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REVIEW ARTICLE

Longevity-modulating effects of symbiosis: insights from *Drosophila–Wolbachia* interaction

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Abstract Microbial communities are known to significantly affect various fitness components and survival of their insect hosts, including *Drosophila*. The composition of symbiotic microbiota has been shown to change with the host's aging. It is unclear whether these changes are caused by the aging process or, vice versa, they affect the host's aging and longevity. Recent findings indicate that fitness and lifespan of *Drosophila* are affected by endosymbiotic bacteria *Wolbachia*. These effects, however, are inconsistent and have been reported both to extend and shorten longevity. The main molecular pathways underlying the lifespan-modulating effects of *Wolbachia* remain unclear, however insulin/insulin-like growth factor, immune deficiency, ecdysteroid synthesis and signaling and c-Jun N-terminal kinase pathways as well as heat shock protein synthesis and autophagy have been proposed to play a role. Here we revise the current evidence that elucidates the impact of *Wolbachia* endosymbionts on the aging processes in *Drosophila*.

Keywords Aging · Lifespan · *Wolbachia* · *Drosophila melanogaster* · Microbiota · Holobiont · Symbiosis

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Introduction

Insect organisms typically harbour large numbers of microbial cells, far exceeding the number of their own cells (Dillon and Dillon 2004). However, the role of microbiota in many aspects of insect the host's physiology has until recently been underappreciated (Russell et al. 2014). The host organism and its microbiota form an integrated "multiorganism" with shared physiology, development, behavior and evolutionary history (Rosenberg et al. 2009; McFall-Ngai et al. 2013; Gilbert 2014, 2016). In this sense, a multicellular organism is rather a consortium of organisms (a "holobiont") that represents a functionally integrated entity (Gilbert 2014). The assemblage of a holobiont's microbial symbionts can be regarded as a specific system of organs integrated into the metabolism and ontogeny of the host. Furthermore, the interplay between the the host and the resident microbial cells can substantially influence gene expression of both (Gilbert 2014). Therefore, the aggregate of microbial and host genomes is sometimes referred to as "hologenome" (Rosenberg et al. 2009; Rosenberg

and Zilber-Rosenberg 2016). Since the organism's fitness is highly dependent on its microbiota, the hologenome is assumed to be a unit of natural selection (Zilber-Rosenberg and Rosenberg 2008). While the applicability of the "hologenome" concept remains debatable (Douglas and Werren 2016), in physiological terms microbiota plays a crucial role in the nutritional status, development, and immune defense of the host (Costello et al. 2012; McFall-Ngai et al. 2013).

The term *symbiosis* refers to a persistent coexistence of dissimilar organisms (De Bary 1879; Saffo 1992). Insects are inhabited by ectosymbiotic (Hughes et al. 2008; Engel and Moran 2013) and endosymbiotic microorganisms (Kikuchi 2009). Ectosymbionts are encountered on body surfaces and inside the cavities of organs, while endosymbiots reside inside of host cells (Martin and Schwab 2012). Age-related alterations in microbiota composition were revealed in *Caenorhabditis elegans* and *Drosophila melanogaster*, as well as in humans (for reviews, see Biagi et al. 2011; Ottaviani et al. 2011; Cheng et al. 2013; Heintz and Mair 2014). Furthermore, since microbiota is essentially involved in homeostatic regulation (McFall-Ngai et al. 2013), it has been suggested that an individual's aging and longevity may be highly dependent on the composition and abundance of its microbial symbionts (Heintz and Mair 2014). It can therefore be assumed that understanding the mechanisms of symbiotic interactions between the host and its microbiota can expand our knowledge of the pathways involved in the control of aging and longevity.

The fruit fly *Drosophila melanogaster* is among the most popular model organisms used to study aging and lifespan (Helfand and Rogina 2003; Paaby and Schmidt 2009; Brandt and Vilcinskas 2013). In *Drosophila*, the basic reservoir of microbial ectosymbionts is the digestive tract, primarily the gut and intestine. The composition of intestinal microbes changes throughout the life cycle depending on the diet and other environmental conditions (Broderick and Lemaitre 2012; Erkosar et al. 2013; Staubach et al. 2013). The most typical bacterial communities harboured by *Drosophila melanogaster* consist of *Lactobacilus brevis*, *Lactobacilus plantarum*, *Enterococcus faecalis*, and *Acetobacter pomorum* (Erkosar et al. 2013). Most of these bacterial species are found in both the larvae and imagoes of all analyzed laboratory stocks of *Drosophila*. Bacterial microbiota gradually changes through both the developmental and adult life stages of the host insect. The amount of *Lactobacillus fructivorans* normally decreases, whereas the amount of *Lactobacillus plantarum* increases throughout the larval development (Wong et al. 2011, 2013). In pupae, *Acetobacter tropicalis* becomes the dominant bacterial species, while *L. fructivorans* and *A. pomorum* are dominant species in young adult and old flies, respectively. The microbiota of the digestive tract varies substantially across various *Drosophila* species (Corby-Harris et al. 2007; Chandler et al. 2011; Wong et al. 2013; Staubach et al. 2013). These differences depend on the diet and other environmental factors (reviewed in Broderick and Lemaitre 2012; Erkosar et al. 2013). The composition of microbiota is also known to vary between laboratory stocks and flies from wild populations (Chandler et al. 2011; Staubach et al. 2013). The gut microbial community undergoes significant changes through the hosts' life course as well (Russell et al. 2014; Zapata and Quagliarello 2015).

Drosophila is known to harbour endosymbiotic bacteria, including *Wolbachia* and *Spiroplasma* (Mateos et al. 2006; Chandler et al. 2011). The number of *Spiroplasma* cells was shown to increase during fly aging, causing more profound manifestations of the so called male-killing phenotype, whereby males are selectively killed (Haselkorn 2010). It is unknown, however, if *Spiroplasma* affects the *Drosophila* lifespan. Therefore, in the present review we will entirely focus on the impact of the *Wolbachia pipientis* endosymbiont on the aging process and longevity in *Drosophila* fruit flies.

Among the bacterial species normally inhabiting *Drosophila*, *Wolbachia* is studied most intensively because of its pronounced effects on the host fitness and its widespread distribution among fruit flies and other arthropods. According to recent data, it infects approximately 52 % of all arthropod species (Weinert et al. 2015). This bacterium is an obligatory intracellular symbiont which is vertically transmitted between generations through the egg cytoplasm and horizontally via the *Drosophila* ectoparasite *Tyrophagus putrescentiae* (Brown and Lloyd 2015).

