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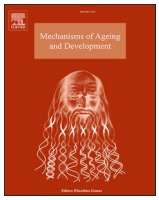

Review

Age-associated anatomical and physiological alterations in *Caenorhabditis elegans*

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ABSTRACT

Since its introduction by Sydney Brenner, *Caenorhabditis elegans* has become a widely studied organism. Given its highly significant properties, including transparency, short lifespan, self-fertilization, high reproductive yield and ease in manipulation and genetic modifications, the nematode has contributed to the elucidation of several fundamental aspects of biology, such as development and ageing. Moreover, it has been extensively used as a platform for the modelling of ageing-associated human disorders, especially those related to neurodegeneration. The use of *C. elegans* for such purposes requires, and at the same time promotes the investigation of its normal ageing process. In this review we aim to summarize the major organismal alterations during normal worm ageing, in terms of morphology and functionality.

1. Introduction

Over time, biomolecules and, in turn, cells and tissues of most organisms gradually accumulate damage, which compromises their physiological functions (Daskalaki et al., 2019; Kirkwood and Austad, 2000). This ageing process is often accompanied by several disorders, including cancer and neurodegeneration, that severely affect the global population. Major scientific efforts are continuously made in order to elucidate the age-related pathophysiological mechanisms towards healthspan extension. Investigation of these conserved mechanisms on mammalian and less-complex invertebrate models constantly reveals numerous molecular targets for anti-ageing treatments.

One such widely-used model, introduced by Sydney Brenner, is the nematode worm *Caenorhabditis elegans* (Brenner, 1974). *C. elegans* is a small and transparent animal, with relatively simple anatomy and a short lifespan of about a few weeks. The main sexual form is the hermaphrodite, that can reproduce by both self-fertilization and mating with rarely-occurring males, generating a large number of offspring (Corsi et al., 2015). Key interventions, including mutagenesis, transgenesis, gene knockdown, as well as CRISPR-based knock in and knock out, are easy to be performed. These properties render both somatic and reproductive age-associated declines fully traceable.

Mammals and the worm share major molecular and cellular similarities. However, the adult *C. elegans* individuals exhibit a key distinctive feature: they consist entirely of post-mitotic cells. Although cellular senescence, which was considered to be a feature of dividing cells, has been recently indicated in post-mitotic mammalian cells (reviewed in von Zglinicki et al., 2021), its possible role in ageing *C. elegans* remains obscure. In any case, progressive organismal dyshomeostasis lies on the base of the nematode ageing.

Age-dependent organismal decline, as manifested by morphological, functional and behavioural defects, originates from alterations at the molecular level. In several worm tissues gene expression undergoes considerable change over time (Wang et al., 2022). Moreover, DNA damage is significantly increased (Klass et al., 1983), whereas proteins become more insoluble and tend to accumulate (David et al., 2010). Additionally, disorganized actin cytoskeleton appears in ageing hypodermis, muscles and intestine (Higuchi-Sanabria et al., 2018). Following molecular changes, several cellular structures are affected. Nuclear morphology and protein composition are altered (Garigan et al., 2002; Haithcock et al., 2005; Palikaras et al., 2023). Mitochondrial networks are progressively degraded in neurons and muscles (Gaffney et al., 2018; Mergoud Dit Lamarche et al., 2018; Morsci et al., 2016; Regmi et al., 2014). Lysosomes become enlarged, tubular, less acidic and more

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dysfunctional, possibly compromising the autophagic flux (Aman and Schmauck-Medina, 2021; Sun et al., 2020). The ageing process is deemed to be governed by a plethora of cascades (reviewed in Daskalaki et al., 2019), including the widely studied insulin/IGF-1 signalling (IIS), that will be discussed in details below.

Ultimately, worms with age-related systemic deterioration are led to death. Several studies have attempted to reveal the mechanisms underlying *C. elegans* organismal death. Importantly, the rate of age-related mortality increase was found to be affected by the current environment, following a shift in temperature or food conditions (Wu et al., 2009). Interestingly though, a stable existing "memory" was indicated to be formed by the previous conditions. A decade ago, it was shown that death is accompanied by the emission of blue fluorescence, originated in the intestine by the necrotic cell death cascade (Coburn et al., 2013). This 'death fluorescence', as termed, was generated by anthranilic acid glucosyl esters, instead of lipofuscin, and was observed to spread systemically. Since blue fluorescence was emitted by gut granules, which are lysosome-related organelles, it was considered possible that it resulted from necrosis, which accompanies, and probably causes, organismal death (Coburn et al., 2013). A later study identified another significant death event, which resembles mammalian *Rigor Mortis*, and was termed 'death contraction' (Galimov et al., 2018). During this phenomenon, body wall muscles contract strongly, which is followed by intestinal necrosis. As indicated above, the alimentary (digestive) system of the worm, is closely related to the process of organismal death. Several additional studies have highlighted the connection of pharyngeal pathology to age-related decline and potential deleterious effects of bacterial food on organismal integrity. These findings will be further discussed below (see 'Alimentary system').

In this review we aim to summarize the major age-related changes in *C. elegans* in a systemic context. For this purpose, we have divided the organism into five functional systems: the hypodermis-cuticle system, the mechanosensory system, the neuromuscular system, the alimentary system and the reproductive system. For each, we provide a brief overview of structure and function, key ageing behavioural-to-subcellular phenotypes, as well as putative underlying mechanisms (see also Table 1). We also highlight key conclusions of studies on transcriptome, proteome and metabolome alterations during ageing.

