Chapter 2
Effects of Ageing on the Basic Biology and Anatomy of *C. elegans*

Laura A. Herndon, Catherine A. Wolkow, Monica Driscoll, and David H. Hall

Abstract Many aspects of the biology of the ageing process have been elucidated using *C. elegans* as a model system. As they grow older, nematodes undergo significant physical and behavioural declines that are strikingly similar to what is seen in ageing humans. Most of the major tissue systems of *C. elegans*, including the cuticle (skin), hypodermis, muscles, intestine, and reproductive system, undergo dramatic physical changes with increasing age. The ageing nervous system undergoes more subtle changes including dendritic restructuring and synaptic deterioration. Many of the physical changes become more apparent near the end of reproduction. In conjunction with tissue ageing, some behaviours, such as locomotion, pumping and defecation, decline substantially during the ageing process. Interestingly, some aspects of physical and behavioural decline are delayed in longevity mutant backgrounds, while other changes are not altered. This chapter provides an introduction to the general features of *C. elegans* anatomy and describes what is currently known about the physical changes that accompany the normal ageing process. It should be noted that some descriptions summarized herein have not been previously published, so that despite the review theme, novel aspects of the ageing anatomy are also featured. Given the common features shared between *C. elegans* and humans during ageing, a greater understanding of the anatomy of this process in *C. elegans* can help illuminate the nature of ageing-related tissue decline across species.

Keywords *C. elegans* • Ageing • Anatomy • Cuticle • Hypodermis • Muscles • Pharynx • Intestine • Germline • Nervous system

L.A. Herndon (✉) • C.A. Wolkow • D.H. Hall
Department of Neuroscience, Albert Einstein College of Medicine,
1410 Pelham Parkway, Bronx, NY 10461, USA
e-mail: laura.herndon@einstein.yu.edu

M. Driscoll
Department of Molecular Biology and Biochemistry, Rutgers University,
Nelson Biological Labs, Piscataway, NJ 08854, USA

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2.1 Introduction

C.elegans is a small, free-living, non-parasitic nematode that feeds on bacteria and fungi growing on decaying fruit and plant matter. Established in the lab in the 1960s, the *C. elegans* model has become a powerful tool for dissecting mechanisms of fundamental processes relevant to human biology and disease. Indeed, the tissue organization of the 959-celled adult *C. elegans* features the same basic anatomical body systems as higher organisms, including a nervous system, skeletal-like and cardiac-like muscle systems, an excretory system, an alimentary system, an epithelial system and a reproductive system (Fig. 2.1a).

The experimental advantages of *C. elegans* are numerous, and include ease of propagation of the hermaphrodite sex, short life cycle, stereotypical development, and simple transparent body plan. The programme of cell divisions that make up the adult *C. elegans* (the cell lineage) has been determined [1–3], and the pattern of connections for the 302 hermaphrodite [4] and 170 posterior male neurons [5] have been mapped. The *C. elegans* genome sequence has been determined [6] and annotated in exquisite detail. Importantly, *C. elegans* genes can be manipulated through forward and reverse genetic approaches, and transgenesis is easy, such that analyses of animals lacking, or overexpressing, almost any gene product is possible. Many human disease genes have homologues in the *C. elegans* genome [7]. Publicly-available electronic resources for *C. elegans* include WormBook, an online review of *C. elegans* biology (wormbook.org), WormAtlas, a database for *C. elegans* structural and behavioural anatomy (wormatlas.org) and WormBase, a genetics database for *C. elegans* and other nematodes (wormbase.org).

C. elegans offers several advantages for studying the basic biology of ageing. First, the lifespan is relatively short (just 2–3 weeks under standard laboratory conditions) and therefore amenable to whole-life survival analyses. Second, large numbers of genetically identical animals can be easily grown under controlled environmental conditions. Interestingly, however, even under controlled conditions, individual lifespans can vary significantly, revealing a stochastic component to ageing [8]. Indeed, the *C. elegans* lifespan exhibits tremendous plasticity, which can be affected by environmental conditions, nutrition, and genetic mutations [9, 10].

Multiple behaviours decline with ageing, such as muscle-regulated locomotion and pharyngeal pumping rates. Early behavioural declines can be better predictors of short life expectancy than chronological age [8, 11–15]. Physical deterioration is a universal feature of the ageing process and a major quality of life issue in human ageing. This introductory chapter describes the basic features of *C. elegans* anatomy and typical physical changes that accompany normal ageing, for both middle – and old-aged animals. This description focuses on the hermaphrodite anatomy, as ageing of the *C. elegans* male is currently less well characterized. Our goal is to provide readers with a basic understanding of *C. elegans* adult anatomy and how ageing affects each of the major tissue types. We emphasize that some of the data included here have not previously been published, and thus this review makes accessible new information in the field. This chapter will provide a background for understanding

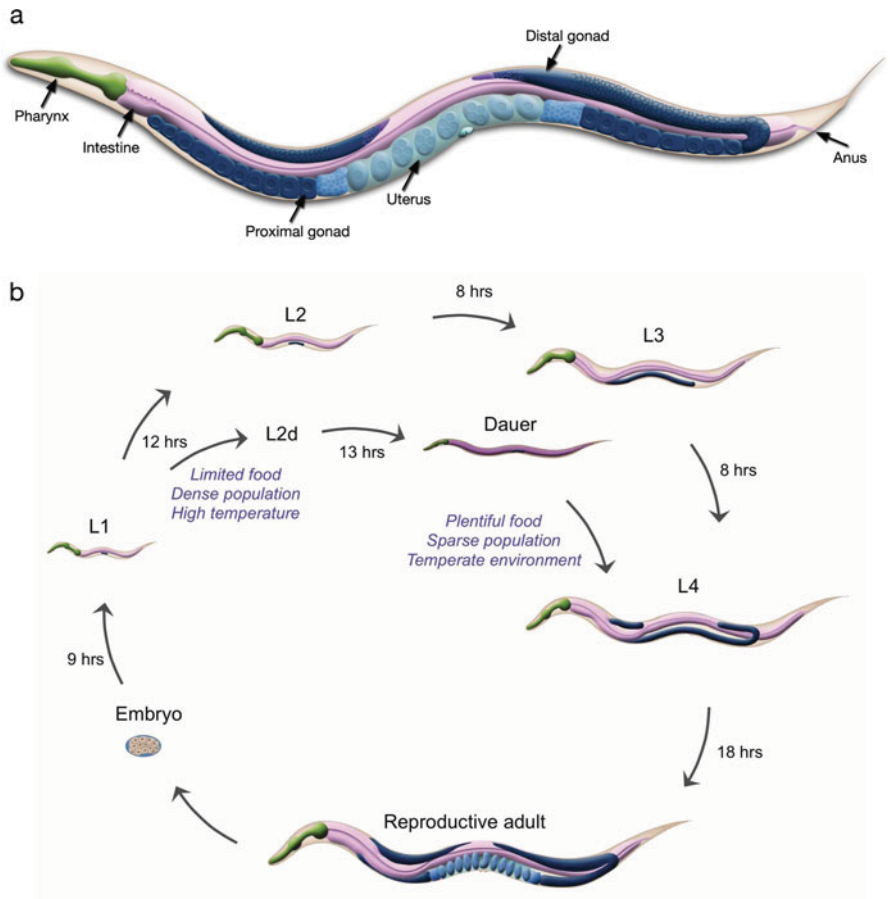

Fig. 2.1 Introduction to *C. elegans* anatomy and life cycle. (**a**) Schematic showing anatomy of an adult *C. elegans* lying on the left lateral side (Image source: [WormAtlas]). (**b**) Life cycle of *C. elegans* at 22 C. Fertilization occurs at time = 0 min. *Numbers along the arrows* indicate the length of time the animal spends at each stage (Image source: [WormAtlas])

the biology of *C. elegans* ageing through a tissue-focused lens, and can speak to the relevance of *C. elegans* ageing to issues of human ageing.