Wolbachia is known to be able to manipulate the reproduction of their host organisms in various ways, such as male-killing (selective killing of male hosts during larval development), sperm-egg cytoplasmic incompatibility (the death of offspring or the absence of fertilization in crosses between infected males and females that are uninfected or infected with a different strain of *Wolbachia*), induction of parthenogenetic

development of females and feminization (conversion of genotypic males into females) (Werren 1997; Werren et al. 2008), and also by influencing mating behavior (Markov et al. 2009; Sharon et al. 2010) and fecundity (Martinez et al. 2015; Serga et al. 2014). Several studies demonstrate that *Wolbachia*, apart from these unfavorable effects on the reproductive functions, can also provide various fitness benefits for their hosts, including antiviral protection (Hedges et al. 2008; Teixeira et al. 2008; Chrostek et al. 2013), generation of ATP for the host (Darby et al. 2012), improved iron utilization (Brownlie et al. 2009), as well as enhanced stem cell proliferation and increased fecundity of the flies (Fast et al. 2011). The phenotypic manifestations caused by *Wolbachia* depend on the strain of the bacteria and the host genotype (Werren et al. 2008). In this way, *Wolbachia* can increase the host's fitness and thus enhance the chance of transmission to the next generations that eventually leads to the spread of *Wolbachia* in natural populations of *Drosophila* (Serga and Kozeretskaya 2013).

Wolbachia primarily resides in the germline cells of the host. Large numbers of this endosymbiont are also distributed across body parts and somatic tissues, residing in the head, wings, salivary glands, hemolymph, thoracic muscles, midgut, Malpighian tubules and fat bodies (Dobson et al. 1999). *Wolbachia* densities vary, depending on the diet of the fly (Serbus et al. 2015), genotypes/strains of the host and the endosymbiont (e.g. Osborne et al. 2009) and the stage of the host's ontogenesis (Yamada et al. 2007). *Wolbachia* titres positively correlate with various phenotypes in *Drosophila*, such as antiviral resistance of the host (Osborne et al. 2009; Johnson 2015), the strength of the lethal phenotype induced by the *w*MelPop strain of *Wolbachia* (Chrostek and Teixeira 2015), and the strength of cytoplasmic incompatibility (Boyle et al. 1993).

This mini-review focuses on molecular mechanisms putatively involved in mediating the link between bacteria, primarily the *Wolbachia* endosymbiont, and lifespan in *Drosophila melanogaster*.

The impact of microbiota on *Drosophila* aging and longevity

Drosophila is a useful model for studying various biological phenomena, including the molecular crosstalk between symbiotic bacterial communities and host cells, as well as the effects of microbiota on the host organism's aging and longevity (Broderick and Lemaitre 2012). The relationship between symbiotic microbiota and *Drosophila* lifespan has been addressed in a number of studies (Brummel et al. 2004; Ren et al. 2007; Carrington et al. 2009). Brummel et al. (2004) have shown that germ-free (gnotobiotic) flies exhibited a shorter lifespan, suggesting that the presence of the symbiotic microbial community may be required for maintaining the normal lifespan. Interestingly, these effects appear to be stage-specific, so that the presence of bacteria in the first week of the imago stage promotes longevity, while later in life bacteria reduce the flies' lifespan (Brummel et al. 2004). In this study, antibiotic treatment extended the lifespan of aged insects. This contradiction may likely be explained by the unfavorable effects of bacterial load later in life (Brummel et al. 2004).

Microbiota-mediated lifespan extension in *Drosophila* is supposed to be associated with the maintenance of homeostasis of intestinal stem cells (Biteau et al. 2010; O'Brien et al. 2011). Microbiota is also believed to influence the flies' aging and longevity via its interaction with signaling pathways that are usually thought to be involved in longevity control. For example, *Acetobacter pomorum* inhabiting the digestive tract and several other organs of *Drosophila* may induce the activation of the insulin/insulin-like growth factor (IIS) pathway through the *Drosophila* insulin-like peptides (DILPs) (Shin et al. 2011). Bacteria with a mutant pyrroloquinoline quinone–dependent alcohol dehydrogenase gene were not able to increase the host insulin/IGF signaling. Flies harboring mutant *A. pomorum* demonstrate extended development, reduced body size and enhanced contents of circulating sugar and triacylglycerides, i.e., phenotypes similar to those observed in IIS mutants. Infection by *Lactobacillus plantarum*, which is known to up-regulate the IIS pathway, exerted growth-promoting effect in fruit flies (Storelli et al. 2011).

The development and longevity of fruit flies have also been demonstrated to substantially depend on eukaryotic microbiota. Yeast species associated with *Drosophila* have been shown to affect the flies' development and fitness by providing essential nutrients, such as sterols and B-vitamins (Anagnostou et al. 2010). In most cases, interrelations between yeast and *Drosophila* are mutually beneficial (Broderick and

Lemaitre 2012). Flies can also be infected with intra- or extracellular trypanosomatid species which are parasitic (Keebaugh and Schlenke 2014). Infection with some microsporidian parasites has been shown to result in adverse fitness outcomes, such as reduced fecundity of the host (Futerman et al. 2006). However, so far the interactions of *Drosophila* with eukaryotic microbes poorly understood. The presumably complex interrelations between the prokaryotic and eukaryotic microbiota might provide one explanation for the differences in the effects of antibiotic treatment among different studies.

Wolbachia infection and lifespan in *Drosophila*

A summary of the impacts of *Wolbachia* infection on fitness-associated traits in *Drosophila* is presented in Table 1. *Wolbachia* infection has repeatedly been reported to significantly influence the lifespan in *Drosophila*; these effects, however, are controversial and include both increased (Alexandrov et al. 2007; Martinez et al. 2015) and decreased (Min and Benzer 1997; Martinez et al. 2015) lifespan (Table 2).The lifespan-modulating effects of *Wolbachia* might depend on the hosts' genetic background (Fry and Rand 2002; Fry et al. 2004) (Table 2). Fry and Rand (2002) used reciprocal hybrid crosses between a fruit fly strain that lived longer with *Wolbachia* (Z53) and one that did not (Z2) and they noted that *Wolbachia* may extend fly lifespan at the expense of reduced reproduction. The positive effect of *Wolbachia* infection on fly lifespan was much more pronounced in hybrids of these two lines than in their parents. Moreover, this beneficial impact of infection was more evident in single-sex cages where courtship and mating did not occur. In these cages, nearly all insects infected with *Wolbachia* lived longer than uninfected flies. In a subsequent study by Fry et al. (2004), female flies from Z53 and Ftf1 strains lived longer if they were infected with *Wolbachia*, while the longevity of Wj9 strain was decreased, and Z2 and Ftf100 fly strains demonstrated no effects on survival associated with *Wolbachia* infection. Ftf1 and Ftf100 are inbred isofemale lines collected in 1992 from Four-Town Farm in RI, USA. The WJ9 inbred isofemale line for this study was provided by Marc Tatar (Brown University). In the research by Min and Benzer (1997), infection with *Wolbachia popcorn* (*w*MelPop) strain was revealed to lead to life shortening in *Drosophila*. This strain has been found to cause degeneration of the retina, muscle and brain tissues, thereby culminating in decreased longevity. Both decreased longevity and degenerative phenotypes were abolished by tetracycline treatment.