2. Hypodermis-cuticle system

In this section we describe how the external layers of the adult worm (i.e. the hypodermis and cuticle) decline with age (Fig. 1). Underneath the cuticle lies the hypodermis (or epidermis), which synthesizes the former, and, in the adult animal is composed of the main body syncytium, as well as some smaller cells anteriorly and posteriorly (Altun and Hall, 2009d). The cuticle is synthesized five times during development, and consists of proteins (namely collagens, cuticulins and glycoproteins) and lipids. The cuticle preserves the body shape, provides protection from external factors and, importantly, enables movements, since it is attached to the musculature (Page and Johnstone, 2007).

The hypodermis has been reported to exhibit several age-related deficits. Of the first ones reported were cell membrane degeneration and accumulation of lipids (Epstein et al., 1972; Herndon et al., 2002). Subsequent studies also showed nuclear deformation (Palikaras et al., 2023) and deterioration of the actin cytoskeleton (Higuchi-Sanabria et al., 2018). With respect to the actin, knockdown of the heat shock transcription factor HSF-1 gene appeared to result in premature cytoskeletal ageing, whereas its overexpression favoured cytoskeletal preservation (Higuchi-Sanabria et al., 2018).

The functionality of hypodermis during life was found to be regulated by the insulin/IGF-1 signalling pathway (IIS), which is a major component of lifespan regulation and ageing (reviewed in Daskalaki et al., 2019). More specifically, reduced IIS, which is a well-known anti-ageing intervention, was shown to promote collagen expression (Ewald et al., 2015). However, thicker cuticle has been reported as a

Table 1
Age-related changes in *Caenorhabditis elegans* systems.

System	Ageing phenotypes	Underlying factors / mechanisms	References
External layers - Hypodermis	Cell membrane degeneration, Lipid accumulation, Nuclear deformation		Epstein et al. (1972); Herndon et al. (2002); Palikaras et al. (2023)
	Actin cytoskeleton deterioration	HSF-1	Higuchi-Sanabria et al. (2018)
External layers - Cuticle	Thickening Wrinkling Cavity and break formation		Herndon et al. (2002); Essmann et al. (2020)
Mechanosensory system - Touch receptor neurons	Outgrowth development, Deformed neuronal body, Abnormal processes	Insulin / IGF-1 signalling; MAPK signalling; PTL-1; Defective neuronal activity	Pan et al. (2011); Tank et al. (2011); Toth et al. (2012)
	Mitochondrial degeneration		Morsci et al. (2016)
Neuromuscular system - Motor neurons	Outgrowth development, Abnormal processes, Defasciculation	Insulin / IGF-1 signalling	Pan et al. (2011)
Neuromuscular system - Body wall muscles	Sarcomere disorganization		Herndon et al. (2002)
	Cytoplasm loss, Cell membrane invaginations, Large lipid droplet accumulation		Herndon et al. (2002)
	Nuclear deformation, Nucleolar enlargement	Insulin / IGF-1 signalling	Herndon et al. (2002)
	Cytoskeletal degeneration	*hsf-1*	Higuchi-Sanabria et al. (2018)
	Mitochondrial network breakdown	Insulin / IGF-1 signalling	Regmi et al. (2014); Mergoud Dit Lamarche et al. (2018); Gaffney et al. (2018); Wang et al. (2019)
Neuromuscular system - Neuromuscular junctions	Spontaneous post-synaptic current decline	Defective synaptic vesicle fusion	Liu et al. (2013)
Alimentary system - Pharynx	Pharyngeal muscle damage	Fast pharyngeal pumping in early life	Chow et al. (2006)
	Pharyngeal cuticle damage	Fast pharyngeal pumping in early life	Zhao et al. (2017)
	Bacterial invasion in the body (occasionally)	Pharyngeal cuticle damage	Zhao et al. (2017); McGee et al. (2011)
Alimentary system - Intestine	Intestinal atrophy coupled with accumulation of yolk in the body	Intestine-to-yolk conversion, promoted by IIS and autophagy	Garigan et al. (2002); Herndon et al. (2002); McGee et al. (2011),

(*continued on next page*)

Table 1 (*continued*)

System	Ageing phenotypes	Underlying factors / mechanisms	References
			Ezcurra et al. (2018)
	Cell membrane disruption		Herndon et al. (2002)
	Nuclear loss (occasionally)		McGee et al. (2011)
	Lipid inclusion accumulation		Epstein et al. (1972); Herndon et al. (2002)
	Lipofuscin accumulation		Klass (1977); Garigan et al. (2002)
Reproductive system – Hermaphrodite germline	Germ cell reduction, Gonadal syncytium atrophy, Proximal oocyte stacking, Terminal oocyte enlargement, Uterinal oocyte-derived tumour formation		Garigan et al. (2002); Jud et al. (2007); Luo et al. (2010); Hughes et al. (2011); McGee et al. (2012); Riesen et al. (2014); de la Guardia et al. (2016)
	Oocyte quality decline	Maternal age, Apoptosis, TGF-β Sma/Mab signalling Insulin / IGF-1 signalling	Andux and Ellis (2008); de la Guardia et al. (2016); Luo et al. (2010); Luo et al. (2009); Hughes et al. (2007)
Reproductive system – Male mating behaviour	Mating behaviour decline	Increased spicule muscle excitability	Guo et al. (2012); Chatterjee et al. (2013); Klass et al. (1983)

feature of ageing in the worm (Herndon et al., 2002), and indicates run-on collagen synthesis with age.