2.2 Introduction to the Life History of *C. elegans*

When food is abundant, temperature is optimal, and over-crowding is not a problem, the *C. elegans* life cycle occurs over ~3 days at 20 °C. After hatching from the egg, *C. elegans* larvae proceed through four larval stages, L1–L4, before becoming

adults (Fig. 2.1b). Most adult *C. elegans* are self-fertile hermaphrodites, although males arise on rare occasions by non-disjunction of the sex chromosome and can then be propagated by crossing. Each larval stage is punctuated by a moult, during which pharynx pumping ceases, and the cuticle is shed and replaced by a newly synthesized stage-specific cuticle.

Under harsh environmental conditions, with limited food, high temperature, or overcrowding, early larvae may reversibly arrest development after the second larval stage as dauer ("enduring") larvae (Fig. 2.1b) [16, 17]. Dauer larvae have a distinct morphology and biology adapted for long-term survival. Recovery from dauer arrest is triggered by food or introduction to a favourable environment. Dauers recover into L4 larvae, which proceed on the same developmental pathway to reproductive adults as larvae that bypassed dauer. For a more detailed discussion of the dauer larva see Chap. 3.

Adult hermaphrodites are self-fertile for approximately 3–4 days and produce about 300 progeny, limited by the number of sperm produced during spermatogenesis. Hermaphrodites inseminated by males receive a fresh supply of sperm and may produce 1200–1400 progeny during an extended reproductive period [18]. After reproduction ceases, animals enter a post-reproductive period lasting 2–3 weeks before death [16, 19]. During the post-reproductive period, feeding and locomotory rates decline, tissues deteriorate, and animals become more sensitive to microbial infection [8, 12–14, 20, 21]. Post-reproductive adults lack stem cells and therefore do not replace cells or tissues damaged by ageing. Thus, apart from the germline, the *C. elegans* model features the ageing of post-mitotic tissues.

2.3 Anatomic Changes That Accompany Ageing

2.3.1 Cuticle

The *C. elegans* cuticle covers the outer surface of the body, providing protection, maintaining body shape, and aiding motility [22, 23]. The cuticle surface is covered by circumferential furrows and ridges called annuli. Bilateral alae, which appear as raised ridges, run lengthwise along the body to facilitate movement (Fig 2.2a).

The cuticle is built from collagens and noncollagenous cuticulins arranged in layers differing in structure and composition (Fig. 2.2b). Over most of the body, the components of the cuticle are secreted by the hypodermis and seam cells, which are the epithelial cells covering the body. The dauer cuticle is thicker and more highly reinforced to protect dauers from environmental threats and desiccation [24]. Cuticle also lines the major body openings, such as the anus and the excretory pore. These "lining" cuticular domains do not appear to be composed of layers, although they can still provide adequate structural support for function. Body openings, including the anus, excretory pore, vulva and pharynx, are lined by interfacial cells that produce the cuticle lining for these structures.

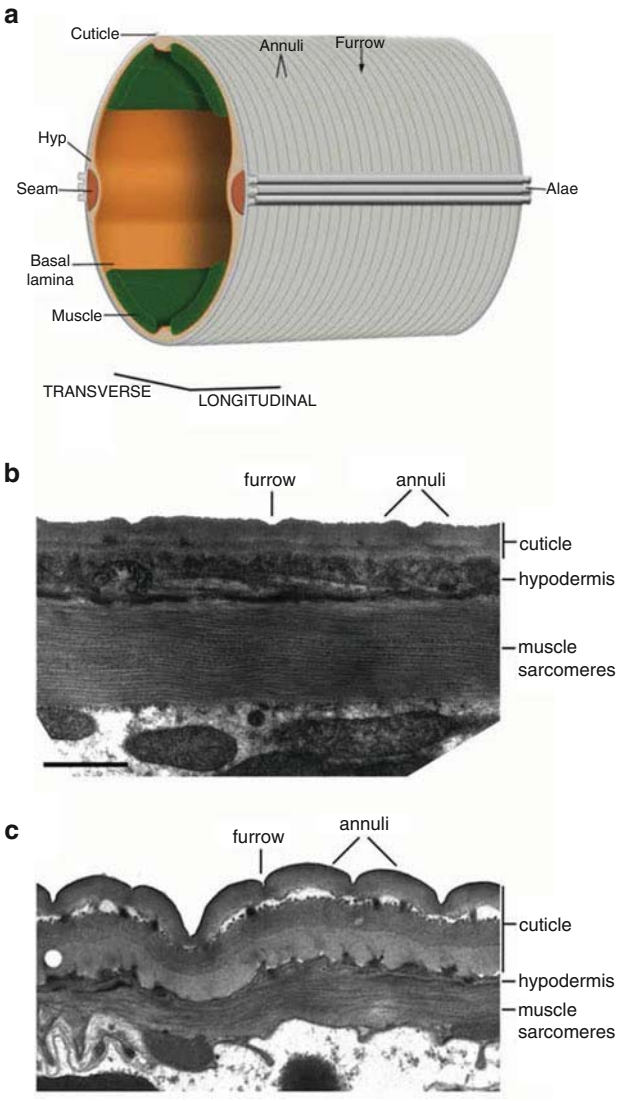

Fig. 2.2 The *C. elegans* cuticle thickens and wrinkles during ageing. (**a**) Schematic showing cuticle structure in *C. elegans*. A thin layer of hypodermal tissue (*orange*) always underlies the body wall cuticle (*grey*), separating the cuticle from the four underlying quadrants of body wall muscles (*green*). The basal laminae of the hypodermis and muscle fuse to make a single layer spanning the extracellular space between the two tissues. Special features of the adult epicuticle include concentric narrow annuli separated by shallow furrows, and several parallel ridges, the "alae", that run for most of the length of the body at the lateral line. The seam cells are a row of specialized epidermal cells underlying the alae (Image source: [WormAtlas]). (**b**) TEM longitudinal section from young adult showing cuticle, hypodermis and adjacent muscle sarcomeres. The annuli and furrows are shown at the outer surface of the body wall (Image source: [Hall] N533 L4 Z915). Bar, 1 μm. (**c**) TEM longitudinal section from 15-day old adult. In the older adult, the cuticle layers are each much thicker, while the furrows and annuli remain visible despite the wrinkling and thickening of the basal and medial cuticle layers. Below the cuticle, the body wall muscle sarcomeres are thinner and disorganized in the older adult (Image source: [Hall] N815 G0713)

2.3.1.1 Ageing of the Cuticle

Adult animals do not moult, so the adult cuticle must persist through the entire adult lifespan. During ageing, the cuticle becomes progressively thicker [8], and cuticle growth may continue until the hypodermis and seam are no longer capable of secreting cuticular components. This continuous growth is likely to result from unregulated biosynthesis of cuticle-related proteins as post-reproductive shut down of overall expression does not transpire (not subject to natural selection pressures, see discussion in [8]). The most prolific age-associated growth occurs in the basal cuticle layers, which can expand in thickness by 10-fold in comparison to young adults (Fig. 2.2c). Concomitant with this thickening, the cuticle becomes progressively more wrinkled overall [8]. Cuticle wrinkles may arise from the combined effects of a weaker, thinner hypodermis, loosened connections between the cuticle and hypodermis, and weakening muscles. In ageing animals, the distinct cuticle linings of body openings remain virtually intact, and are possibly reinforced (Herndon et al. unpublished data).