These findings suggest that in most cases *Wolbachia* can provide fitness benefits for the host insects. Lifespan effects of *Wolbachia*, however, appear to depend on the genotypes of both the symbiont and the host (Table 2). Flies infected with the *w*MelCS and *w*MelCS-like genotypes of *Wolbachia* have shorter lifespans compared to flies infected with, for example, the *w*Mel genotype (Chrostek et al. 2013). *w*MelPop is likely a unique virulent variant of *Wolbachia* that proliferates massively in the host fruit flies and shortens their lifespan. Remarkably, the *w*MelPop strain has only been found in laboratory stocks and is unknown from natural populations of fruit flies. The manifestation of the life-shortening phenotype triggered by *w*MelPop infection has been shown to be associated with the bacteria density in the affected tissues (Chrostek et al. 2013). Interestingly, *w*MelPop strain is known to protect *D. melanogaster* from viruses even better than other closely related *w*Mel variants, and at the same time it reduces the host's overall survival, suggesting that there can be a trade-off between the symbiont-mediated protection and other components of their fitness. In their recent research aimed to identify the genetic mechanisms of *w*MelPop pathogenicity, Chrostek and Teixeira (2015) have demonstrated that *w*MelPop virulence depends on the amplification of a DNA region containing eight *Wolbachia* genes, called *Octomom*.

Decreased lifespan is a commonly observed outcome of elimination of *Wolbachia* from *D. melanogaster* (Table 2), which suggests that *Wolbachia* may play a significant role in determining the lifespan of the host. The rationale for this hypotheis is provided by studies where lifespan phenotype was estimated in infected *Drosophila* hosts of different genotypes with variable lifespans. Grönke et al. (2010) and Ikeya et al. (2009) have found that *Wolbachia* interacts in a complex way with the insulin/IGF signaling pathway, which might cause perturbed mutant phenotypes in regard to this pathway and lead to an extended lifespan in *Drosophila* (Fig. 1; Table 3). *Wolbachia* also appears to be capable to amplify the lifespan-extending effect of mutant *Indy* gene that encodes an exchanger for Krebs cycle intermediates (Toivonen et al. 2007).

Table 1 Effects of *Wolbachia* on some commonly studied fitness-associated and life history traits of *Drosophila*

Wolbachia strain	*Drosophila* species[a]	Effects observed	References
Strain is unknown	*D. recens*	CI (cytoplasmic incompatibility)	Werren and Jaenike (1995)
	D. innubila	Increased fecundity in infected females compared to uninfected or tetracycline-treated	Unckless and Jaenike (2012)
	D. borealis	MK (male-killing)	Sheeley and McAllister (2009)
	D. innubila		Dyer and Jaenike (2004)
	D. subquinaria		Jaenike (2007)
	D. bifasciata		Hurst et al. (2000)
	D. auraria	Partial CI (partial cytoplasmic incompatibility)	Bourtzis et al. (1996)
	D. sechellia		Giordano et al. (1995), Bourtzis et al. (1996)
	D. annanasae		Bourtzis et al. (1996)
	D. bifasciata		Hurst et al. (2000)
	D. innubila	Resistance/tolerance to RNA viruses	Unckless and Jaenike (2012)
*w*Au	*D. simulans*	Resistance/tolerance to RNA viruses	Osborne et al. (2009)
*w*Au-like, *w*Mel-like	*D. paulistorum*	Altered mating behaviour	Miller et al. (2010)
		Increased fecundity in infected females compared to uninfected or tetracycline-treated	Miller et al. (2010)
*w*Ha	*D. simulans*	CI	O'Neill and Karr (1990)
*w*Mel	*D. melanogaster*	Altered mating behaviour	Markov et al. (2009), Sharon et al. (2010)
		Altered survival in cold/warm conditions	Versace et al. (2014)
		Increased fecundity in infected females compared to uninfected or tetracycline-treated	Olsen et al. (2001), Fry et al. (2004), Serga et al. (2014)
		Increased lifespan in females infected with *Wolbachia* compared to tetracycline-treated	Alexandrov et al. (2007)
		Partial CI	Hoffmann (1988)
		Resistance/tolerance to RNA viruses	Hedges et al. (2008), Teixeira et al. (2008)
		Restored fertility in *Wolbachia*-infected *Sxl* mutants	Starr and Cline (2002)
*w*MelPop	*D. melanogaster*	Degradation of the nervous and muscle tissues; decreased lifespan	Min and Benzer (1997)
*w*No	*D. simulans*	CI	Mercot et al. (1995)
*w*Ri	*D. simulans*	CI	Hoffmann et al. (1986), Ballard (2004)
		Increased fecundity in infected females compared to uninfected or tetracycline-treated	Kriesner et al. (2013)
		Resistance/tolerance to RNA viruses	Osborne et al. (2009)

[a] *Drosophila* species were not included in this table if the phenotypic impacts of *Wolbachia* infection were absent or unknown

Molecular pathways underlying cross-talk between *Wolbachia* and *Drosophila*

Wolbachia, like other endosymbiotic bacteria, secretes various molecular factors through the Type IV Secretion System to interact with the host organism (Masui et al. 2000). Among other candidate factors, ankyrin domain-containing proteins likely play a crucial role. Ankyrin repeats, a repeating sequence of 33 amino acids, mediate protein–protein interactions in eukaryotes (Caturegli et al. 2000). The genome of *Wolbachia* contains 23 genes which encode proteins with ankyrin domains (Wu et al. 2004). The most probable candidate mediators of the *Wolbachia*–

