meal also results in the increase of midgut microbiota [14]. These data indicate that heme derived from a blood meal help regulate ROS levels and local immunity that affect the midgut microbial community.

Nrf2 signaling in oxidative stress response

The nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element (ARE) is a critical regulatory circuit for defense against oxidative stress in various organisms [16]. The Keap1/Nrf2 signaling is conserved in *Drosophila* [17–22], and oxidant induced Nrf2 activation regulates antioxidant and detoxification responses [23]. Relevant genes in the pathway are present in the genome of mosquitoes as well [24]. The metabolites of polyunsaturated fatty acids (PUFAs), in particular, electrophilic lipid oxidation and nitration products, can induce Nrf2 activation [25]. Recently, a linoleic acid derivative, 10-Oxo-trans-11-octadecenoic acid (KetoC) generated by *Lactobacillus plantarum*, a bacterial resident in the human gut, has been shown to be an activator of Nrf2-ARE signaling in response to oxidative stress [26]. Interestingly, taxa in genus *Lactobacillus* have been identified in mosquito microbiota [7, 27]. The possibility of bacteria-derived metabolites in mosquito redox homeostasis is open to investigation.

Heme sequestration, storage, and transport

The peritrophic matrix (PM) is a semi-permeable, extracellular structure that separates the blood bolus from gut epithelial cells [28]. The PM has a large capacity for heme sequestration [29]. Heme aggregates are present within or near the PM [30]. AeIMUC1, a PM protein with chitin-binding domains and mucin-like domains, is a heme binding protein [31]. Xanthurenic acid (XA) is a metabolite in the kynurenine pathway of tryptophan degradation. XA is produced in large quantities in the midgut of *Aedes aegypti* after a blood meal. XA is an iron chelator that binds to heme and iron, therefore reducing the amount of free heme for use in the Fenton reaction [32]. Interestingly, certain *Pseudomonas* bacteria produce quinolobactin, an efficient iron scavenger derived from XA in the tryptophan-kynurenine-xanthurenic acid pathway [33, 34]. This suggests there may be a contribution of bacteria-derived heme scavengers involved in maintaining midgut redox homeostasis.

Ferritin is a primary iron storage and transport protein in mosquitoes [8, 35–37]. In *Ae. aegypti* ferritin transcription is induced by blood feeding, H_2O_2, and heme feeding [36, 38]. In *Anopheles gambiae*, ferritin gene is responsive to a blood meal as well [39, 40]. Iron in

vertebrate host blood is present in two forms, hemoglobin and ferric-transferrin. In *Ae. aegypti*, ferritin is present in the midgut, fat body, ovaries, and eggs. Iron from both ferric-transferrin and heme is loaded into ferritin and is then secreted into the hemolymph [8, 35]. The ferritin likely serves a dual function, to transfer iron to eggs and to sequester excessive free iron from being used in the Fenton reaction [8]. Interestingly, in *Ae. aegypti* GSTX2 has an affinity for hematin [41], and this gene is transcriptionally responsive to heme [13]. GSTX2 belongs to the GST class that is associated with hematophagous insects [41], the transcriptional response to heme and affinity for hematin suggest that GSTX2 may play a protective role in coping with heme toxicity during blood digestion. Enzymatic degradation of heme is another detoxification method. In *Ae. aegypti*, heme oxygenase catalyzes heme degradation [42]. Apparently, multilayer protective mechanisms have evolved in mosquitoes to cope with the massive heme load from a blood meal.

Host antioxidant defense systems

Antioxidant systems are well developed during evolution. Cu, Zn and Mn superoxide dismutases (SOD) catalyze the conversion of superoxide anion into hydrogen peroxide, and catalase is responsible for detoxifying hydrogen peroxide to oxygen and water. Based on a gene expression dataset of *An. gambiae* [43], *catalase* gene is constitutively expressed at a moderate level in unfed mosquitoes and shows an early two-fold upregulation at 3 hours after blood feeding in the whole body. At 24 hours after feeding, the expression level in the whole body subsides to a level similar to that in the unfed mosquitoes, but the expression in the midgut is approximately four times higher than that in the fat body, the tissue that plays various roles in metabolism and immunity [44]. This expression pattern suggests that blood feeding triggers an early production of catalase systemically, which prepares mosquitoes for the elevation of ROS associated with the following blood digestion. Higher expression in the midgut only at 24 hours when blood digestion is at a peak indicates the local need of defense against ROS. Consistently, in *Ae. aegypti, catalase* is inducible by a blood meal [45] as well as a chemically defined artificial diet including hemoglobin [46]. Silencing *catalase* and *sulfhydryl oxidase* increased the mortality of *An. gambiae* after a blood meal [47], which highlights the role of the catalase pathway in mitigation of oxidative stress after blood feeding.

