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The main actors involved in extending the invertebrate life span / Ottaviani, Enzo; Franchini, Antonella; Mandrioli, Mauro. - In: INVERTEBRATE SURVIVAL JOURNAL. - ISSN 1824-307X. - ELETTRONICO. - 11:(2014), pp. 73-78.

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## MINIREVIEW

**The main actors involved in extending the invertebrate life span****E Ottaviani, A Franchini, M Mandrioli***Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy**Accepted February 3, 2014***Abstract**

Classical invertebrate models, *i.e.*, *Drosophila melanogaster* and *Caenorhabditis elegans*, have provided the keys to understand the life span regulation. In the present paper we summarize the mechanisms involved in this process with particular emphasis on the role of the fly fat body. It is interesting to note that pathways which lead to an extension of life span are highly conserved in animals so that “longevity pathways” identified in invertebrates provide templates for the identification of genes and drugs that regulate longevity and diseases also in other animals, including mammals.

**Key Words:** *Drosophila melanogaster*; insulin/IGF-1-like pathway; fat body; gut microbiota; longevity

**Introduction**

Aging is a well-conserved process during evolution that involves different actors including dietary restriction (DR), fat body and/or adipose tissue and insulin/insulin growth factor-1 (IGF-1)-like pathway (Klötting and Blüher, 2005). The regulation of life span in different organisms starts from glucose (as observed in yeasts) or insulin/IGF-1-like (as described in worms and flies). The increase of growth and mortality occurs through the downregulation of antioxidant enzymes and heat shock proteins, together with the reduction of the accumulation of glycogen and/or fat. Vice versa, the decrease of these pathways prolonged the life span by simulating the DR (see for review, Katic and Kahn, 2005).

**Insulin/IGF-1-like pathway and DR**

The insulin/IGF-1-like (IIS) pathway probably plays a central role in the evolution of multicellularity (Skorokhod *et al.*, 1999). It is involved in several processes, including growth and longevity and a reduced activity of the pathway extends life span (Partridge and Gems, 2002; Tatar *et al.*, 2003; Kenyon, 2005). The regulation of growth and size in *Drosophila melanogaster* requires the following components: the insulin/IGF-1 receptor INR (insulin-like receptor), the INR substrate CHICO, the PI3K, the PI3K target PKB (also known as Akt1) and dFOXO, the fly forkhead transcription factor

phosphorylated and inactivated in response to IIS (Weinkove and Leevers, 2000) (Fig. 1).

Studies performed in invertebrates, such as *D. melanogaster*, *Caenorhabditis elegans* and *Trechus angusticollis*, demonstrated that a DR was able to extend life span at the expense of the fecundity (Chippindale *et al.*, 1993; Partridge *et al.*, 2005; Heestand *et al.*, 2013). However, it is still unclear if the observed increase in life span is due to a specific nutrient or dietary (Tatar, 2011) and the trade-off between longevity and fecundity related to DR is not always observed (Heestand *et al.*, 2013).

In flies the extension of life span by DR involves the rapamycin (TOR) signaling pathway (Kapahi *et al.*, 2004), and the increase of triacylglycerols (TAG) (Bohni *et al.*, 1999; Zhang *et al.*, 2000). Furthermore, DR intervenes in the fatty acid metabolism, a process required for the life span extension (Katewa *et al.*, 2012).

**Fat body**

Fat body is an important source of energy that is stored as glycogen and TAG (Leopold and Perrimon, 2007; Arrese and Soulages, 2010). TAG are the core of the so-called lipid particles, also known as lipid droplets or lipid bodies (Ottaviani *et al.*, 2011a). At a phylogenetic level, TAG are present in yeasts, such as *Saccharomyces cerevisiae* (Zweytick *et al.*, 2000; Daum *et al.*, 2007) and *Candida parapsilosis* (Neugnot *et al.*, 2002), the nematode *C. elegans* (Watts, 2009), the molluscs *Ifrimeria nautilei* (Saito and Hashimoto, 2010) and *Haliotis fulgens* (Nelson, 2002), the insect *D. melanogaster* (Grönke *et al.*, 2005), the sea star *Asterias rubens* (Allen, 1998), the sea urchin

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*Echinus esculentus* (Allen, 1998) and the sea cucumber *Holothuria forskali* (Allen, 1998).

It has been reported that the reduction of adipose tissue influences the extension of the life span in different invertebrates (Hwangbo *et al.*, 2004; Kenyon, 2005; Klötting and Blüher, 2005). For instance, the reduction in fat mass in *D. melanogaster* provokes an overexpression of dFOXO with the consequent extension of the life span.