While the thickened ageing cuticle generally remains intact and capable of protecting the animal from outside insults until death, the cuticle cannot provide protection from *internal* causes of death, such as internally-hatching embryos or vulval muscle breakdown that allows gonad or gut extrusion. Thus internal injuries can ultimately induce cuticle lapses, although cuticle failure itself does not appear to be a major cause of death.

2.3.2 Hypodermis

The *C. elegans* hypodermis is composed of a large syncytium, named hyp7, which encloses most of the body and provides a barrier for the pseudocoelomic cavity (Fig. 2.3a) [23, 25]. Additional hypodermal cells are located in the head and tail. The hypodermis serves several functions, including the deposition of basement membrane components, secretion of certain cuticle components and direct formation of specialized cuticle structures. In addition, the hypodermis establishes the basic body plan during embryogenesis and guides migration of certain cells during development.

Fig. 2.3 (continued) right edge of the panel show debris filling the pseudocoelom (Image source: [Hall] N801 E565). Bar, 1 μm. (**d**) TEM cross-section from a healthier day 15 adult showing the lateral hypodermis filled with cellular detritus, including abundant lipid droplets. The hypodermis cytoplasm is less electron dense than in the young adult (**b**) and organelles are altered or missing. The thickened cuticle has pulled away from the hypodermis during fixation, indicating structural weakness in cuticle attachment. In addition, acellular material has been shed into the space beneath the thickened cuticle (Image source [Hall] N812 U3 M784). Bar, 1 μm

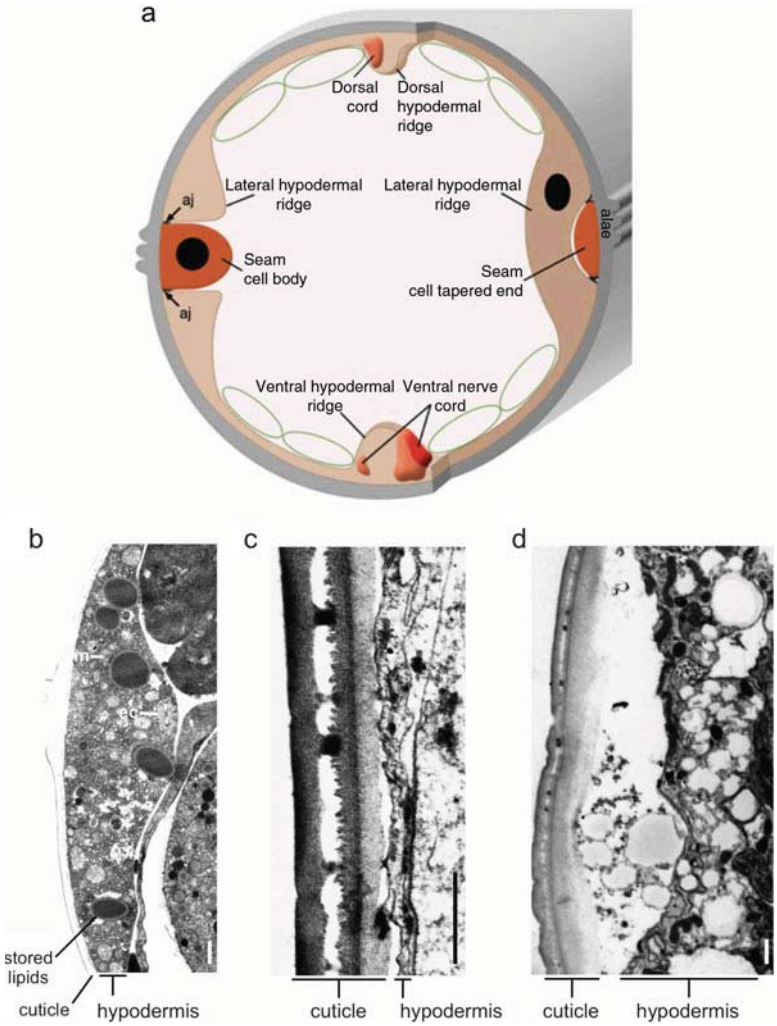

Fig. 2.3 The *C. elegans* hypodermis becomes thinner and fragile during ageing. (**a**) Schematic view at the midbody shows the hypodermis (*tan*) as it encloses the animal just below the cuticle (*grey*). The hypodermal syncytium is quite thin where is underlies the body wall muscles (*empty green circles*), but is enlarged along the lateral borders and where it provides support to the longitudinal nerve cords (*red*). Specialized seam cells (*dark orange*) lie in rows just under the cuticle alae at the lateral borders, linked to the neighbouring hypodermis by adherens junctions (*aj*) (Image source: [WormAtlas]). (**b**) TEM cross-section of the hypodermis at the lateral line in a young adult. In young animals, the hypodermis is filled with organelles, including abundant RER, mitochondria (m), and stored lipids and yolk. A clear internal space, the pseudocoelom, lies between the hypodermis and the tissues within the body cavity, such as the distal gonad (*upper right*) and uterus (*lower right*). The excretory canal is visible (ec) at the edge of the pseudocoelom (Image source: [Hall] N506 M700). Bar, 1 μm. (**c**) TEM cross-section of an older (day 15) adult showing extremely thinned hypodermis, devoid of most cytoplasmic components, and much less electron dense. The thickening of the cuticle is apparent in comparison to (**b**). Brighter areas at the

2.3.2.1 Ageing of the Hypodermis

Growth continues for several days after the final moult, stretching the hypodermal hyp7 syncytium [8]. As animals age, the hypodermal cylinder becomes exceedingly thin in all regions and loses the capacity to maintain its shape. Viewed by electron microscopy, the ageing hypodermal cytoplasm contains fewer organelles than young hypodermis, such as smooth and rough ER and mitochondria, and those that are present often appear damaged (Fig. 2.3c, d compared to Fig. 2.3b). The cytosol also becomes progressively less electron dense. In very old animals, the hypodermal cytoplasm is nearly empty and the tissue thins to the breaking point, particularly on the basal pole facing the pseudocoelom. Ageing may disrupt "clean up" functions of hypodermal cells, which normally clear damaged cells and other materials from the pseudocoelom by engulfment. Old-age accumulation of debris materials in the pesuedoceolom (see below) suggests loss in efficacy of this process. Compared to other tissues, the hypodermis may be a particularly weak link during ageing, and its physical breakdown may have fatal consequences for old animals. Loss of hypodermal cylinder integrity would allow the pseudocoelom to mix with apical contents, which could damage anchorage of the muscles and cuticle, leaving cells or debris to float inside the cuticle. That components of the cuticle and/or basement membranes may be critical in healthy ageing is supported by recent findings that extracellular matrix gene expression is enhanced in multiple long-lived mutants and modulated expression of particular individual collagens can impact lifespan [26].

2.3.3 Muscle

The two main types of muscle cells in *C. elegans* are the single sarcomere/non-striated muscles and the multiple sarcomere/obliquely striated muscles [27]. Single sarcomere/non-striated muscle cells include the muscles of the pharynx, the somato-intestinal muscle, the anal sphincter and depressor, the contractile gonadal sheath, and the sex-specific muscles of the uterus, vulva and male tail. Of these, the pharynx muscle has been best studied in the context of age-associated structural and functional change and is discussed in greater detail in a later section.