Table 2 Effect of elimination of *Wolbachia* on lifespan in *D. melanogaster*

Strain of *D. melanogaster*	Lifespan of infected flies compared to uninfected ones	Reference
w^{1118}	Decreased	Min and Benzer (1997)
Z53	Conditionally increased	Fry and Rand (2002)
Z2	No difference	
Z53 and Ftf1	Increased	Fry et al. (2004)
Wj9	Decreased	
Z2 and Ftf100	No difference	
95	Increased	Alexandrov et al. (2007)
CantonS-Indy^{206}	Increased	Toivonen et al. (2007)
w^{Dah}	No difference	Ikeya et al. (2009)
w^{Dah} daGAL/InRDN	Increased	
w^{Dah} daGAL	Increased	
w^{Dah} InRDN	No difference	
w^{Dah} *dilp2–3,5*	Increased	Grönke et al. (2010)
CantonS	No difference or conditionally decreased	Ponton et al. (2015)

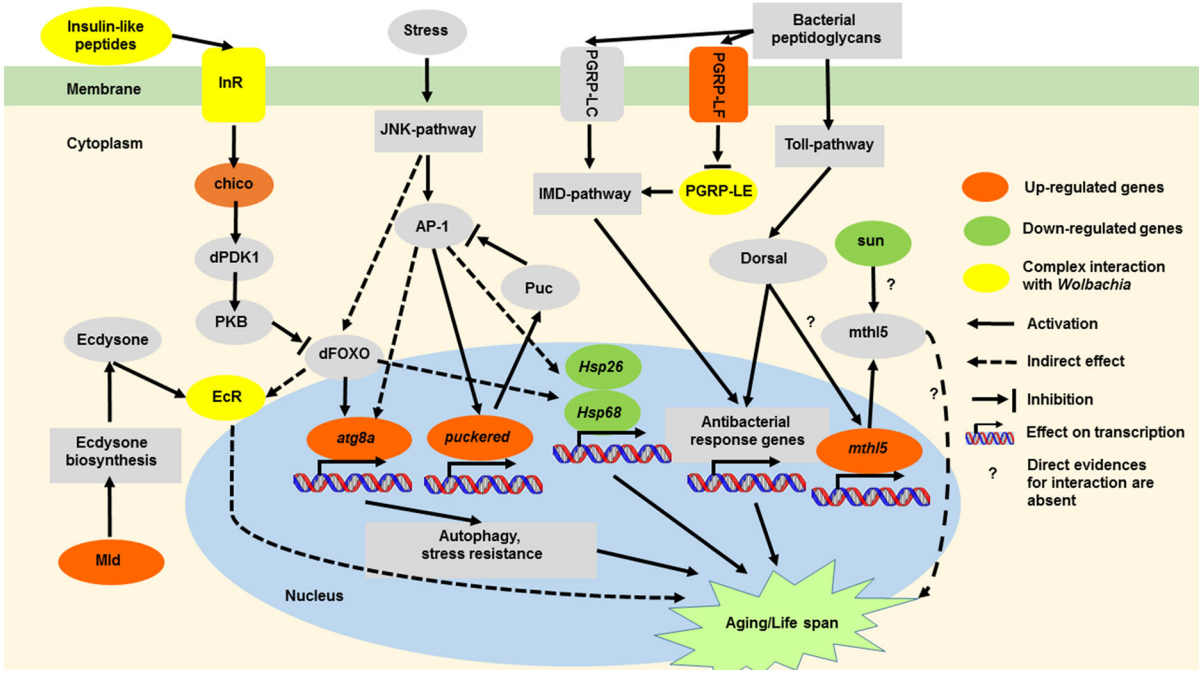

Fig. 1 A hypothetical scheme for the molecular cross-talk between *Wolbachia* and host regulatory networks contributing to *Drosophila* lifespan (for details, see Table 3)

Drosophila interactions are the WD0285, WD0636, and WD0637 proteins (Wu et al. 2004, Foster et al. 2005). These proteins have been detected in both facultative and obligate endosymbiotic bacteria (Siozios et al. 2013), and they have been shown to be involved in the induction of cytoplasmic incompatibility (CI) in *D. simulans* and *D. melanogaster* (Papafotiou et al. 2011). Since ankyrin domain-

Table 3 List of genes of *Drosophila* that are presumably involved in interactions with *Wolbachia*

Model/stage	Gene name[a]	Function of gene product	Effects observed	*Wolbachia* effects on expression	Reference
Adult females	*Atg8a*	Autophagy	Activation of host autophagy	Up-regulated	Voronin et al. (2012)
Larval testes	*chico*	Binding with insulin-like peptide (Ilp) receptor; Ilp signal trasmission	Regulation of organism growth	Up-regulated	Zheng et al. (2011)
N/A[b]	*EcR* (*Ecdysone receptor*)	Ecdysteroid hormone receptor; regulates RNA-polymerase II activity and the structure of chromatin	Regulation of autophagy, ecdisis and metamorphosis	N/A[c], ecdysone receptor possibly is a target of *Wolbachia* inducing altered reproduction	Negri and Pellecchia (2012)
S2 cell line	*Hsp22* (*Heat shock protein 22*)	Protein refolding	Response to heat and oxidative stress	Down-regulated	Xi et al. (2008)
Larval testes				Down-regulated	Zheng et al. (2011)
S2 cell line	*Hsp26*	Myosin binding	Response to heat and cold acclimation	Down-regulated	Xi et al. (2008)
S2 cell line	*Hsp27*	Protein refolding	Response to bacteria and fungi, behavioral response to starvation	Down-regulated	Xi et al. (2008)
S2 cell line	*Hsp68*	Protein refolding	Involved in neurogenesis; response to starvation	Down-regulated	Xi et al. (2008)
Adult males and females	*Ilp* (*Insulin-like peptide*)	Binding with insulin receptor	Carbohydrate and triglyceride homeostasis	N/A[c], flies carrying *dilp2-3,5* mutant alleles and *Wolbachia* had an extended lifespan	Grönke et al. (2010)
Larval testes	*Indy* (*"I'm not dead yet"*)	Citrate/succinate transmembrane transport	Involved in the regulation of metabolism of lipids and carbohydrates	Up-regulated	Zheng et al. (2011)
Adult males				N/A[c], flies carrying $Indy^{206}$ mutant allele and *Wolbachia* had an extended lifespan	Toivonen et al. (2007)
Adult males and females	*InR* (*Insulin receptor*)	Insulin binding; insulin signaling transmission	Organism growth; carbohydrate metabolism; gonad development and mating behaviour	N/A[c], flies carrying InRDN allele and *Wolbachia* had extended lifespan	Ikeya et al. (2009)
S2 cell line	*mld* (*molting defective* (DTS-3)	Ecdysone biosynthesis	Regulation of circadian rhythm	Up-regulated	Xi et al. (2008)
S2 cell line	*mthl5* (*methuselah-like 5*)	G-protein coupled receptor	Response to starvation	Up-regulated	Xi et al. (2008)
S2 cell line	*mys (myospheroid)*	Cell-substrate adhesion	Muscle and nervous system development	Up-regulated	Xi et al. (2008)