Apart from being thickened, *C. elegans* aged cuticle may also become wrinkled (Herndon et al., 2002). A more recent study aimed to reveal the properties of the ageing cuticle by using atomic force microscopy (AFM) (Essmann et al., 2020). In this study, the cuticles of aged animals were shown to possess several abnormalities, namely breaks and cavities, and bacterial nutrients were suggested to support cuticle preservation (Essmann et al., 2020).

3. Mechanosensory system

In this section we discuss the ageing process in the sensory system of the worm, comprised of neurons that respond to various types of stimuli and regulate behaviour. In general, sensory ability to gentle touch has appeared to be preserved during ageing, although the output behavioural responses might be affected by defective neuromuscular coordination (Glenn et al., 2004). However, several studies have reported age-associated changes in sensory neurons.

Among sensory neurons, touch receptor neurons have been extensively studied during ageing. These neurons, placed either anteriorly or posteriorly, are engulfed by cells of the hypodermis, and, therefore, lie in close proximity to the cuticle, innervating almost half of the worm's length (Goodman, 2006). Touch receptor neurons sense light touch and, in turn, modulate locomotion.

Locomotion, driven by the neuromuscular system (discussed below), but regulated by the sensory system, was one of the very first aspects in *C. elegans* biology observed to decline upon ageing (Bolanowski et al., 1981; Croll et al., 1977; Herndon et al., 2002). Intriguingly, isogenic populations of same-aged worms appeared to include differentially motile individuals. Due to this endogenous variability, they were classified into three categories, from highly mobile to severely defective animals (Herndon et al., 2002). However, even in the latter, all neuronal structures appeared morphologically intact during ageing.

Subsequent studies, though, revealed that both the body and the processes of such neurons may acquire abnormal features over time (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012) (Fig. 2). For example, neuronal body in ALM touch receptor neurons loses its round/oval shape and exhibits aberrant protrusions, without, however, undergoing cell death (Pan et al., 2011). The processes of ALM and PLM neurons obtain bubble-like lesions and blebs, the latter giving rise to branches in late ages (Pan et al., 2011). ALM processes also appear to lose their mitochondrial content gradually (Morsci et al., 2016). Of note, neuronal mitochondria of aged animals acquire abnormal features, such as indistinguishable cristae, indicating mitochondrial degeneration (Morsci et al., 2016).

The integrity of these neurons is affected by several pathways, one of which is the IIS. Mutations in the DAF-2 receptor and downstream transcription factor DAF-16 appeared to delay and worsen, respectively, the aforementioned phenotypes (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012). Within IIS, heat-shock transcription factor HSF-1 (Pan et al., 2011; Toth et al., 2012), or in synergy with IIS, protein MEC-1 (Pan et al., 2011), function against neuronal ageing. Apart from IIS, MAPK signalling is also implicated in the maintenance of touch receptor neurons, since loss of two kinase encoding genes, *jnk-1*, or the upstream *jkk-1*, promoted neurite branching (Tank et al., 2011). In similar features

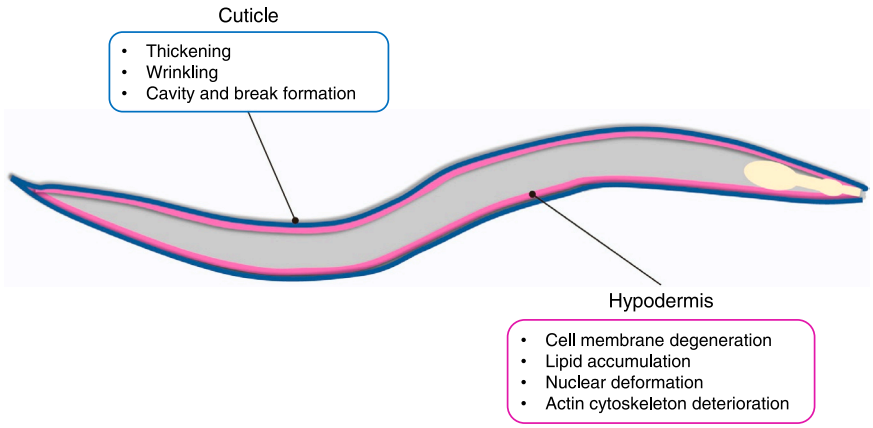

Fig. 1. Age-related changes in the *C. elegans* hypodermis and cuticle. The aged hypodermis and cuticle in *C. elegans* are characterized by several abnormalities. The hypodermis exhibits degenerated cell membranes, accumulated lipids, deformed nuclei and deteriorated actin cytoskeleton. The cuticle becomes thicker and wrinkled, and forms cavities and breaks.