In the redox metabolism of most aerobic organisms, glutathione is an essential agent [48, 49]. Glutathione exists in both reduced (GSH) and oxidized (GSSG) states. GSH reduces H_2O_2 or other peroxides catalyzed by the glutathione peroxidase (GP) resulting in GSSG. GSSG can be regenerated by glutathione reductase (GR) using NADPH as a reducing agent. GR is absent in both *Drosophila* and mosquitoes, thioredoxin reductase (TrxR) takes the place of GR instead [50–52]. In a *Walbachia* infected *Ae. albopictus* cell line, antioxidant proteins SOD, peroxiredoxin (Prx) and GP is upregulated [53]. In *An. stephensi*, gene encoding 2-Cys peroxiredoxin is induced for self-protection when mosquitoes launch an oxidative and nitrosative defense against malaria parasite infection [54].

Oxidative stress-induced metabolic reconfiguration

The glutathione system and the thioredoxin system both require NADPH as reducing equivalents [49]. To maintain a functional GSH/GSSG couple buffer, it is critical to have a sufficient NADPH supply. The pentose phosphate pathway (PPP) is a major pathway to reduce NADP to NADPH. In yeast, nematodes, and humans it has been shown that upon oxidative stress induction, glucose catabolism is routed from glycolysis to the PPP [55, 56]. This metabolic reconfiguration increases the flux of NADPH generation, therefore providing sufficient reducing power for combating oxidative challenges [57, 58]. In the migratory locust, *Locusta migratoria*, a genome-wide transcription analysis revealed that under hypobaric hypoxia-induced oxidative stress, glycolysis was suppressed, and PPP was enhanced [59]. In a *Drosophila* model, a knockdown of the gene encoding ribose-5-phosphate isomerase (RPI) increases glucose-6-phosphate dehydrogenase (G6PD) activity, which results in an elevated level of both NADPH and GSH. The manipulated flies exhibit increased resistance to oxidative stress and prolonged lifespan [60]. In addition to the enzymes G6PD and 6-phosphogluconate dehydrogenase (6PGD) in PPP, cytosolic isocitrate dehydrogenase (IDH) and cytosolic malic enzyme (MEN) are two other enzymatic players in a concerted metabolic network for the reduction of $NADP^+$ to NADPH. This network coordinates metabolic responses to various environmental stress, such as oxidative stress, starvation, and desiccation [61]. In *Aedes* mosquitoes, metabolic genes are responsive to blood intake. The gene encoding pyruvate kinase is downregulated and genes encoding 6PGD, catalase, hexokinase, thioredoxin, and heat shock proteins are upregulated [45]. In summary, a sufficient production of reducing power is necessary to ensure a successful oxidative defense. Any disturbance in the NADP-reducing network would make an organism more susceptible to stress conditions. Further studies are needed to understand the metabolic responses to stress associated phenotypes in mosquitoes, which may reveal new molecular targets for intervention. These attributes may be used to develop novel mosquito control strategies.

Community structure in mosquito-associated microbiota

The mosquito gut ecosystem is inhabited by a complex and dynamic microbial community along the mosquito life cycle. In recent years, gut-associated microbiota has been characterized for *Anopheles*, *Aedes* and *Culex* mosquitoes in various habitats in different geographic regions around the world. The advances in microbiota structure, as well as their impact in different mosquito traits in physiology and immunity, have been reviewed [62–65]. Structurally, dominant taxa have been identified to belong to three phyla: Proteobacteria, Bacteroidetes, and Firmicutes. The alpha-proteobacteria are represented by genera *Asaia, acetobacter* in family Acetobacteriaceae; the gamma-proteobacteria are represented by genera *Serratia, Enterobacter, Pantoea*, etc. in family Enterobacteriaceae, genus *Pseudomonas* in family Pseudomonadaceae, genus *Acinetobcter* in family Morexellaceae and genus *Aeromonas* in family Aeromonadaceae; the Bacteroidetes are represented by genera *Elizabethkingia* and *Chryseobacterium* in family Flavobacteriaceae; and the Firmicutes are represented by genus *Bacillus* in family Bacillaceae. The genomes of several bacterial isolates derived from mosquitoes have been sequenced, including *Asaia* sp., *Elizabethkingia anophelis*, *Enterobacter* sp., *Pseudomonas* sp., *Serratia* sp.,