The multiple sarcomere muscles, more commonly known as somatic or body-wall muscles, control movement and locomotion. These 95 skeletal muscle-like cells constitute the most abundant muscle group. The body wall muscles are arranged as staggered pairs in four longitudinal bundles situated in four quadrants lining the body cylinder (Fig. 2.4a–c). Evenly-distributed attachment points bind the body-wall muscle bundles along their length to the hypodermis and cuticle. The basic unit of the contractile apparatus is the sarcomere, and these contractile units are repeated in body muscle, giving the cells a "striated" appearance (Fig. 2.4a).

A typical somatic muscle cell has three parts: the contractile myofilament lattice or spindle, a noncontractile body, called the muscle belly, containing the nucleus and the mitochondria-filled cytoplasm, and the muscle arms, slender processes extending towards the nerve cords or the nerve ring where neuromuscular junctions (NMJ) are

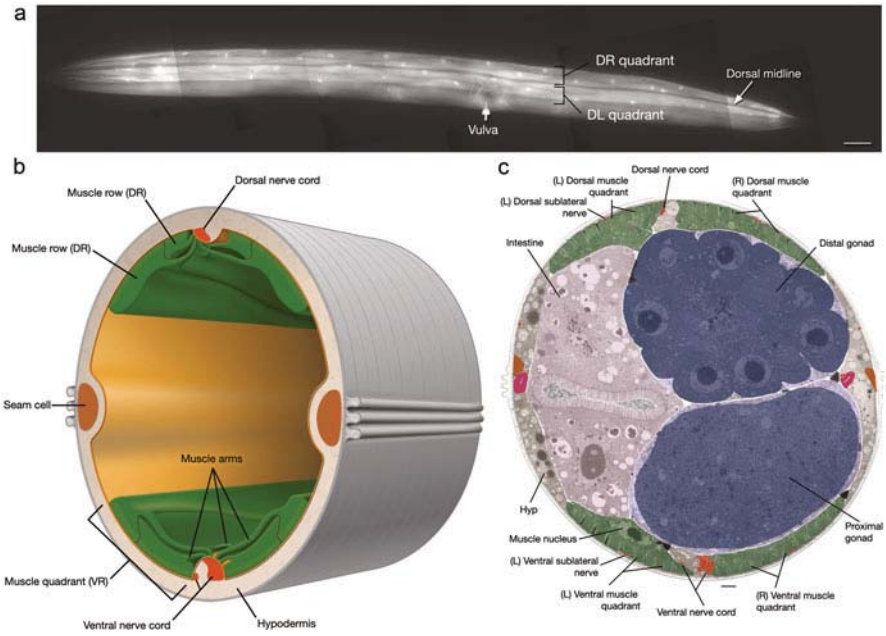

Fig. 2.4 Organization of the *C. elegans* body wall muscles. (**a**) Epifluorescence image (dorsal view) of a body wall muscle-specific GFP reporter expressed in a young adult hermaphrodite (*unc-27:GFP* reporter; Strain source: Jia, L and Emmons, SW). This view shows the full extension of the two dorsal muscle quadrants from nose to tail (*left* to *right*). Polarized light helps to visualize the myofilament lattice which runs virtually parallel to the body axis, separated by the narrow ridge of dorsal hypodermis at the midline. Each quadrant consists of two parallel rows of muscle cells. Nuclei of the muscle cells can be seen as *white circles*, lying near the of each spindle-like cell. Bar, 50 μm (Image source: [WormAtlas]). (**b**) Diagram of the midbody region. Each muscle cell (*green*) along the midbody extends one to three thin "muscle arms" inward to reach the nearest nerve cord where it receives innervation. Thus four dorsal rows in two dorsal quadrants extend arms to the dorsal nerve cord, and four rows in two ventral quadrants extend arms to the ventral nerve cord. Basal lamina (*light orange line*) separates the muscle from the nerve cords and the hypodermis. Hypodermis, which is stylized in this diagram for illustration purposes, separates muscle from cuticle (Image source: [WormAtlas]). (**c**) TEM thin section of the young adult midbody has been false-coloured to show the layout of the muscle quadrants (*green*) in finer detail. Note that all myofilament sarcomeres lie close to the cuticle, while each muscle has its cell body, the "muscle belly", lying more central, containing the nucleus, RER, mitochondria and other organelles. Muscle arms extend away from the muscle belly. Bar, 1 μm (Image source: [WormAtlas])

situated (Fig. 2.4b). Somatic muscle nuclei in young adults are oblong, intermediate in size between neuronal and hypodermal nuclei, and have spherical nucleoli.

2.3.3.1 Ageing of Body Wall Muscle

Body wall muscle sarcomeres become strikingly disorganized in aged animals (Fig. 2.5) [8]. Most sarcomere bundles contain fewer myosin thick filaments than in young muscle, with individual filaments sometimes appearing to bend and break. Muscle cells appear to shrink overall (Fig. 2.5 c, d), possibly due to cytoplasmic

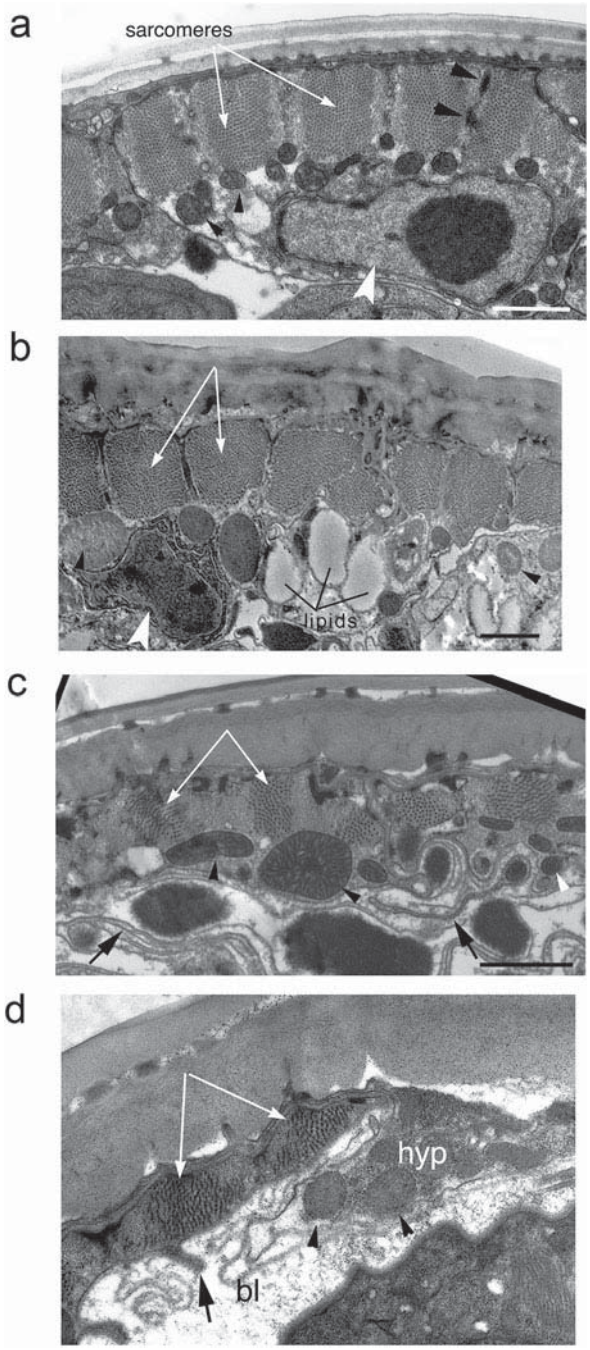

Fig. 2.5 Body wall muscles become disorganized and deteriorate during ageing. (**a**) Young adult body wall muscle cell showing five sarcomeres running side by side beneath the cuticle. The muscle myofilaments are anchored to darkly-staining "dense bodies" (*large black arrowheads*) connecting to the muscle's plasma membrane. The plasma membrane is linked to the cuticle by intermediate filaments extending across the thin hypodermal layer and connecting to wispy

loss, and lipid droplets can accumulate within the muscle cells (Fig. 2.5b) [8]. Body wall muscle nuclei also show pronounced changes with nuclei becoming misshapen and nucleolar size increased. A few nuclei in ageing muscle have been observed to become electron-dense and appear to undergo autophagy. The degree of nuclear change appears to correlate with locomotory ability of the individual animal, though there is still much variation in individual muscle cells within a single animal, suggesting a stochastic component in the decline of single cells [8].