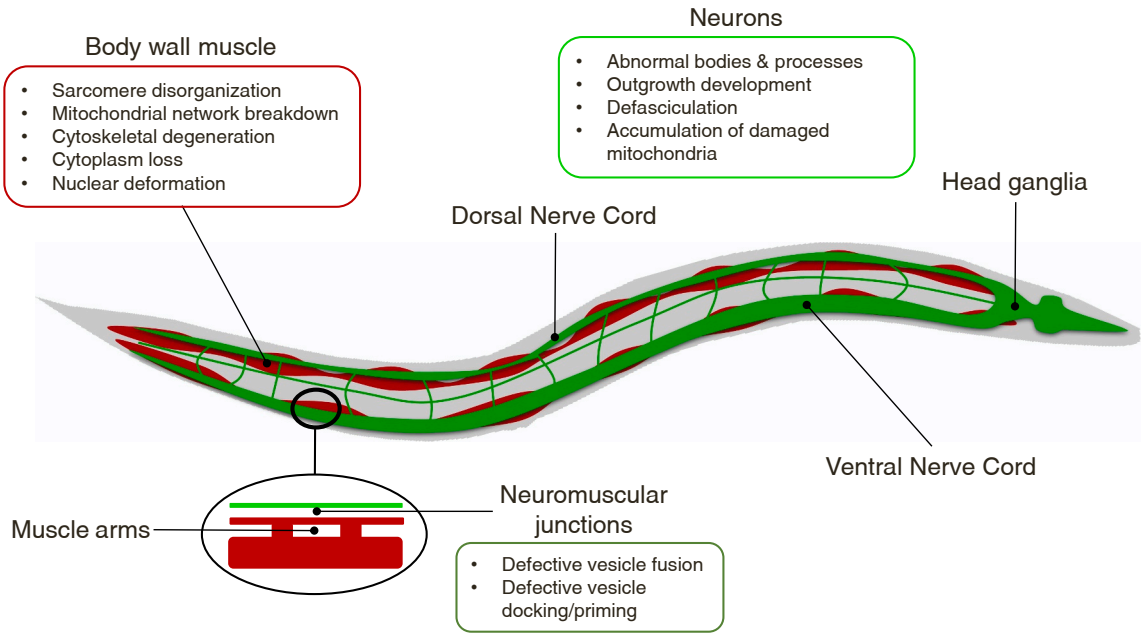

Fig. 2. Age-related changes in the *C. elegans* neurons and body wall muscles. In ageing *C. elegans* both neuronal and muscular system are deteriorated. Neuronal bodies and processes are deformed, outgrowths are developed and mitochondria are degenerated. Ventral axons of cholinergic neurons additionally undergo 'defasciculation', that is they lose their tight packaging inside the fascicles. Body wall muscles are severely affected: sarcomeres become disorganised and numerous alterations occur in the sub-cellular level (mitochondrial network breakdown, cytoskeletal degeneration, cytoplasmic loss and nuclear deformation). Lastly, synapses between neurons and muscles are impaired due to defects in vesicle fusion and, in later life, in docking/priming.

resulted total loss of the mammalian tau homolog PTL-1 (Chew et al., 2013), which is highly expressed in touch receptor neurons. Lastly, these cells may also depend on their activity in order to be preserved. Gain-of-function mutations in the hyperpolarizing channel SLO-1, that inactivate neurons, are reported to cause neuronal body degeneration, bubbling and axon beading phenotypes (Pan et al., 2011).

Conclusively, touch receptor neurons, that modulate locomotion, are morphologically deteriorated with age. IIS, MAPK signalling and other factors, as well as reduced electrical activity, are possible contributors to this decline. Defective sensory ability might, in turn, be detrimental for the worm's motor ability.

4. Neuromusculatory system

In this section we discuss the ageing process in motor neurons and body wall muscle cells (Fig. 2), both involved in *C. elegans* locomotion. Motor neurons, the body of which is located in the ventral nerve cord, innervate dorsal and ventral body wall muscles (Altun and Hall, 2011). These muscles consist of single-nuclear cells, organized into four quadrants (Altun and Hall, 2009e). Their characteristic striated form is attributed to the multiple contractile units, called sarcomeres, that are structured by myosin thick and actin thin filaments. Muscle arms are extended into the dorsal or ventral nerve cord and form gap junctions as well as chemical synapses with the processes of the neurons (Altun and Hall, 2011).

Motor neurons exhibit similar deficits with touch receptor neurons. For example, GABAergic motor neurons exhibit process outgrowth during ageing, promoted, notably, by the loss of *jnk-1* (Tank et al., 2011). In regards specifically to the axons of GABAergic DD and VD motor neurons in the dorsal and ventral nerve cord, a beading phenotype is developed over time (Pan et al., 2011). Their morphological and functional integrity are also dependent on PTL-1 (Chew et al., 2013). Cholinergic axons in the ventral nerve cord lose, in addition, their tight packaging inside fascicles, which is known as 'defasciculation' and becomes more severe under *hsf-1* loss-off-function mutation (Pan et al., 2011). Also, mutation in *daf-2* was shown to delay the ageing phenotypes, hence indicating the importance of the IIS for cholinergic neurons as well (Pan et al., 2011).

Musculature is one of the first systems observed to highly degenerate with age, resembling human sarcopenia (Herndon et al., 2002). Muscle cells accumulate large lipid droplets, acquire invaginated membranes and lose their cytoplasm gradually (Herndon et al., 2002). Nuclei are reduced in number and altered in shape, whilst nucleoli grow in size (Herndon et al., 2002). Nuclear alterations appear to be delayed upon mutation of *age-1*, a PI(3) kinase encoding gene involved in IIS. As revealed by electron microscopy, sarcomeres become misarranged, with fewer myosin filaments (Herndon et al., 2002). Actin cytoskeleton, a major component of muscle structure, was, lastly, shown to become thinner and age prematurely upon *hsf-1* knockdown (Higuchi-Sanabria et al., 2018). Thus, decline of muscular integrity preservation mechanisms is considered to underlie age-related sarcopenia. A recent transcriptome analysis study also revealed an ageing-promoting role for the mammalian myelin regulatory factor orthologue *myrf-2*, which is upregulated in old muscles, and its muscle-specific knockdown ameliorated both sarcomere morphology and worm motility (Wang et al., 2022).