Stenotrophomonas maltophila, and *Staphylococcus hominis* [66–72]. Overall, the microbial diversity is high in mosquito-associated microbiota. At an individual level, variation can also be high [73]. The community structure is significantly influenced by the sources where mosquitoes acquire microbes in the environment, i.e., aquatic larval habitats and terrestrial habitats during adulthood [74]. In general, the gut community in larval stage is different from that in the adult stage; larval gut bacteria are largely expelled in meconium through the intestine remodeling during metamorphosis. Newly emerged mosquitoes acquire new bacteria from nectar or other natural sugar sources from plants. For example, *Pseudomonas, Asaia*, and *Acetobacter* are often associated with nectar [17, 75], and *Acinetobacter* and many enteric bacteria are prevalent in the plant rhizosphere and soil [76]. Nectar may become contaminated with these soil bacteria due to proximity. A significant impact on the microbial structure in the female mosquito gut occurs when a blood meal is taken. The bacterial abundance increases while diversity decreases. Bacteria in Enterobacteriaceae proliferate favorably in the blood-fed midgut, but bacteria in Sphingomonadaceae and Xanthomonadaceae are not well adapted to the altered environment and become less abundant or undetectable [7, 77]. It is an open question that how the drastic structural shift happens, which involves an interplay between the mosquito host and microbes. One of the possible drivers of this shift may be the change of nutrient provision from carbohydrate-rich diet to protein-rich diet and a corresponding alteration of metabolic architecture in the midgut. Oligotrophic bacteria may not be well fit in the nutrient enriched metabolic architecture in the blood-fed midgut. Besides, the fluctuation of oxidative states in the gut niche before and after a blood meal may play a critical role in shifting the microbial structure (see next section).

Duox mediated ROS and gut microbial homeostasis

Gut bacteria contribute significantly to the host fitness in many ways. The maintenance of microbial homeostasis is a research area that has attracted much attention recently [78, 79]. In the gut of fruit fly *Drosophila melanogaster*, the Dual oxidase (Duox) dependent ROS generation system is essential for maintaining gut microbial homeostasis. Duox belongs to the NADPH oxidase family proteins with a function dedicated to the production of ROS. The NADPH oxidase domain of the Duox catalyzes the transfer of one electron from NADPH to O_2 to form superoxide and subsequent H_2O_2; then the peroxidase domain converts H_2O_2 into hypochlorous acid (HOCl) in the presence of a chloride ion [80]. In the *Drosophila* model, autochthonous gut microbiota is largely monitored by Duox-ROS pathway [81–85]. IMD-AMP pathway likely acts as an immune mechanism complementary to the Duxo pathway, which remains inactive unless pathogens proliferate in the gut. The activity of the Duox system is tightly controlled at two different levels. MAPK p38/ATF2 transcription controls Duox gene expression, and the enzymatic activity of Duox is controlled by intracellular calcium concentration, which is modulated by signaling endosomes that require at least Cadherin 99C, phospholipase C-β (PLCβ) and protein kinase C (PKC) [81, 83]. Pathogen-secreted uracil has been shown to be able to fully activate the Duox-ROS system by inducing *duox* transcription as well as enhancing Duox enzyme activity [86]. The uracil is assumed to be recognized by a yet-unidentified receptor [83]. Usually, commensal bacteria do not release uracil, which may be an evolutionary

outcome that adaptive symbionts modify the uracil secretion mechanism to avoid an induction of host ROS production [82]. Interestingly, uracil can modulate *Drosophila* defecation [87]. This process requires both Duox pathway and transient receptor potential (TRP) channel TRPA1. TRPA1 is a HOCl receptor and promotes defecation. Pathogen-derived uracil activates Duox system to generate ROS and HOCl, the latter triggers TRPA1 to promote defecation, resulting in the expulsion of pathogens in parallel to the microbicidal effects of ROS and HOCl [87].