Table 3 continued

Model/ stage	Gene name[a]	Function of gene product	Effects observed	*Wolbachia* effects on expression	Reference
N/A[b]	*PGRP-LE* (*Peptidoglycan recognition protein-LE*)	Peptidoglycan binding	Defense response to Gramm-negative bacteria	N/A[c], PGRP-LE is hypothesised to bind *Wolbachia* peptidoglycan	Kaneko et al. (2006), Lemaitre and Hoffmann (2007)
S2 cell line	*PGRP-LF* (*Peptidoglycan recognition protein LF*)	Peptidoglycan binding	Antibacterial response	Up-regulated	Xi et al. (2008)
S2 cell line	*puc (puckered)*	Negative regulation of JNK-pathway	Regulation of development and stress responses	Up-regulated	Xi et al. (2008)
3rd instar larvae	*Sod* (mitochondrial and cytosolic *superoxide dismutase*)	Antioxidant and superoxide dismutase activities	Response to oxidative stress, regulation of mitophagy	N/A[c], *Wolbachia* decreased SOD activity	Wang et al. (2012)

[a] Gene names are given according to http://flybase.org/ release of November 20, 2015 (Attrill et al. 2015)

[b] Direct experimental research was not performed. The interaction of this gene with *Wolbachia* is rather hypothetical

[c] In this study changes in gene expression in the presence of *Wolbachia* infection were not measured

containing proteins are involved in the *Wolbachia*-induced CI, they can likely play a role in other aspects of *Wolbachia–Drosophila* interactions, including the flies' aging. Apparently, *Wolbachia* can also influence the synthesis of some other proteins involved in aging. The association between *Wolbachia*-induced transcriptional profiles and *Drosophila* aging process, however, has not been systematically studied so far.

In this analytical review, we summarize research findings regarding 175 genes that are thought to be involved in *D. melanogaster* aging (according to the FlyBase Gene Ontology (ID: GO:0007568) release of November 20, 2015), and 165 genes involved in adult lifespan determination (GO:0008340) and implicated in the molecular cross-talk between *Drosophila* and *Wolbachia* (Attrill et al. 2015; Ashburner et al. 2000). The list of these genes is presented in Table 3.

Wolbachia infection and immune response in *Drosophila*

Wolbachia-mediated lifespan effects appear to be significantly influenced by the flies' immune system protecting the organism against pathogenic bacteria, but also regulating the bacterial community required for normal functioning and survival (Eleftherianos et al. 2013). Chronic high-load infections and over-activated immune responses have also been suggested to cause lifespan shortening (Paik et al. 2012). In *Drosophila*, the signaling pathways regulating anti-microbial peptide gene expression are the immune deficiency (IMD) and Toll pathways (Aggarwal and Silverman 2008; Myllymaki et al. 2014) (Fig. 1). Activation of these pathways protects insects from pathogenic bacteria, resulting in increased survival. Chronic inflammation, on the contrary, is associated with accelerated aging rate and lifespan shortening (Myllymaki et al. 2014). In *Wolbachia*-infected fruit flies, up-regulated IMD and Toll pathways genes, alongside with up-regulated antimicrobial peptides genes, have been repeatedly observed (Eleftherianos et al. 2013).

The IMD pathway represents a crucial component in the response to infection in the *Drosophila* gut. This pathway has been shown to be activated by both the gut microbiota and ingested microorganisms, and it can also be induced by microbial infection (Buchon et al. 2013). In *Drosophila*, the bacterial load is known to increase over lifetime. This process is accompanied with the up-regulation of antimicrobial effector genes (Pletcher et al. 2002; Seroude et al. 2002) and IMD pathway target genes (Eleftherianos and Castillo 2012; Combe et al. 2014). Moreover, several lines of evidence indicate that the capacity of the *Drosophila* immune system to eliminate bacteria remains apparently unchanged in advanced ages (Eleftherianos and Castillo 2012). These data, however, contradict the findings by Brummel et al. (2004) who observed lifespan extension in fruit flies after bacteria had been removed after the age of 4 weeks. However, this discrepancy may also be due to different methods of obtaining the germ-free flies (Ridley et al. 2013) and variation in microbiota between different laboratory and wild-type strains of *Drosophila,* given the high complexity of the molecular interactions between bacterial symbionts and their hosts.

The most important components of innate immunity in *Drosophila* are innate immunity proteins, namely the peptidoglycan recognition proteins (PGRPs) playing a key role in defense against pathogenic organisms (Gupta 2008). The main functions of these proteins include the activation of major *Drosophila* immune pathways, such as the Toll and IMD pathways, as well as peptidoglycan recognition of the bacterial cell wall (Royet and Dziarski 2007). Among the 13 PGRP family members in *Drosophila*, the PGRP-LE protein is known to play a crucial role in peptidoglycan recognition and the activation of the IMD pathway (Fig. 1). In contrast to other important activators of IMD, the transmembrane protein PGRP-LE functions as an intracellular receptor for peptidoglicans (Kaneko et al. 2006) and thus is a likely candidate to interact with *Wolbachia* endosymbionts (Lemaitre and Hoffmann 2007). Overexpression of PGRP-LE in the fly fat body causes a constitutive immune up-regulation and enhanced pathogen resistance. However, chronic activation of PGRP-LE appears to induce permanent inflammation and shorten lifespan (Libert et al. 2006). Another important negative regulator of the IMD pathway belonging to this protein family is PGRP-LF, which most likely acts by inhibiting PGRP-LC through concurrent interactions with bacterial peptidoglycans (Maillet et al. 2008). Remarkably, the *PGRP-LF* gene has been reported to demonstrate a 1.26-fold up-regulation in *Drosophila* S2 cell lines infected with *Wolbachia* relative to the uninfected control cells (Xi et al. 2008).

Since the activity of immune pathways, including IMD, appears to be important for *Drosophila* survival, the effects of *Wolbachia* on both the *PGRP-LE* and *PGRP-LF* expression levels can likely affect fruit fly longevity (Paik et al. 2012).