Much attention has been paid to muscle mitochondria. Interestingly, the mitochondrial network starts losing its tubular form in early adulthood (Gaffney et al., 2018; Mergoud Dit Lamarche et al., 2018; Regmi et al., 2014), and around the 4th day (at 20 °C) fragmentation begins. At 10 days of adolescence severe fragmentation is observed (Gaffney et al., 2018). Possibly, however, fragmentation characterizes only immobile animals of a same-age population (Mergoud Dit Lamarche et al., 2018). According to swim assays, movement deficits associate more with mitochondrial network breakdown, rather than with sarcomere disruption (Gaffney et al., 2018). Functional muscles in *daf-2* animals have been related to preserved mitochondrial structure, higher mitochondrial mass and increased intracellular ATP, all of which are *daf-16*-dependent (Wang et al., 2019). Defective mitophagy is another important factor underlying ageing. An interesting study used a premature ageing mutant strain for *wrn-1* (which encodes a DNA repair-involved protein), characterized by shorter lifespan and lower

basal mitophagy than N2 (Fang et al., 2019). This study showed that the cause of accelerated ageing in these worms was NAD+ depletion, since treatment with NAD+ precursors increased their lifespan, but also ameliorated mitochondrial network morphology and restored mitophagy in the muscles (Fang et al., 2019).

The contribution of motor neuron and muscle deterioration in locomotory decline was remarkably studied by Liu and colleagues. In this study, initiation of motility defects in early adulthood (day 5 at 20 °C) was temporally associated with a decline in spontaneous post-synaptic currents at neuromuscular junctions, possibly attributed to problematic synaptic vesicle fusion (Liu et al., 2013). Treatments with fusion-triggering arecoline ameliorated the worm speed, reversal frequency and other parameters, further supporting the contribution of synaptic decline in locomotory incapability. Other ageing-associated deficits, namely defective docking or priming of vesicles at synapses, as well as muscle degeneration occur in later life, so their effects on locomotion are believed to be subsequent (Liu et al., 2013).

5. Alimentary system

One of the most complex components of the nematode body, highly associated with the ageing process, is the alimentary system, also known as digestive system or gastrointestinal tract (Fig. 3). Bacteria are taken up and ground by the pharynx, the nematode's food pump. The pharynx is formed by epithelial cells, intrinsically active musculature, made up of syncytial and non-syncytial cells, and a small nervous system that regulates the muscular function (Altun and Hall, 2009b). The organ is divided into procorpus, anterior bulb, isthmus and the terminal bulb, that contains the grinder structure (Altun and Hall, 2009b). Posteriorly to the pharynx lies the intestine, comprised of epithelial cells that enclose its lumen. The intestinal cells send small projections, called 'microvilli', into the lumen, that support digestion and nutrient absorption (Altun and Hall, 2009a). The caudalmost edge of the alimentary system includes the rectum and the anus (Altun and Hall, 2009c).

A number of studies connected ageing to a decline in alimentary system function, and to a detrimental effect of *E. coli*, the worm's bacterial food, on organismal integrity. The first reported ageing behaviours with regards to the digestive tract were reduced defecation frequency (Bolanowski et al., 1981; Croll et al., 1977) and decline of the pharyngeal pumping rate (Croll et al., 1977; Hosono et al., 1980; Huang et al., 2004). Young animals exhibit a high pumping rate that is reduced over time. It was considered possible that the observed 'plugging' of undigested bacteria in some older worms affects the pumping rate (Chow et al., 2006; Garigan et al., 2002; McGee et al., 2011). However, this notion was largely challenged since non-plugged animals were also shown to pump slowly (Chow et al., 2006). Hence, it appeared rather possible that fast pumping in early life causes damage to pharyngeal muscles (Chow et al., 2006), leading to subsequent pathologies. Supporting this, *eat-2* mutants, which are characterized by decreased pumping rate, exhibited less damaged pharynges than same aged control worms (Chow et al., 2006). Moreover, in *daf-2(e1370)* mutants (that adjust pumping rate depending on the temperature) pumping repression at 25 °C for 15 days resulted in higher pumping potential, following re-exposure at 20 °C (Chow et al., 2006).

Several years later, another study provided evidence that fast pumping in young adults injures the pharyngeal cuticle, thus increasing susceptibility of bacterial invasion (Zhao et al., 2017). It was already known that food bacteria can become detrimental for the intestinal microvilli and invade cells and cavities (such as the uterus) (McGee et al., 2011). Bacterial invasion and proliferation was related to occasional worm corpses with enlarged terminal bulbs, named 'P deaths' (Zhao et al., 2017). In the exclusive presence of killed bacteria, P deaths were eliminated and lifespan was extended (Garigan et al., 2002; Gems and Riddle, 2000; Podshivalova et al., 2017; Zhao et al., 2017).

Age-associated immunodeficiency (Youngman et al., 2011) and toxic overproduction of metabolites by accumulated microbes could favour worm death (Podshivalova et al., 2017). However, not all aged worms are equally vulnerable to colonization or P death-prone. Transmission Electron Microscopy revealed scars in the pharyngeal cuticle of old individuals, indicative of post-injure healing that limits invasion (Zhao et al., 2017). Intriguingly, effects of bacteria are not restricted on the alimentary system. A recent study supported the necessity of bacterial nutrients for the preservation of the cuticle, which also loses its integrity during ageing (Essmann et al., 2020).

The digestive tract undergoes several additional age-related changes. The intestine exhibits structural and compositional alterations, including disruption of the plasma membrane (Herndon et al., 2002) and accumulation of fluorescent material ('lipofuscin') (Garigan et al., 2002; Klass, 1977) as well as lipid inclusions (Epstein et al., 1972; Herndon et al., 2002). Nuclei of the intestinal cells may be reduced drastically during ageing, albeit nuclear loss is remarkably variable in aged worms (McGee et al., 2011). This nuclear loss appears to be apoptosis-independent (McGee et al., 2011).