As the body wall muscles deteriorate with age, the normally sinusoidal locomotory behaviour also declines [8, 12, 13, 20, 28–30]. Older animals move only when stimulated and display increasingly irregular patterns of movement. The most decrepit animals can no longer move forward or backward, and only slightly twitch their head or tail regions when touched. A closer look at the muscle cells in individual animals with movement defects suggests coincident levels of sarcomere deterioration, indicating that muscle cell deterioration may contribute to ageing-related locomotory declines (compare Fig. 2.5b–d, which show progressive loss of myofilaments in animals with increasingly impaired movement). Indeed, analysis of individual ageing animals showed that decline in locomotory ability more closely predicted time of death than did chronological age [8]. Recent studies suggest that that the earliest detectable locomotory declines reflect changes in neuronal signalling at the neuromuscular junction

Fig. 2.5 (continued) filaments in the basal layer of the cuticle. A prominent nucleus (*large white arrowhead*) containing a large nucleolus lies beneath the sarcomeres in the muscle belly, surrounded by large mitochondria. A row of mitochondria (*small black arrowheads*) lie in the muscle belly, close to the sarcomere (Image source: [Hall] N513 G607). Bar, 1 μm. (**b**) A similar cross-section of a body wall muscle in a relatively motile 15 day adult. In this adult, the muscle cell retains intact sarcomeres with many myofilaments per unit volume, though reduced somewhat compared to the young adult (**a**). The nucleus is present in this view and the nucleolus appears less electron dense. The muscle belly remains fairly large with numerous mitochondria, but contains large lipid droplets and is less electron dense. Thickening of the overlying cuticle is also apparent in this animal (Image source: [Hall] N810 R443). Bar, 1 μm. (**c**) Cross-section from a slow-moving 15 day old adult shows dramatic muscle cell changes. The myofilament lattice is smaller and disorganized, including a dramatic decline in myosin filaments per sarcomere (*long arrows*). Although mitochondria are still present (*black arrowheads*), the cell has shrunken and the cytoplasm is devoid of most organelles, including RER or lipid storage. At the basal pole, wispy pieces of membrane may be shedding into the pseudocoelom (*short black arrows*), sometimes containing small mitochondria, and coated on the outside by basal lamina. The pseudocoelom itself has gained volume and contains basal lamina fragments and large dark yolk granules. The basal layer of the cuticle is now extremely thick compared to a young adult (Image source: [Hall] N813 G506). Bar, 1 μm. (**d**) TEM cross-section of a paralysed 15 day adult showing extreme loss of muscle integrity. Sarcomeres have lost most myosin and actin filaments, although the remaining filaments are well positioned between smaller dense bodies. The muscle belly is virtually absent except for a thin projection (*short arrow*), indicating continuing shedding into the pseudocoelom, with a concomitant loss of mitochondria and cytoplasm from the belly. Whorls of basal lamina (*bl*) and other debris are floating in the huge volume of pseudocoelom. The cuticle is intact and vastly enlarged, especially the basal layer. *Arrowheads* indicate the presence of intact mitochondria inside the neighbouring hypodermis (*hyp*) (Image source: [Hall] N829 R157)

(NMJ) [12, 31, 32]. Later in life, muscle deterioration adds to this early impairment, enhancing locomotory declines in old animals. Regardless of the important question of initiating mechanism, substantial sarcopenia accompanies *C. elegans* ageing.

2.3.4 Pharynx

The *C. elegans* pharynx is a neuromuscular organ (20 neurons and 20 muscles) located in the head through which food is ingested and crushed for intestinal absorption (Fig. 2.6). In a young adult, the pharynx pumps 200–300 times per minute to draw suspended food particles (bacteria and fungus) into the alimentary tract. Food particles are concentrated in the corpus region (the most anterior region of the pharynx) and pass through the isthmus to the terminal bulb, where a cuticular structure called the grinder pulverizes food for digestion in the intestine. As animals age, the pharynx exhibits both structural and functional declines [8, 13, 21, 28, 29, 33].

2.3.4.1 Ageing of the Pharynx

Pharyngeal cells deteriorate in older adults and prominent vacuoles often appear within the organ (Fig. 2.6d). The elongated muscle cells of the isthmus become weakened with age, as they often appear to be bent or kinked in EM images (Fig. 2.6b) [33]. Finally, the pharynx itself becomes less efficient at crushing bacterial cells, and intact bacteria are more likely to be observed in the pharyngeal lumen of older adults (Fig. 2.6e). The stress of pumping over adult life may damage the pharynx, as mutations that limit contractions can slow functional decline [33].

The rate of pharynx pumping decreases progressively with age, such that pumps are rare in animals older than 8 days, which is a striking senescence feature in a ~21 day lifespan [13, 28]. Considerable heterogeneity in pump rate of individuals has been reported [34] and this heterogeneity increases with age [33]. Exogenous serotonin can stimulate pumping in young adults [35] and can also stimulate pumping in old animals, although pumping rates +/− serotonin still progressively decline over adult days 2–8 [33]. Since neurotransmitter response is maintained, but functionality declines, the structural deterioration of the pharynx muscle with age appears likely to limit its functional capacity. There is a paucity of information on how the ageing of the 20 pharyngeal neurons impacts organ function.

The live bacterial food upon which *C. elegans* nematodes are maintained in the laboratory is at best a minor cause for decreased pharynx pumping with ageing, as pump rates declined similarly when animals were raised on bacterial food sources