Wolbachia-mediated stress response and lifespan in *Drosophila*

Oxidative stress response

The observed *Wolbachia*-mediated antiviral protection is suggested to be related to the elevated oxidative stress levels in endosymbiont-infected flies. High levels of reactive oxygen species (ROS) generated in the mitochondria are well known to cause cellular damage and promote aging. Recent findings, however, indicate that ROS act as essential signaling molecules to promote healthspan and longevity, and their moderate levels may induce an adaptive response and thus improve the systemic defense mechanisms (Simonsen et al. 2008; Ristow and Schmeisser 2014). In flies harboring the protective *Wolbachia* strains, concentrations of hydrogen peroxide have been found to be 1.25- to 2-fold higher than those in flies cured of this infection, and flies with high levels of endogenous hydrogen peroxide have been demonstrated to be less susceptible to virus-induced mortality (Wong et al. 2015).

Superoxide dismutase (SOD) is an important enzyme in the organismal antioxidant defense system and it is implicated in protecting the cells from the superoxide radicals generated through the aerobic metabolism (Fukai and Ushio-Fukai 2011). This enzyme is increasingly recognized for its regulatory functions in metabolism, growth and oxidative stress response (Che et al. 2015). Overexpression of SOD has repeatedly been demonstrated to correlate with reduced levels of oxidative damage and extended longevity in *Drosophila* under both normal metabolism and stress conditions (Fleming et al. 1992; Landis and Tower 2005). Presence of the *w*Mel *Wolbachia* strain has been shown to be associated with decreased superoxide dismutase (SOD) activity compared to tetracycline-treated *Wolbachia*-free flies (Wang et al. 2012). The *w*Ri strain, however, causes increased SOD levels in *Drosophila* spermatocytes (Brennan et al. 2012). Decreased SOD activity possibly explains the elevated levels of ROS in the study by Wong et al. (2015), in which *Wolbachia* infection was associated with increased viral resistance.

Jun N-terminal kinase pathway

In *Drosophila*, the stress-responsive Jun N-terminal Kinase (JNK) signaling pathway has been shown to play an important role in inducing autophagy under heat stress (Gonda et al. 2012), oxidative stress (Wu et al. 2009) and antibacterial responses (Maillet et al. 2008). This pathway is also believed to be a crucial genetic factor in the control of fruit fly longevity (Wang et al. 2003, 2005). Up-regulation of the JNK pathway via overexpression of its components, such as JNK/Bsk (Biteau et al. 2010) and JNKK/Hep (Libert et al. 2008), has been demonstrated to extend *Drosophila* longevity under both normal and stress conditions. A significant lifespan extension was observed in flies heterozygous for loss-of-function alleles of the *puckered* gene encoding a VH1-like phosphatase which play a key role in negative regulation of JNK activity (Wang et al. 2003, 2005). Moreover, flies bearing mutations that promote JNK signaling have been found to accumulate less oxidative damage and live significantly longer than wild-type ones (Wang et al. 2003). The forkhead transcription factor FOXO appears to be required for the JNK-mediated lifespan extension in *Drosophila* (Wang et al. 2005) (Fig. 1). The authors concluded that JNK signaling antagonizes the IIS pathway, resulting in the nuclear localization of FOXO and inducing its targets, such as stress defense and growth control genes. In addition, the JNK pathway has been shown to up-regulate the transcription of *Hsp* genes, such as *Hsp68* and *Hsp26*, via the *Drosophila FOXO* ortholog *dFOXO* (Wang et al. 2003, 2005).

In the *Drosophila* S2 cell system, *Wolbachia* infection has been found to up-regulate the expression of the *puckered* gene (1.3-fold change) (Xi et al. 2008), thereby potentially causing life extension in vivo. Moreover, a 1.3-fold up-regulation of *dJun* has been found in infected S2 cells, potentially inducing the up-regulation of *puckered* expression (Xi et al. 2008). Thus, *Wolbachia* infection can likely cause down-regulation of *Hsp* genes by up-regulating the *puckered* gene which, in turn, negatively regulates the JNK pathway.

Heat shock proteins

Another mechanism playing a significant role in *Drosophila* survival under stressful conditions is the synthesis of heat shock proteins (HSPs) (Karunanithi and Brown 2015). Many studies show that up-regulation of *Hsp* genes is associated with increased lifespan, while down-regulation results in life shortening in fruit flies (reviewed in Tower 2011). The main function of the chaperone proteins encoded by these genes is to assist in the processes of folding and refolding of other proteins, especially under stress conditions.

In research conducted on *Drosophila* S2 cells, 11 out of 27 *Hsp* genes demonstrated 1.14- to 1.36-fold down-regulation in *Wolbachia*-infected cells relative to the uninfected control cells (Xi et al. 2008). Among these genes, *Hsp22* and *Hsp68* have been previously shown to be crucial to longevity in *D. melanogaster* (Tatar et al. 1997; Wang et al. 2003; Morrow et al. 2004). Zheng et al. (2011) also revealed 1.55-fold down-regulation of the *Hsp22* gene in the larval testes of *Wolbachia*-infected flies. Importantly, the JNK signaling pathway has been shown to be involved in up-regulation of the *Hsp26* and *Hsp68* genes via dFOXO (Wang et al. 2003, 2005). Therefore, since the *puckered* gene negatively regulates the JNK pathway, *Wolbachia* infection may likely down-regulate the expression of *Hsp* genes.

Autophagy pathway

Autophagy is the cellular self-cleaning process responsible for elimination of damaged cellular components that protects cells against stressful conditions. The autophagic response allows to mobilize the cellular energy resources from recycled organelles in order to cope with stress, particularly in conditions of hypoxia, amino acid and/or glucose deprivation, genotoxic stress and viral infection (Feng et al. 2015; Filomeni et al. 2015). As a consequence, autophagy can enhance cellular fitness and survival. At the organismal level, the induction of autophagy is suggested to contribute to the beneficial effects of calorie restriction and exposures to low-dose radiation, toxins and other stressors (Szumiel 2012; Moore et al. 2015). It also plays an important role in lifespan extension in various experimental models, most likely because it prevents the age-associated accumulation of damaged proteins and organelles (Gelino and Hansen 2012; Martinez-Lopez et al. 2015; Madeo et al. 2010, 2015).