Another remarkable aspect of intestine-related ageing is associated with yolk, which normally originates from the gut and ends up to the germline (Hall et al., 1999; Kimble and Sharrock, 1983). Yolk production and transfer is the 'meeting point' of the alimentary system with the reproductive system. Several years ago, yolk particles were observed to accumulate in the body cavity of aged animals (Garigan et al., 2002; Herndon et al., 2002; McGee et al., 2011). Moreover, it was supported that pseudocoelomic lipoprotein pools (that consist of yolk) accumulate because of the lipoprotein production being temporally extended after

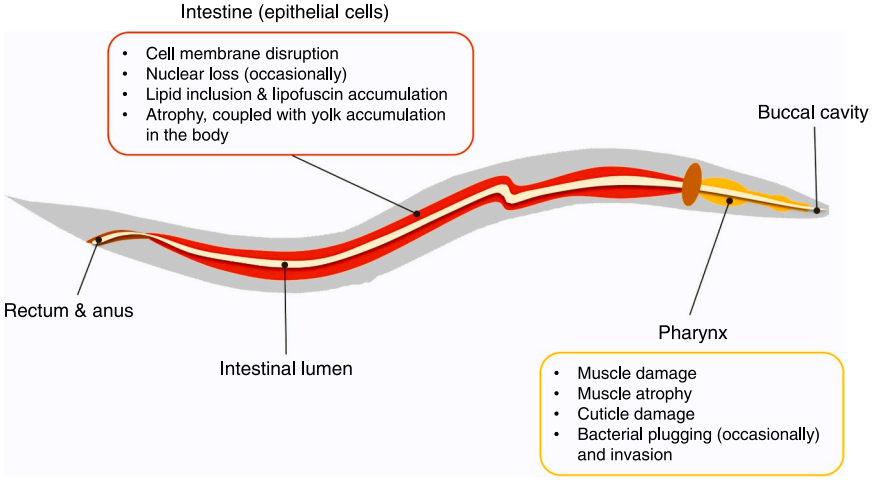

Fig. 3. Age-related changes in the *C. elegans* alimentary system. The ageing-related decline of essential pharyngeal structures is possibly attributed to the fast pumping during early life. If not repaired, cuticular injures may enable bacterial invasion to the worm's body. The intestinal cells are also structurally impaired: cell membrane disruption, lipid inclusion and lipofuscin accumulation and occasional nuclear loss have been reported. Lastly, intestinal atrophy has been observed, coupled with yolk accumulation in the body, due to intestine-to-yolk conversion (more information is included in the text).

egg laying cessation (Herndon et al., 2002). Such an accumulation, being coupled with intestinal atrophy, indicated that lipoprotein is derived from the intestinal biomass (Ezcurra et al., 2018). According to the same study, this conversion process, promoted by IIS and autophagy, also explained the extracellular accumulation of yolk. Impressively, another recent work showed that the excess production of yolk in later life is in fact beneficial for the worms' progeny (Kern et al., 2021). The yolk was observed to be vented by the parental worms and support larval growth. This venting was found to be promoted by the IIS (Kern et al., 2021).

6. Reproductive system

Reproductive decline is another major aspect of ageing, besides somatic degeneration. In *C. elegans* the reproductive system consists of somatic tissue components (somatic gonad), that host the germline (Lints and Hall, 2009d). In adult hermaphrodites, two gonad arms enclose the female gametes, that gradually mature from mitotically proliferating nuclei in the distal gonad syncytium to completely cellularized enlarged oocytes in the proximal edge (Lints and Hall, 2009c). Each gonad arm is connected to a spermatheca, in which the male gametes, originated from either the hermaphrodite or a male, are stored. After passing through the spermathecas and becoming fertilized, the eggs end up in the uterus, where embryonic development is already in progress (Lints and Hall, 2009b). Subsequently, the eggs pass through the vulva to the external environment, and ultimately hatch. Adult male gonads, on the other hand, consist of a single arm connected to the cloaca (Lints and Hall, 2009a). The cloaca hosts the copulatory spicules, special structures which facilitate the mating procedure. The reproductive system in both sexes is supported by neuronal and muscular activities.

6.1. Ageing of the gonad and germline

During a hermaphrodite's reproductive period, offspring production follows an increasing and, subsequently, a decreasing trend (Hughes et al., 2007). In *C. elegans*, reproduction ceases around the fourth day of adulthood. Post-reproductively, the gonad has been observed to acquire degenerative features, including atrophy and fragmentation (de la Guardia et al., 2016). Of note, again, these phenotypes vary among individuals (de la Guardia et al., 2016). Moreover, during the same period, germ cell abundance is lowered, the gonadal syncytium becomes atrophic, and cellularized oocytes become stacked in the proximal gonad (Fig. 4). The terminal oocyte (i.e. the one being just before entering spermatheca) is swollen, and the intra-uterinal oocytes form a large tumour (de la Guardia et al., 2016; Garigan et al., 2002; Hughes et al., 2011; Jud et al., 2007; Luo et al., 2010; McGee et al., 2012; Riesen et al., 2014).