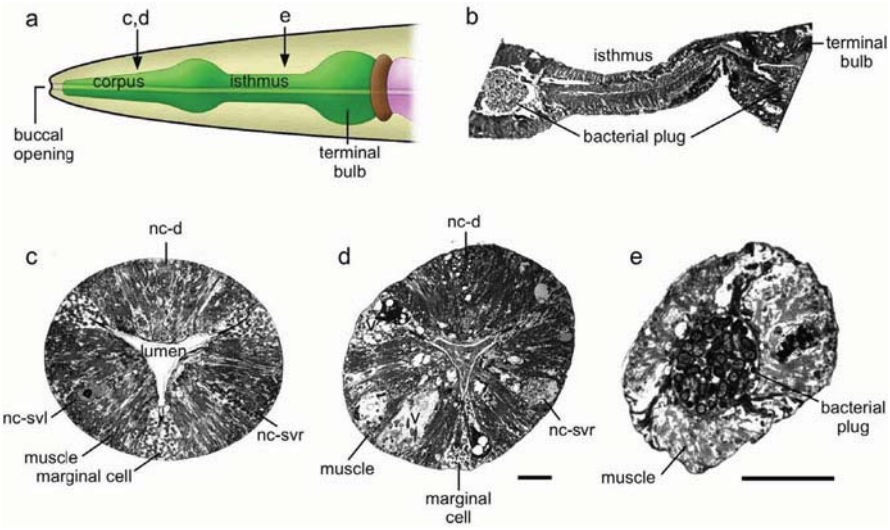

Fig. 2.6 Deterioration of the pharynx during ageing. (**a**) Schematic layout of the adult pharynx (*green*) with major regions labelled and showing the relative positions of the pharyngeal valve (*brown*) and the intestine (*pink*). (**c**–**e**) indicate approximate positions of TEM thin sections shown in lower panels (Image source: [WormAtlas]). (**b**) Longitudinal TEM section of the pharynx isthmus between the corpus and terminal bulb in a 7 day old adult. In this middle-aged adult, the isthmus is already weakened and kinked near the terminal bulb. Bacterial cells are seen as densely packed plugs in the lumen of the corpus and terminal bulb. In some animals, the bacterial plugs can be seen extending into the isthmus region (Image source: [Hall] N824 N4924). (**c**) Cross-section of the corpus in a young adult. The tissue has threefold symmetry with a row of marginal cells lying at each apex of the internal lumen, and two rows of fused pharyngeal muscles whose sarcomeres are oriented radially on each side of the lumen. Narrow rows of pharyngeal neurons lie between the pharyngeal muscles, named the dorsal, subventricular left and subventricular right nerve cords (*nc-d*, *nc-svl* and *nc-svr*). One neuronal cell body (and its nucleus) is visible here in the ventral left pharyngeal nerve (Image source: [MRC] N2U 411 0238-06). (**d**) A similar region of the pharynx in a 15-day old adult which had maintained its locomotory behaviours in movement assays [8]. In this animal, many pharynx myofilaments remain intact, although muscles, marginal cells and nerve cords are vacuolated and sometimes disorganized. Intact bacteria have entered a vacuole in one muscle cell at the lower left (V). The central lumen is filled with electron-dense material which may be debris from partially ground up bacteria (Image source: [Hall] N812 F828). Bar, 5 μm. (**e**) TEM cross section of pharyngeal isthmus region of a paralysed 15-day old adult showing extensive muscle deterioration and accumulation of intact bacteria in the lumen (bacterial plug), forcing the lumen to open widely. The three marginal cells are identifiable by thick bundles of intermediate filaments connecting radially to the lumen, but most nerve cords are difficult to identify. The tissue is distorted in overall shape, muscle myofilaments are twisted, and the muscle cytoplasm has become much less electron dense (Image source: [Hall] N807 G905). Bar, 5 μm

that were growth arrested due to antibiotic treatment [33]. Still, in the absence of strong pumping the pharynx can become plugged with bacteria as animals age (Fig. 2.6e), either as a cause or consequence of pharynx decline.

2.3.5 Nervous System

The *C. elegans* hermaphrodite nervous system is composed of 302 neurons arranged throughout the body (Fig. 2.7a) for sensory, locomotory, and other behavioural functions mediated by an array of neurotransmitters including acetylcholine, GABA, serotonin, dopamine and glutamate. At the tip of the nose, sensory neurons detect gustatory and olfactory stimuli such as food and other nematodes. Thermal stimuli are detected by thermosensory neurons in the head. Mechanosensory neurons, distributed in the lips and along the body's length (Fig. 2.7b), alert the animal to physical stimuli in their environment. Signals from sensory neurons are transmitted to interneurons, many of which are bundled in the main ganglion of the nerve

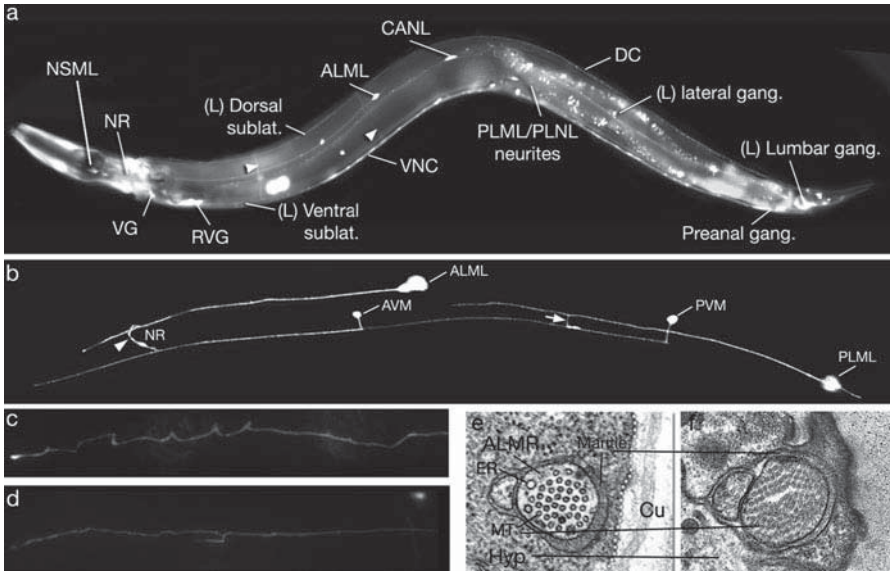

Fig. 2.7 Neurons (**a**) Epifluorescence image of panneuronal GFP reporter in an adult hermaphrodite showing distribution of neurons throughout the body. This is a left lateral view with anterior to the left. *NR* Nerve ring, *RVG* retrovesicular ganglion, *VG* ventral ganglion, *VNC* ventral nerve cord, *DC* dorsal cord. Motor neurons are scattered along the VNC and send processes to the DC via commissures (*arrowheads*). Magnification, 400× (Image source: [WormAtlas]). (**b**) Epifluorescence image of the touch receptor neurons expressing the cell-type specific GFP reporter, (*mec-4:GFP*) in a young adult hermaphrodite, left lateral view. The neuron processes are straight and evenly labelled by the reporter in the young adult (Image source: [WormAtlas]). (**c**), (**d**) Ageing-related morphological abnormalities in touch neurons. Touch neurons in older adults visualized with the same GFP marker as in (**b**) appear wavy (**c**) or branched (**d**) (Image source: Toth and Driscoll). (**e, f**) TEM cross section view of the ALM touch neuron from a young adult (**e**) and a 15-day-old adult (**f**). In young adults, the touch neurons are embedded in the hypodermis just beneath the cuticle. An electron-dense ECM, called mantle, surrounds the touch receptor processes and attaches them to the body wall. Touch receptor processes are typically filled with 15-protofilament MTs. While the touch neuron in **f** appears healthy and well structured, the cell process is filled with dozens of adventitious microtubules. *ER* Endoplasmic reticulum (Image source: [Hall] N501 S3 N517; N810 M782)

ring in the head. Interneurons, in turn, signal to motorneurons in the dorsal and ventral nerve cords to mediate behavioural responses, such as touch avoidance and egg-laying. Neuromuscular junctions (NMJ) are clustered in synaptic regions where neurons appose the muscle cell arms (Fig. 2.8a). Motor behaviours are controlled by stimulatory cholinergic and inhibitory GABAergic inputs.