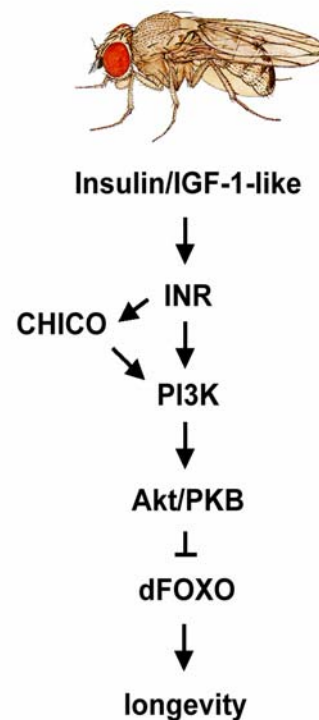
Recent findings showed that the addition of *Escherichia coli* to the diet of *D. melanogaster* females significantly increase the longevity of both the two strains examined [a short-life strain (Bloomington *Drosophila* Stock Center (FBst0006971) with an average adult life span of 10 days and a long-life standard lived R strain with an average adult life span of 50 days] (Franchini *et al.*, 2012). In the short-life flies the lengthening of lifespan was particularly evident at days 7 and 9 when the survived flies grown in presence of bacteria were four and three times more numerous than controls. Moreover, 5 % of flies fed with *E. coli* were still alive at day 11, whereas controls were all dead. In the long-life strain, an extension of the life span was also observed: at days 45 and 48 the percentages of survived flies were three and five times higher in the bacteria fed samples than in controls and about 20 - 25 % of flies grown in presence of *E. coli* was still alive while controls were all dead (between the 49<sup>th</sup> and 51<sup>st</sup> day).

The comparison of structural and histochemical observations from flies fed with different diets, demonstrated that the presence of *E. coli* induced modifications in the fat body. This organ was characterized by a loose tissue of both layers of cells close to the integument and different sized lobes surrounding the internal organs in thorax and abdomen cavities. The most abundant cell type consisted of large polygonal cells containing different amounts of stored materials. At day 4, when no difference in survival of females from short-life strain was found, the morphology and histochemical reactivity of fat body from samples fed by *E. coli* were similar to those of most of the controls. It was well developed with the main cell type rich in glycogen and few empty unstained vacuola of different size (Fig. 2a). However, the network of loose perivisceral lobes of cells poor in glycogen and rich in lipid droplets, observed in some control flies, were not detected in bacteria fed samples. At day 9, the fat body of control survived flies appeared reduced in its perivisceral lobes that were constituted by vacuolated cells mainly storing lipid droplets (Fig. 2b). In contrast, fat bodies from most flies grown in the presence of bacteria were formed by well-developed islets of cells, full of glycogen (Fig. 2c). In some flies, the perivisceral cell aggregates contained lipid droplets (Fig. 2d). The fat body in control and bacteria fed samples from the long-life strain, similar to that of short lived flies, did not show relevant differences in structure and histochemical reactivity when no difference in survival was detected. In contrast, when the longevity was significantly extended, an higher percentage of controls contained reduced glycogen