Several studies have aimed to elucidate the underlying mechanisms of age-related germline decline. Oocyte quality depends on maternal age, since egg viability appears to be reduced during the reproductive period (Andux and Ellis, 2008). In mated worms incapable of sperm production (called 'females'), blockage of cell death by mutation in *ced-3* or *ced-4* genes, encoding an executioner caspase and its activator, respectively, resulted in an earlier-onset (3–4 days post sexual maturation) decrease in egg viability, indicating a role of apoptosis on oocyte quality preservation (Andux and Ellis, 2008). In the same study, *ced-1* and *ced-6* mutations, that prevent cell corpse engulfment without inhibiting cell death, resulted in similar, but milder phenotypes compared to the previous two, supporting that apoptosis does not remove defective oocytes, but rather modulates the oocyte abundance towards proper resource allocation (Andux and Ellis, 2008). A subsequent study indicated that physiological apoptosis is continued post-reproductively (day 8 of adulthood) in the germline, and that its total blocking delayed gonad deterioration at the same period (de la Guardia et al., 2016). It thus appears possible that the effects of apoptosis in later life are detrimental for gonadal integrity.

Oocyte quality is also influenced by two reproductive span regulating cascades, namely TGF-β Sma/Mab and IIS (Hughes et al., 2007; Luo et al., 2010; Luo et al., 2009). In spermless *fem-1* worms, mutations in *sma-2* or *daf-2* appeared to protect against chromosomal missegregation within oocytes (Luo et al., 2010). Reduction of either pathways also eliminated unfertilized oocytes, decreased oocyte malformation post-reproductively, and appeared to favour continuation of germline proliferation during ageing (Luo et al., 2010). Interestingly, both cascades were shown to regulate oocyte quality preservation non-autonomously (Luo et al., 2010).

One of the most well-known theories of ageing, that of 'disposable soma', suggests the existence of an evolutionary trade-off between reproduction and the somatic maintenance, that leads to organismal

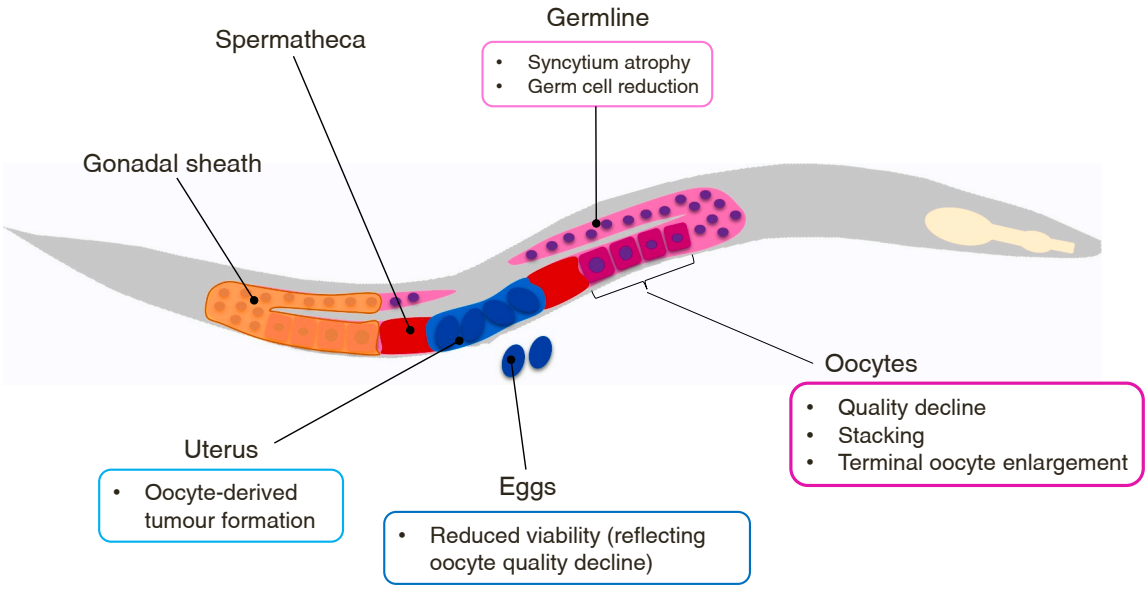

Fig. 4. Age-related changes in the *C. elegans* hermaphrodite reproductive system. The *C. elegans* gonad undergoes substantial alterations over time. The gonad syncytium becomes atrophic, germ cells are reduced, and the quality of oocytes is decreased, as reflected by increased embryonic lethality. Inside the uterus oocytes may form tumour-like structures as well.

decline (Kirkwood, 1977; Kirkwood and Austad, 2000). In consistency with this theory, germline ablation in *C. elegans* favours longevity because of resource allocation to the somatic maintenance (Arantes-Oliveira et al., 2002; Berman and Kenyon, 2006). For the relationship between soma and the germline in the context of somatic and reproductive ageing, the reader is referred to other instructive and more focused review articles (Gaddy et al., 2021; Maklakov and Immler, 2016).

6.2. Ageing in male worms

Ageing in male individuals has also been investigated. Compared to hermaphrodites, males have a shorter lifespan. This difference is attributed to TRA-1, a protein involved in sex determination, which positively regulates *daf-16* in hermaphrodites (Hotzi et al., 2018). Another considerable difference is the lack of germline apoptosis (Gumienny et al., 1999) and gonad degeneration in males, consistent with the hypothesis that the former contributes to the latter in hermaphrodites (de la Guardia et al., 2016).

Increasing male age has been correlated with increased embryonic death and decreased production of fertilized eggs, which results by both reduced sperm transfer per mating, as well as reduced mating behaviour (Chatterjee et al., 2013; Klass et al., 1983). Mating behaviour decline was found to begin from the early adult life (3rd day of adulthood) (Guo et al., 2012). Interestingly, this was not related with muscle deterioration, that occurs in later life, but rather with higher spicule muscle excitability, that compromises male mating performance. In accordance with this, reduced expression of acetylcholine receptor genes (*unc-29*; *acr-16*; *acr-18* and *gar-3; which are expressed in sex muscles*) was observed to ameliorate mating performance (Guo et al., 2012).