2.3.5.1 Ageing of the Nervous System

In contrast to the striking ageing-related physical decline of the body muscles, nervous system changes are more subtle [8]. There is no indication that any *C. elegans* neurons undergo cell death or necrosis in the course of normal ageing. Sensory specializations, such as cilia and dendrites, remain well preserved. Touch neurons, as analysed by electron microscopy, maintain their basic ultrastructure in aged animals (Fig. 2.7e, f). Most other aspects of the nervous system decline progressively, with the predominant pattern of change evident from EM data involving progressive losses of synaptic integrity and shrinkage of the neuron soma. At the cellular level, morphological abnormalities in processes can increase with age, to a degree that depends on individual neuron type. Further discussion of ageing in the nervous system can be found in Chap. 8.

2.3.5.2 Synaptic Decline During Ageing

At the nerve cords and nerve ring, the numbers and size of intact synaptic contacts decline substantially with age [36]. Surviving synapses are smaller and contact zones often contain very few synaptic vesicles compared to young synapses (Fig. 2.8). The absolute cross-sectional diameter of presynaptic zones, which occur *en passant* along the axons, is generally a function of the number of synaptic vesicles and mitochondria that are locally collected near the presynaptic dense bar. In some older synapses, the presynaptic zone shrinks to enclose only the presynaptic bar, which itself may be flexed and shortened to fit within a tiny axonal process [36]. These findings suggest that neuronal signalling is greatly reduced in older adults, unless electrical synapses can compensate for the loss of chemical signals. Interestingly, 15-day-old animals that age gracefully by locomotory criteria maintain greater synaptic integrity than same-age, same-environment 15-day-old animals that have aged poorly (earlier onset locomotory decline) [36]. Recent electrophysiological studies suggest that decline in presynaptic neurotransmitter release at the *C. elegans* neuromuscular junction is the earliest detectable decline in locomotory ageing, preceding detectable muscle deficits [32].

2.3.5.3 Changes in the Neuronal Processes During Ageing

EM data support that most axons shrink in diameter during ageing. The nerve cords remain intact and the axons appear unbroken, despite diameter shrinkage. In touch neurons, microtubule networks appear important for adult structural maintenance [37]

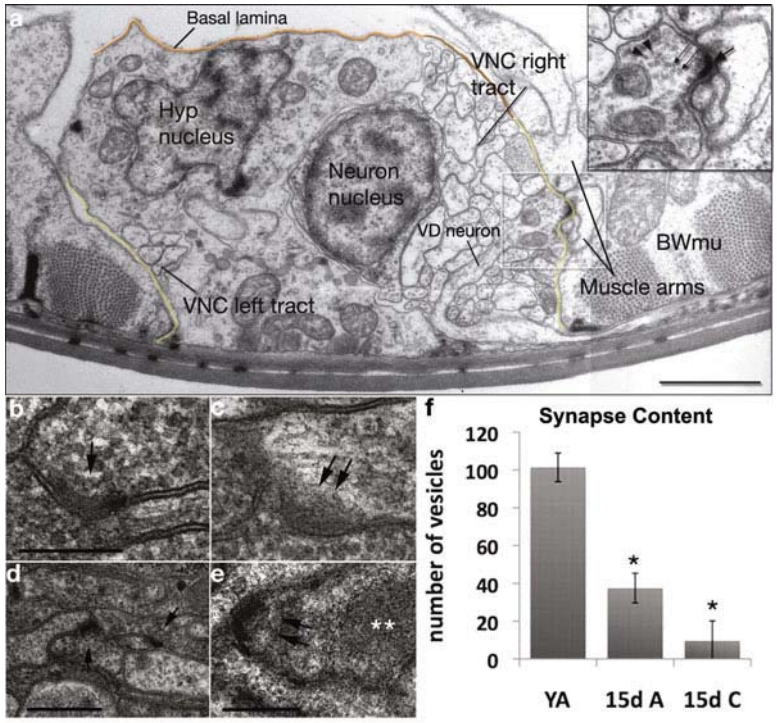

Fig. 2.8 Synapses (**a**) TEM of the neuromuscular junction (NMJ) between a ventral motor neuron and several muscle arms, transverse section. (*Inset*) The same synaptic region, magnified. At the point of contact with the post-synaptic elements, the presynaptic process enlarges into a varicosity with a specialized darkly staining bar at the active zone (*thick arrow*) and contains many synaptic vesicles (*thin arrows*) close to microtubules (*arrowheads*). Bar, 1 μm (Image source: [WormAtlas]). (**b–e**) Comparison of synapses in young (**b, c**) and 15-day-old (**e, f**) adult animals. Older adults were divided into three classes based on mobility. Class A animals were highly mobile while Class C animals moved only when prodded and primarily moved just their head and tail regions. (**b**) A young adult animal exhibits a prominent presynaptic bar along the plasma membrane and the process is swollen with synaptic vesicles. Vesicles lying close to the bar are somewhat smaller in diameter than vesicles away from the release zone. Bar, 0.25 μm (for **b** and **c**). (**c**) A depleted synapse (*double arrows*) in the same young adult displays a normal presynaptic bar, but a paucity of synaptic vesicles close to the bar or at a distance. (**d**) In a Class A (mobile) adult at 15 days, chemical synapses (*arrows*) remain well organized but have fewer vesicles near the presynaptic bar and the presynaptic process is therefore smaller in diameter. Note that many nearby axons (away from the synapse) remain almost the same diameter as in a young adult. Many axons still contain clusters of synaptic vesicles and small bundles of microtubules. Bar, 0.5 μm. (**e**) Closeup of a depleted synapse (*double arrows*) in a Class A animal at 15 days of age. A fuzzy electron dense inclusion (*white asterisks*) lies close to the depleted synapse. This may represent pathological deposition of cytoplasmic proteins. Bar, 0.25 μm. (**f**) Quantitation of synaptic features in ageing *C. elegans*. YA, young adult; 15d A, 15 day old class A animal that is relatively vigorous for its same-age counterparts and considered to have aged gracefully; 15d C, 15 day old class C animal that is decrepit, barely mobile, and considered to have aged poorly. Data include measurements of 51 synapses from six young adults; 52 synapses from three Class A animals; 28 synapses from three Class C animals. Synapses were from the nerve ring and lateral ganglia. "*Number of vesicles*" indicates counts of all vesicles within 300 nm from the synaptic density. *Asterisks* indicate $p < 0.02$ as compared to young adult values; repeated measures analysis of variance test (SAS programme) (Data and images in (**b–f**) from [36])

and can become disorganized with age, at least near the soma [38]. Mitochondria travel within touch neuron processes at progressively slower speeds in both anterograde and retrograde directions [39], consistent with a declining cytoskeletal transit network.

Fluorescent reporters that allow visualization of neuronal processes have revealed dramatic structural changes in neurons that increase in frequency with age. Neurite branching, axon beading, axon swelling, axon defasiculation, "bubble" formation, waviness, and new growth from the soma have been reported for touch receptor neurons, PVD sensory neurons, PDE dopaminergic neurons, GABAergic neurons and others (Fig. 2.7c, d) [36, 38, 40–42]. Longitudinal observations suggest these structures are dynamic—appearing, disappearing, and progressing to different structures [36, 38]. The age-dependent occurrence of some of these features can be modulated by insulin-like signalling, MAP kinase and heat shock stress response signalling and neuronal attachment. The functional significance of morphological changes in ageing *C. elegans* neurons remains to be experimentally defined.