The microbial composition in the *Drosophila* gut is simple. Commonly found bacteria are aerotolerant taxa in genera *Acetobacter* and *Lactobacillus* [88–90]. Oriental fruit fly *Bactricera dorsalis* harbors a more complex gut microbiota, dominated by taxa in family Enterobacteriaceae, such as *Klebsiella*, *Enteorbacter, Pectobacterium* and *Serratia* [91]. The diverse microbiota in the fly makes it a better model to study the role of the Duox system in microbial homeostasis. Indeed, the Duox-ROS system is functional as well in the fly [92]. The *BdDuox* gene is inducible upon ingestion of non-gut resident bacteria and the minor gut resident *Bacillus cereus*. However, the gene is not responsive to the dominant symbionts. RNAi-mediated knockdown of *BdDuox* led to a bacterial expansion with altered taxonomic composition in the gut microbial community. The disturbed community structure restored when the *Duox* RNAi effect subsided [92]. Apparently, the host gut Duox-ROS system is forbearing to the autochthonous microbial structure, but sensitive to the compositional change. Such an effective surveillance mechanism maintains the stability and resilience of the microbiota.

As mentioned above, the mosquito gut accommodates a much more diverse community [7, 27, 93, 94]. After blood feeding the microbial abundance increases and the taxonomic composition changes. Taxa in family Enterobacteriaceae are greatly enriched. The bacterial expansion is allowed due to several adaptive mechanisms. First, the proliferated bacteria are encased inside the blood bolus surrounding by the PM. Second, as shown in *An. gambiae*, the peroxidase/Duox system mediates protein cross-linking to form a dityrosine network (DTN) on the luminal surface of epithelial cells of the midgut. This extracellular network reduces the permeability to soluble molecules in the blood bolus. Thus, microbial immune elicitors are separated from direct contact with epithelial cells, which avoids immune activation [95]. Similarly, the DTN in ticks prevents the invasion of the pathogen *Borrelia burgdorferi* [96]. Third, heme mediates a reduction of ROS in the gut epithelial cells. In *Aedes aegypti*, a certain level of ROS is present in the epithelial cells of the sugar-fed midgut. This ROS generation is Duox dependent. Knockdown of *duox* resulted in the reduction of ROS levels and the increase of bacterial abundance in the sugar-fed midgut. However, in the blood-fed gut, the ROS level is significantly reduced, and heme is responsible for the ROS reduction [15]. Fourth, heme also downregulates anti-bacterial activity, as shown in a heme-treated *Aedes* Aag2 cell line [13], which was corroborated by a reduced abundance of immune gene transcripts [15]. Collectively, these adaptive mechanisms restrain the proliferation of bacteria in the blood bolus.

In summary, the Duox-ROS system plays multiple roles in shaping a dynamic microbiota in the mosquito gut. In the sugar-fed gut, Duox mediated ROS is critical for modulating the gut community; once a blood meal is taken, peroxidase/Duox mediated DTN acts as a physical

shield to block microbial electors from interacting directly with epithelial cells, which avoids an overactivation of immune responses to the symbiotic residents. In the meantime, expanding microbes are restrained in the PM wrapped bolus, and will be excreted with digested blood waste via defecation, which involves Duox/TRPA1 as well.

Microbial capabilities to harmonize oxidative stress appear to be a critical factor in shaping symbiosis in insects. A genomic comparison of symbiotic strains of *Acetobacter* in *Drosophila* revealed a gene cluster involved in oxidative stress detoxification [97]. Some alpha-proteobacteria appear to have specific responses to heme. Louse-borne pathogenic bacteria *Bartonella quintana* experiences a host transition from a heme restricted niche in the bloodstream of the human host to a heme-rich niche in the gut of body louse. It is essential for *Bartonella* to have a survival strategy to adapt to the niche switch. *Bartonella* produces a family of hemin binding proteins (Hbp) that are responsive to the temperature switch from human to louse, changed hemin concentration, and oxidative stress [98–100]. It has been demonstrated that *B. quintana* sigma factor rpoE, a member of the sigma factor group ECF15, is involved in the adaptation to conditions in lice [98]. The ECF15 family consists of master regulators of the general stress response in alpha-proteobacteria to combat various environmental stresses, see recent reviews [101–104]. Similar studies are needed for understanding adaptation mechanisms that mosquito symbionts use to thrive in the heme-enriched blood-fed gut environment. The availability of mosquito derived bacterial strains and their genomes [66–72] enables such studies in bacterial ecology in the mosquito gut microbiome.