7. Transcriptome, proteome and metabolome alterations during ageing

Knowledge derived from studies on transcriptome, proteome and metabolome alterations during ageing is essential for a complete and valid overview of the latter in *C. elegans*. In this section we aim to summarize the major observations of such studies, which contribute to the elucidation of molecular mechanisms underlying the ageing process.

Informatively, the 'signature' of ageing is detected in all of the aforementioned levels (transcriptome, proteome and metabolome). As already implied above, ageing can be viewed either by the time of birth ('chronologically') or by the organismal condition ('physiologically'). Given the high physiological variability observed among synchronized worms, it becomes clear that these two aspects of ageing do not necessarily coincide. A recent study, aiming to dissect the difference between them at the level of transcriptome, revealed that chronological ageing is associated with upregulation of intron-derived transcripts and non-coding RNAs (Ham et al., 2022). Physiological ageing, on the other hand, was correlated with downregulation of mRNAs encoding proteins which are involved in mRNA post-transcriptional fate (Ham et al., 2022). For such conclusions, this study used *daf-2* mutants, which are characterized by delayed physiological ageing. At the same time another group studied tissue-specific transcriptomic alterations on the basis of chronological ageing (Wang et al., 2022). This work provided evidence that several tissues of the worm discussed above, i.e. the hypodermis, neurons, body wall muscles and intestine, undergo distinct transcriptomic alterations over time, and each of them is represented by several unique ageing-regulated differentially expressed genes (Wang et al., 2022). Therefore, each tissue is differentially affected by the ageing process in terms of gene expression.

With respect to the proteome, N2 worms have showed significant shifts on the abundance of one-third of proteins during chronological ageing (Walther et al., 2015). This 'proteome disruption', which was additionally associated with protein aggregation, appeared to be ameliorated or worsened in *daf-2* or *daf-16* mutants, respectively, indicating again the importance of IIS on ageing (Walther et al., 2015). Informatively, IIS mutant worms had been shown to share considerable similarities on metabolome with another long-lived mutant, characterized by defective translation (Fuchs et al., 2010). It is therefore possible that longevity cascades converge to regulate the same metabolic processes.

Proteome and metabolome analyses have also indicated a decline of the molecular machinery with chronological age. Importantly, proteins associated with transcription, translation, as well as mRNA and protein turnover, have been shown to decrease during ageing (Copes et al., 2015). Moreover, the abundance of essential molecular building blocks, such as free amino acids, free fatty acids and nitrogenous bases is considerably altered with age (Copes et al., 2015). Reduction of hydrophobic amino acids, such as methionine, or of adenine, guanine and cytosine are some key alterations detected during ageing (Copes et al., 2015). However, these experiments were performed on a strain with germline proliferation defects at restrictive temperature in order to avoid 'offspring contamination'. This intervention should be, therefore, considered during interpretation of the results.

8. Concluding remarks

In the previous sections we summarized the major age-related changes in the nematode, *C. elegans*. Decline of 'higher', more complex organismal functions, such as behaviour, result from deterioration of somatic systems being involved and, occasionally, from failed coordination between them. Defective systems are associated with various sub-cellular alterations, and regulatory mechanisms. Among these mechanisms, the insulin/IGF-1 signalling appears to dominate the regulation of both somatic and reproductive ageing. Our knowledge on ageing, viewed either chronologically or physiologically, has been enriched by transcriptomic, proteomic and metabolomic studies. Such approaches enable both whole-organism and tissue-specific observations, which are essential in order to understand the complex molecular background of ageing.

Worm-based studies often trace a whole population's progression over time. As highlighted above, many ageing phenotypes are surprisingly variable among individuals, regardless of homogeneity in age, genetic background and environmental conditions. 'Stochasticity' is thus suggested to be a major component of ageing, in line with 'disposable soma theory', which attributes organismal ageing to random damage events (Herndon et al., 2002; Kirkwood, 1977). Apart from stochasticity, another putative ageing component is organismal hyperfunction, which may ultimately lead to trauma (e.g. by increased pharyngeal pumping) (Chow et al., 2006; Zhao et al., 2017) or behavioural deterioration (e.g. by increased male muscle excitability) (Guo et al., 2012). It is, lastly, of high importance to note that ageing can be regulated 'trans-generationally'. An interesting example is that defects in a histone modifier, the H3K4me3 complex, increased the lifespan of future worm generations (Greer et al., 2011). It is possible that such defects cause heritable epigenetic alterations which would be undone over a number of generations (Benayoun and Brunet, 2012). Another, more recent study connected longevity-promoting mutation in *wdr-5* (encoding a methyltransferase complex component) with inter-generational enrichment of the H3K9me2 heterochromatin factor (Lee et al., 2019). Therefore, existence of such transgenerational memories should be highly considered during the ageing studies.

Clearly, the properties of *Caenorhabditis elegans* render it a powerful model in the field of ageing. Anti-ageing studies are highly based on molecular and cellular mechanisms being conserved from nematodes to mammals. Modelling of age-related disorders in worms has become achievable due to these conserved mechanisms, and several studies have highlighted major similarities in ageing mechanisms between mammals and nematodes. Model-based studies have indicated numerous putative interventions, such as mitophagy enhancement, against age-related human pathology (Fang, Hou, Palikaras et al., 2019). Lastly, other key

properties of *C. elegans*, namely self-fertilization and high reproductive yield would greatly facilitate large-scale population studies, towards the elucidation of the biological and evolutionary significance of ageing.

Data availability

No data was used for the research described in the article.

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