In *Drosophila,* autophagy was shown to be regulated by crosstalk between the TOR, IIS, IMD and JNK pathways (Gelino and Hansen 2012). One of the *Drosophila* genes that is closely related to the autophagy process, namely, *autophagy-specific gene 8a* (*Atg8a*), is known to encode a protein involved in the control of the intracellular *Wolbachia* density in many invertebrate species (Voronin et al. 2012). *Atg8a* was found to be three times higher expressed in *D. melanogaster* infected with the pathogenic *Wolbachia* strain *wMelPop* relative to uninfected flies (Voronin et al. 2012). Elevated expression of *Atg8a* in the nervous system of adult flies has been suggested to be implicated in life extension through inducing oxidative stress resistance and eliminating damaged cell components (Simonsen et al. 2008), thereby leading to cell population rejuvenation.

IIS pathway

Drosophila longevity is known to be substantially dependent on nutritional conditions, such as the balance between dietary proteins and carbohydrates (Simpson and Raubenheimer 2012). *Wolbachia* infection has been shown to substantially influence the impact of the host nutritional status on lifespan. In particular, *Wolbachia* has been demonstrated to modulate the effect of the protein/carbohydrate (P:C) ratio on *Drosophila* longevity (Ponton et al. 2015). Flies fed with the ratio of 1:16 P:C in their diet lived longer than those who fed with 1:1 P:C ratio. No differences were observed in survival curves for infected and non-infected insects fed with 1:16 P:C and when flies were allowed to choose between 1:1 P:C and 1:16 P:C food. However, under the 1:1 P:C ratio uninfected flies lived longer. The authors explain these findings by competition for carbohydrates between *Wolbachia* and the host, resulting in a decreased lifespan of infected flies. Moreover, in this study infected flies reared on substrates with the 1:1 P:C ratio demonstrated higher reproduction rates than uninfected ones. When flies were allowed to select between yeast and sucrose solutions, the protein intake was higher in uninfected flies compared to infected. The carbohydrate intake was about the same in infected and uninfected flies. The average P:C ratio in infected and uninfected flies

was 1:20 and 1:9, respectively. The authors suggested that *Wolbachia*-infected flies can modify their nutritional behavior to ameliorate the life-shortening effect of infection at the cost of decreased reproduction.

The link between the flies' dietary status and their longevity is likely mediated by the IIS signaling pathway known to play a crucial role in the regulation of nutrient uptake and metabolism (Nässel et al. 2015). This pathway is thought to play a central role in growth, stress resistance, reproduction, metabolism and lifespan determination of all multicellular organisms, including *D. melanogaster* (Junnila et al. 2013; Sadagurski and White 2013; Wang et al. 2014). Moderate tissue-specific and/or whole-organism reduction in the IIS pathway activity has been found to be associated with life extension in fruit flies (Broughton and Partridge 2009). DILPs are triggers of the insulin signaling cascade that act through binding to the insulin receptor (InR) (Fig. 1). There are 7 DILPs that are expressed in *D. melanogaster* in a tissue- and developmental stage-specific manner (Brogiolo et al. 2001). Grönke et al. (2010) have found that homozygous *dilp2* null mutants and homozygous *dilp2,3*- null double mutants have a significantly extended median lifespan, and homozygous *dilp2-3,5* null triple mutants have a slightly extended maximum lifespan. The extended mean lifespan has also been observed in flies with ablated median neurosecretory cells that produce DILPs 2, 3 and 5 (Broughton et al. 2005).

Wolbachia infection has been demonstrated to substantially influence the IIS pathway (Grönke et al. 2010). Its up-regulation has been found in infected flies with null-mutations in some genes within this pathway. Grönke et al. (2010), by examining how *Wolbachia* interacts with the *Drosophila* IIS pathway, found that loss of DILPs produced in the brain significantly extended lifespan, but only in the presence of *Wolbachia*. Specifically, *wDah dilp2-3,5* mutants that carried *Wolbachia* had increased median and maximum lifespans compared to *wDah* wild type lines with and without *Wolbachia* and *Wolbachia*-free *wDah dilp2-3,5* mutants. However, *Wolbachia* infection did not contribute to the observed lifespan extension in *dilp2* single mutants and *dilp2-3* double mutant flies. *Wolbachia* infection also contributed to DDT resistance of *dilp2-3,5* triple mutants, but had no effect on the survival of flies under starvation and peroxide treatment. The authors suggest that moderate down-regulation of IIS can cause life extension. A simultaneous loss of DILPs 2, 3, or 5 may, however, lead to deleterious phenotypic effects, which *Wolbachia* infection might attenuate by up-regulating IIS signaling. In the Ikeya et al. (2009) study, dominant negative reduction of insulin receptor (InRDN) activity in the presence of *Wolbachia* led to reduced growth and fecundity and extended lifespan. In uninfected InRDN flies, extreme dwarfism, sterility, increased fat content and decreased lifespan were observed compared to infected and uninfected control flies and infected flies with mutant receptor InRDN. Removal of *Wolbachia* from control flies caused a moderate reduction in weight and fecundity but did not affect lifespan. Expression of InRDN in the fat body had no effect on lifespan in *Wolbachia*-infected flies, whereas removal of *Wolbachia* resulted in lifespan extension. These data suggest that *Wolbachia* may interact with IIS downstream of InR. No differences in expression of the IIS downstream target 4E-BP have been found, however, in infected and uninfected *dilp2-3,5* flies (Grönke et al. 2010). There were also no differences between infected and uninfected *dilp2-3,5* mutants in egg-to-adult development time, stress resistance, survival, fecundity and energy storage. Taken together, these data suggest that life extension observed in infected flies might proceed via other pathways than IIS.

To summarize, although the interaction of *Wolbachia* with various components of the *Drosophila* IIS pathway is still obscure, the presence of this endosymbiont in many cases seems to alleviate IIS pathway mutant phenotypes (Fig. 1).

Life extension was also obtained in flies with a *chico* gene dose reduction. This gene is known to encode the ligand of InR. An extended median lifespan has for instance been observed in flies carrying a loss-of-function allele *chico1* (Clancy et al. 2001). *Wolbachia*-infected flies demonstrate up to 1.53-fold up-regulation of the *chico* gene expression in the larval testes (Zheng et al. 2011). These data indicate that the *chico* gene may be implicated in mediating the *Wolbachia*-induced longevity phenotypes.

dFOXO is another important factor mediating the effects of IIS on lifespan. This factor is maintained in the cytoplasm via the phosphorylation by IIS. While dFOXO is localized in the cytoplasm, JNK-activated stress response genes are repressed. IIS-mediated phosphorylation therefore operates antagonistically to JNK-mediated phosphorylation (Partridge and Brüning 2008). Giannakou et al. (2004) have found

that up-regulation of *dFOXO* is associated with lifespan extension in flies. Slack et al. (2011) using a newly generated null allele of *dFOXO* have demonstrated that the IIS-mediated lifespan extension was almost completely blocked by the removal of dFOXO. However, unlike *C. elegans*, the lack of dFOXO did not suppress fecundity, oxidative stress resistance and body size phenotypes in IIS-compromised fruit flies.