Mosquito C-type lectins and microbial homeostasis

C-type lectins, a family of carbohydrate binding proteins, mediate various cross-kingdom interactions in the host-microbiota interface [105–107]. In the gut of *Ae. aegypti* and *Culex pipiens pallens*, certain mosquito C-type lectins bind to bacterial glycans, which provides a protective shield to interfere with the deposition of antimicrobial peptides onto commensal bacteria [108]. It has recently been shown that O-antigen, a glycan polymer at the outermost domain of bacterial lipopolysaccharide (LPS), is involved in the stable inhabitation of *Enterobacter* sp. in the midgut of *Anopheles* mosquitoes [109]. The O-antigen may participate in the interplay with host lectins in the gut. The interaction of microbial glycan and mosquito lectins is an understudied area; more investigations should be encouraged in this direction.

Plant derived polyphenols and microbiota

Nectar is an essential energy and nutrient source for mosquitoes. Nectar is composed of various ratios of hexoses such as glucose, fructose, and sucrose with minor constituents such as amino acids, lipids, phenolic content, and esters [110, 111]. Nectar harbors a microbial community as well [112, 113]. Mosquitoes have a preference to certain plants as their energy source. *Ricinus communis* is one of the plants *An. gambiae* prefers to take nectar from [114–116]. The extracts containing phenolics from *Ricinus communis* showed significant free radical scavenging activity [117]. Plant derived dietary polyphenolics are able to modulate the gut microbiota in mammals [118], and gut microbes make significant

contributions to the biotransformation of dietary polyphenols to bioactive derivatives, some of which have strong antimicrobial and antiparasitic activities [119]. Resveratrol, a polyphenolic compound, has a broad range of biological activities including antioxidative capacity and ability to activate signaling molecules adenosine monophosphate-dependent protein kinase (AMPK) and sirtuins (reviewed in [120]). It has been shown that resveratrol is able to extend lifespan in *Caenorhabditis elegans* and *Dr. melanogaster* by activating Sir2, a member of the sirtuin family of NAD^+-dependent deacetylases, likely through a mechanism related to caloric restriction [121]. Recently, it has been shown that polyphenol-rich diets (including resveratrol) enhanced longevity of *Ae. aegypti*, which was mediated by activating AMPK. Besides, resveratrol feeding led to a reduced bacterial load in the mosquitoes. Consistent with this, AMPK inhibition resulted in increased bacterial proliferation. The AMPK mediated midgut autophagy was involved in the microbial modulation [122]. Interestingly, resveratrol feeding had inconsistent effects on lifespan of *An. stephensi* [123]. Likely, effects of polyphenols on mosquito phenotypes depend on the complex interactions of all parties in the gut environment. Since plant sugar based diets are the daily source for mosquito energy and nutrient requirements, nectar composition, nectar microbiota, and phytochemicals may play a critical role in the metagenomic homeostasis in the gut ecosystem. This direction remains to be explored in the future.

Conclusion and perspectives

In this review, we summarize recent advances in the impact of metagenomic interactions in the redox homeostasis in the mosquito gut. In the responses to the oxidative stress during blood digestion, heme plays an essential role as a signaling molecule. Heme-induced transcriptional responses coordinate systemic responses to harness oxidative stress. To ensure a sustainable redox balance, metabolic reprogramming to maintain the generation of reducing agent NADPH is behind the oxidative defense mechanisms. Increasing evidence suggests the oxidative environment is a driving force for guiding a symbiotic relationship. The host Duox-ROS system plays a vital role in maintaining a dynamic symbiotic microbiota, and the microbiome-derived metabolites contribute to the redox homeostasis as well. At the emerging stage of characterizing microbial genetic repertoire, many areas are open to exploration to gain a better understanding of metagenomic interplay in the mosquito gut ecosystem.

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Highlights

- Blood digestion leads to oxidative stress in mosquitoes.
- The redox homeostasis is maintained by oxidative defense mechanisms.
- Mosquito Duox-ROS pathway contributes to shaping the symbiotic microbiota.
- Microbial metabolites may participate in redox homeostasis.