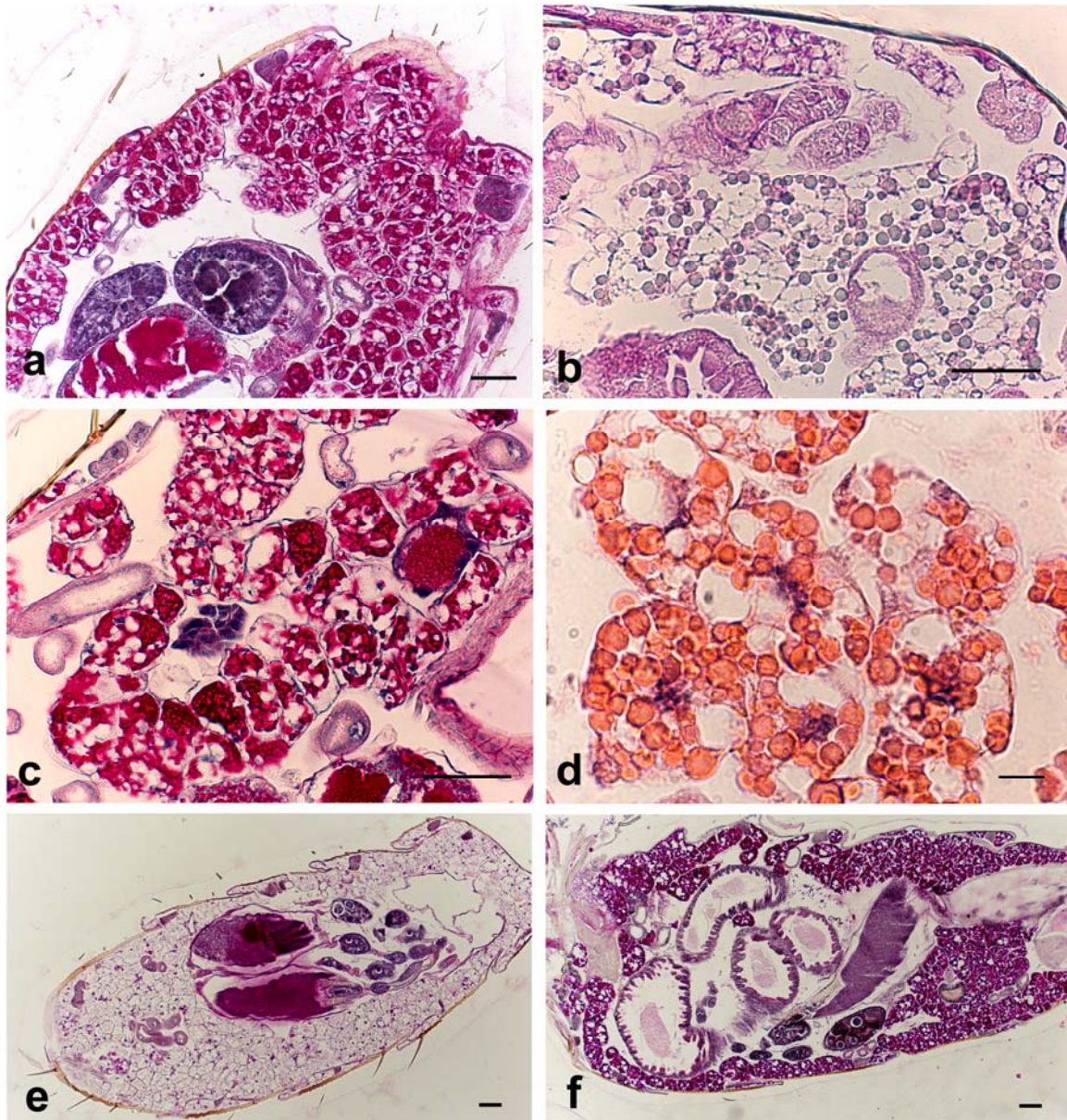
stores in comparison with bacteria fed flies (Figs 2e, f). The fat body cells were PAS-negative in 85 % of control survived flies against a 55 % of bacteria fed flies at day 48. In contrast to the short lived strain, no lipid droplets have seen to accumulate in empty vacuolated cells in the course of fly aging.

### Gut microbiota and insulin signaling pathway

In recent years, the presence of a reduced microbiota (less than 30 species) made *D. melanogaster* an intriguing model to understand the principles that govern host-microbiota interactions (Kostic *et al.*, 2013). Indeed, *Drosophila* represents an experimentally tractable system to discover the molecular underpinnings the host-commensal interactions also in other insects, including those that act as vectors of infectious diseases or are of importance to agriculture (Douglas *et al.*, 2011). In other cases, the implications of *Drosophila*-microbiota interaction allowed to uncover broader concepts of mutualism that are conserved among higher-order organisms (Kostic *et al.*, 2013).



**Fig. 1.** Regulative pathway of life span in flies. A reduction of the insulin/IGF-1- pathway activates a cascade resulting in dFOXO overexpression and an extended longevity. INR: Insulin-like receptor. CHICO: INR substrate. PI3K: phosphatidylinositol-3 kinase. Akt/PKB: serine/threonine kinase B. dFOXO: fly homologue of the mammalian forkhead (FOXO) family of transcription factors.