Ecdysteroid biosynthesis and signaling

Drosophila lifespan is known to be significantly dependent on the signaling pathway of ecdysone, a steroid hormone which is a major regulator of insect development. This pathway is also involved in the manifestation of *Wolbachia*-induced reproductive phenotypes (Negri et al. 2010; Negri 2011). Flies heterozygous for a mutation in the *EcRV559fs* gene encoding ecdysone receptor have been found to exhibit increased lifespans and stress resistance, with no evident deficit in locomotor activity or fertility (Simon et al. 2003). Female flies of the DTS-3/+ strain that are mutant for the *moulting defective (mld)* gene implicated in ecdysone biosynthesis also demonstrate increased longevity when cultivated at 29 °C. Remarkably, a 1.67-fold up-regulation of this gene has been detected in the S2 cell line infected with *Wolbachia* (Xi et al. 2008). Consequently, it has been suggested that *Wolbachia* produces specific regulators able to interact both directly and indirectly with the ecdysone receptor, thereby modulating the ecdysteroid signaling (Negri and Pellecchia 2012). These findings suggest that the ecdysteroid pathway can be involved in *Wolbachia*-mediated lifespan modulations in *D. melanogaster* (Fig. 1).

Indy gene

Another gene playing an important role in the energy metabolism and longevity regulation in fruit flies is the *Indy* (*I'm not dead yet*) gene encoding an exchanger for Krebs cycle intermediates. Reduced expression of this gene leads to metabolic alterations similar to those induced by calorie restriction, such as increased mitochondrial biogenesis as well as altered metabolism of lipids and carbohydrates (Frankel and Rogina 2012), and also causes life extension in *Drosophila* (Rogina and Helfand 2013; Rogers and Rogina 2015). Variations of this gene have been demonstrated to confer fitness advantage and lifespan extension in natural populations via a transposon insertion (Zhu et al. 2014). Specifically, flies heterozygous for a *Hoppel* transposon insertion substantially outlive homozygous insects lacking the insertion in the *Indy* gene.

In the *CS-Indy*^{206} line, its extended longevity phenotype was inhibited by tetracycline treatment (Toivonen et al. 2007). The authors suggest that the life-extending effect could have likely been mediated by *Wolbachia* infection, and this effect could have been abolished by antibiotic treatment. Remarkably, in the *CS-Indy*^{206} line, the extended longevity phenotype was abolished by tetracycline treatment (Toivonen et al. 2007). The authors speculate that the extended longevity phenotype has been induced by *Wolbachia* or other bacteria which might be removed by tetracycline. Noteworthy, the expression of the *Indy* gene has been found to be up-regulated in the larval testes by the presence of *Wolbachia* (Zheng et al. 2011).

Methuselah gene

The *methuselah* (*mth*) gene encoding a G-protein-coupled receptor, is known to be among the genes linked to lifespan and stress resistance in fruit flies (Paaby and Schmidt 2008; Petrosyan et al. 2014). Mutations reducing the transcriptional activity of this gene have been found to extend *Drosophila* longevity (Lin et al. 1998). *The methuselah-like (mthl)* genes are also believed to play a substantial role in determining lifespan (Araújo 2012). *Wolbachia*-infected and uninfected S2 cell cultures have not been found to demonstrate any differences in the expression levels of the *mth* gene; the *mthl5 gene*, however, has been found to be 1.26-fold up-regulated. The *stunted* (*sun*) gene known to encode a peptide agonist of Mth and whose mutation resulted in lifespan extension in *Drosophila* (Cvejic et al. 2004), was, on the contrary, 1.18-fold down-regulated in infected S2 cells (Xi et al. 2008).

Interactions between pathways

The inconsistency of *Wolbachia* effects on lifespan phenotypes in *Drosophila* among different studies (Table 2) can possibly be explained by a complex regulatory network with which *Wolbachia* interacts

(Fig. 1). The JNK and IIS pathways are in an antagonistic relationship (Karpac and Jasper 2009). The ecdysone pathway is regulated by IIS (Orme and Leevers 2005). The JNK pathway splits from the IMD pathway at the level of JNKKK(Tak1): the IMD and Toll pathways branches lead to the activation of antimicrobial response, while the JNK pathway activates stress response genes (Fig. 1) (Myllymaki et al. 2014). The presence of *Wolbachia* appears to cause down-regulation of some genes which when overexpressed promote lifespan (such as for example *Hsp26* and *Hsp68*) (Fig. 1; Table 3), up-regulation of other genes that promote lifespan when overexpressed (*atg8a*, *PGRP-LF*), and up-regulation of genes that promote lifespan when less expressed (*puc*, *mld*, *chico*, *mthl5*). Identification of the main pathway/gene that interacts with *Wolbachia* and contributes to the lifespan phenotype in *Drosophila* is complicated by at least three facts: (1) alterations of gene transcription levels do not predict the quantity of functional protein from those genes (Vogel and Marcotte 2012), (2) the summarized gene expression changes (Table 3) under the influence of *Wolbachia* have been observed on different model systems (e.g. S2 cell line by Xi et al. 2008, dissected larva testes by Zheng et al. 2011, and adult flies by Voronin et al. 2012), and (3) an apparently complex crosstalk between pathways involved in lifespan determination in *Drosophila*.

Conclusion

The research findings reviewed in this article suggest that the molecular cross-talk between *Drosophila* and its microbiota can have an important impact on the host's lifespan. In particular, the *Drosophila* endosymbiont *Wolbachia* has been shown to be able to substantially affect the expression of some key longevity-associated genes. These genes are known to be responsible for a variety of pathways and processes essential for the flies' viability, such as stress resistance, immune response, autophagy, energy metabolism, oxidative stress defense, and other key survival functions. It is not clear, however, why infection with *Wolbachia* can in some cases promote longevity, while in other cases it causes life-shortening effects.

These impacts of *Wolbachia* infection should therefore be taken into account in the research of aging processes using the *Drosophila* model. Further investigation is also required for a more precise identification of the molecular pathways by which *Wolbachia* modulates the aging process and lifespan in *Drosophila*.

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