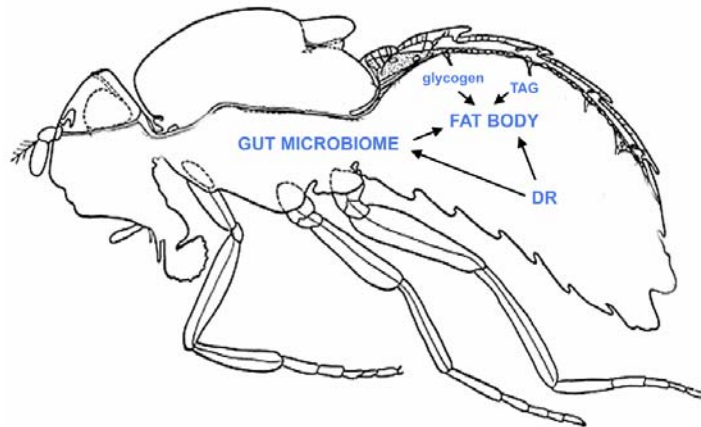


**Fig. 2** Longitudinal sections of the fat body from short- (a-d) and long (e, f) life *Drosophila* females fed with standard diet (controls) and in the presence of *E. coli* (PAS/hematoxylin, a-c, e, f; hematoxylin-eosin, d). At day 4, when no survival differences were found between controls and bacteria fed flies, the structure and histochemical reactivity of fat body were similar (a). At day 9, in control survived flies the perivisceral lobes were reduced and the vacuolated cells mainly stored lipid droplets (b). In contrast, in most flies grown with addition of bacteria to the diet, well developed islets of cells were full of glycogen (c) while in some females the cells of perivisceral aggregates contained lipid droplets (d). The fat body from 45 day old long-life control females, with strongly reduced glycogen stores (e), is compared with that from bacteria fed flies (f). Bars: 50  $\mu\text{m}$  (a-c, e, f); 10  $\mu\text{m}$  (d).

According to previous studies, the microbiota regulates the accumulation of fat by promoting the storage of TAG in the adipocytes (Bäckhed *et al.*, 2004), and regulates the life span in flies (Seung *et al.* 2011). Interestingly, the fly commensal bacterium *Acetobacter pomorum* modulates the IIS expression in the fat body affecting host homeostatic programs controlling developmental rate, body size, energy metabolism and intestinal stem cell activity. This

result is due to the ability of *A. pomorum* to induce the activation of the PI3K suggesting that this commensal bacterium also have an effect on the fly life span (Shin *et al.*, 2011).

The possible regulative role of *A. pomorum* in fly life span is not surprising taking into account that the digestive tract of many insect species harbours several bacteria that perform different beneficial functions to their host and may affect host longevity



**Fig. 3.** The circuitry of longevity in flies. TAG: triacylglycerols. DR: dietary restriction.

(Ottaviani *et al.*, 2011b). For instance, *Ceratitis capitata* life span was extended after feeding with Enterobacteriaceae due to direct effects of these symbionts on medfly metabolism and development (Behar *et al.*, 2008). Similarly, non-virulent strains of *Wolbachia* can extend *Drosophila* life span (Fry and Rand, 2002) and experiments performed using axenic cultures and antibiotic treatment revealed that exposure to bacteria during the first week of adult life increased longevity by 30 - 35 % in flies (Pletcher *et al.*, 2002; Seroude *et al.*, 2002; Brummel *et al.*, 2004).

A second set of intriguing data have been published by Storelli *et al.* (2011) evidencing a reduced insulin signaling in germ-free *Drosophila*, while the addition of the commensal bacterium *Lactobacillus plantarum* is sufficient on its own to restore the natural *Drosophila* microbiota growth-promoting effect. According to the published data, *L. plantarum* exerts its benefit by acting genetically upstream of the TOR-dependent host nutrient sensing system controlling hormonal growth signaling. The key implication of this study, together with data of Shin *et al.* (2011), is that different bacterial products, derived from taxonomically divergent bacteria, can affect insulin signaling in *Drosophila*.

As a whole, these data on *Drosophila* open some intriguing questions, since as suggested by Douglas (2011), multiple bacterial products may interact (competitively, additively and/or synergistically) with the *Drosophila* insulin signaling networks, so that the “standard” setpoint of the fly insulin signaling could be a titration between the high and low preferred setpoints of the bacteria and fly respectively (Douglas, 2011). Interestingly, if the intrinsic set point of flies is calibrated constitutively to account for bacterial manipulation (as is likely because bacteria are always present in naturally occurring *Drosophila*), then the signaling would be depressed in the germ-free flies, which lack the manipulative up-regulation by the bacteria (Douglas, 2011). The insulin signaling may be therefore the

result of an evolutionary “agreement” between *Drosophila* and its microbiota, explicable not only in the context of fly ecology, but also in terms of the long evolutionary history of fly-microbiota interactions. Bacterial intervention in animal signaling networks can be considered as part of how the resident microbiota keeps flies healthy and also mediate the life span extension (Douglas 2011).

### Conclusive remarks

The data here reported show that several components, such as insulin pathways, DR, fat body and gut microbiota are deeply interconnected in insect aging and interact for extending life span (Fig. 3).

The life history of each animal is therefore a trade-off resulting from the complex evolutionary history of each species that should face different kinds of competitively, additively and/or synergistically interactions. This scenario opens an intriguing perspective for human health and aging since if our life span has been defined by an heterogeneous set of interactions among our genome, diet, microbiota and environment, we can try to disentangle this evolutionary settled equilibrium (for instance through the supplementation of nutraceuticals) looking for a different, and artificially defined, new state aimed to shape our health and aging with beneficial effects. In this context, the presence of conserved “longevity pathways” from invertebrate to vertebrates could provide templates for the identification of genes and drugs that regulate longevity and diseases in mammals making evolutionary medicine able to complement other approaches to issues in medical research and practice.

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