2.3.5.4 Changes in Neuronal Soma

Cytoplasmic contents change dramatically during neuronal ageing. Virtually all neurons in older adults display some degree of cytoplasmic shrinkage and increased electron density. In some neuron somata, the plasma membrane barely accommodates the nucleus and the remaining organelles, such as mitochondria and vesicles, are squeezed out into the nearby axon or dendrite. In ageing touch neurons, mitochondria redistributions in the soma occur as fewer and fewer mitochondria are identified in this compartment [39].

2.3.6 Glia

The nematode has a relatively small number of glial cells (56), most of which are specialized to create special environments for protecting the ciliated endings of sensory neurons (Fig. 2.9a) [43, 44]. These glial cells are known as the socket and sheath cells, and in the adult male, the structural cells of the ray neurons in the tail. Nematode axons are not myelinated. Only the CEP sheath cells and the GLR cells form larger wrapping processes to enclose portions of the nerve ring neuropil, a role similar to those of human astrocytes or microglia.

2.3.6.1 Ageing of Glia

Glial cells appear to remain viable into old age in the nematode, still enclosing sensory endings (Fig. 2.9b, c) ([8] and unpublished data) and are not normally required for neuronal viability [45]. Much like that of neurons, the glial cell cytoplasm becomes progressively more electron dense as the cells shrink in volume, and

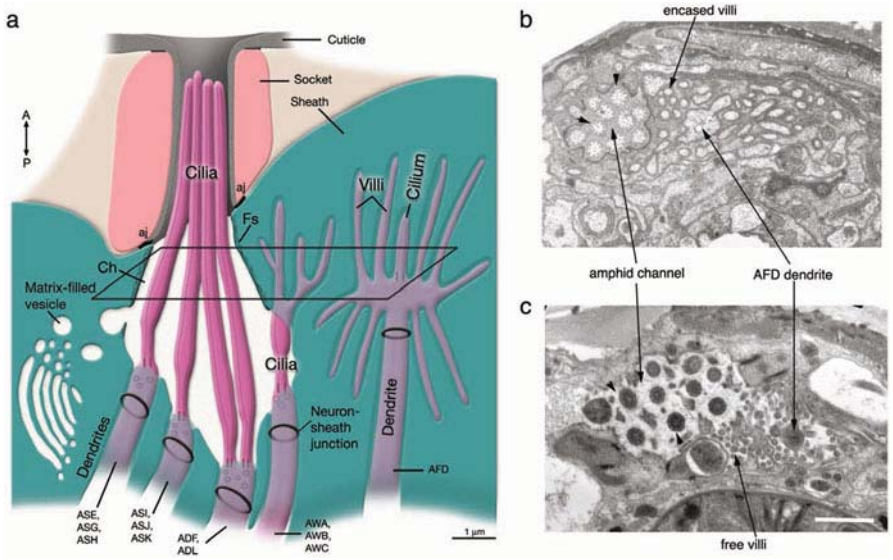

Fig. 2.9 Anatomy and decline of the amphid sensillum. (**a**) Structure of the amphid opening in a young adult, seen longitudinally, anterior to the top. The amphid channel (*Ch*) is lined by the lip cuticle in the distal (socket) part and an electron-dense lining supported by a scaffold of cytoskeletal filaments (*Fs*) in the anterior sheath. The socket cell is connected to the hypodermis and the sheath cell by adherens junctions (*aj*). Circular adherens junctions are also seen to tightly seal the dendrites to the sheath cell (neuron-sheath junction) proximally to the level where the dendrites enter the channel. A large Golgi apparatus located at the base of the sheath-cell process (*left*) gives rise to matrix-filled vesicles bound towards the channel. Several specialized neuron dendrites embed into the sheath cell with little or no exposure to the amphic channel (*AWA*, *AWB*, *AWC*, *AFD*). Mitochondria (not shown) are also present in this region (Image source: [WormAtlas] modified, with permission, from Perkins et al. [59]). Bar, 1 µm. (**b**, **c**) TEM of amphid channel cilia and AFD in young and old adults. Transverse sections through middle segments of cilia (area from boxed region in **a**). In a young adult (**b**), the distal portions of the "channel cilia", characterized by nine doublet microtubules, sit inside the amphid channel lumen (*black arrowheads*), while the AFD villi and its thick dendrite are encased inside individual thin channels of the amphid sheath, away from the channel. By comparison, the 15 day adult animal (**c**) shows the AFD villi are unsheathed and some have entered the main amphid channel to mix between the channel cilia. While there doesn't appear to be neuronal or glial cell loss, a shrinkage of glial sheath cytoplasm has led to a wider and more open amphid channel inside the sheath cell. All cilia and dendritic segments in the 15 day animal are more electron dense than in the young adult, and the AFD villi appear to have shrunken in diameter (Image source: [Hall] **b** SW8; **c** N813 537). Bar, 1 µm

sometimes accumulates vacuoles and small dark endosomes. As individual glial cells shrink, the narrow extracellular channel around the cilia can become progressively wider, but the opening to the exterior environment remains patent, and even very old cilia are likely to remain exposed to environmental signals. One sensory neuron, named AFD, contains sensory fingers ("villi") that are enclosed by the peripheral portion of the amphid sheath cell in young animals (Fig. 2.9a, b). During ageing, this region of the amphid sheath shrinks dramatically, so that it can no longer provide separate narrow channels for the AFD finger cells—the intact AFD villi eventually lie unsheathed inside the main amphid channel (Fig. 2.9c). Little is

known about the viability of the wrapping processes of the CEP sheath cells and GLR cells during ageing. Much remains to be learned about contributions of *C. elegans* glia to ageing and healthspan.

2.3.7 Intestine

After food is pumped and pulverized by the pharynx, it enters the intestine where it is digested and nutrients are absorbed. Additionally, the intestine functions to synthesize and store macromolecules, initiate immune responses, and nurture germ cells by producing and secreting yolk [46–49]. The intestine is comprised of 20 large epithelial cells that are mostly positioned as bilaterally symmetric pairs to form a long tube around a lumen (Fig. 2.10). The intestine is not directly innervated and has only one associated muscle (the stomatointestinal muscle) at its posterior extreme. Intestinal cells are large and cuboidal with distinct apical, lateral and basal regions (Fig. 2.10b). Intestinal cells contain one or often two large nuclei with prominent nucleoli, many mitochondria, extensive rough endoplasmic reticulum, many ribosomes and an extensive collection of membrane-bound vesicles and vacuoles. Adherens junctions seal each intestinal cell to its neighbours on the apical side and gap junctions and septate-like junctions connect them on the lateral sides (Fig. 2.10b, c). Microvilli extend from the apical face into the lumen forming a brush border (Fig. 2.10b, c). The terminal web, a network of intermediate filaments, anchors the microvilli.

2.3.7.1 Ageing of the Intestine

Progressive degradation of the intestine includes loss of intestinal microvilli and nuclei as well as changes in the size, shape and cytoplasmic contents of the organ. In young adults, the intestinal lumen is nearly uniform in size and shape along its entire length. In older adults, the lumen becomes thinner along most of its length, winding, and swollen in various sections. Microvilli on the lumenal face of the intestinal cells become shorter and sparser (Fig. 2.11). Microvilli shortening has a stochastic component as normal, shortened, or completely absent microvilli can be found on the intestinal cells of the same animal.

In addition to changes within the intestinal lumen, there are dramatic age-related changes in the intestinal cell cytoplasm. Although variable within a single animal, some intestinal cells contain abundant lipid droplets or vacuoles not found in cells of younger animals (Fig. 2.11a, b) [8]. Some cells can appear to have a lytic cytoplasm or have reduced cytoplasmic volume (Fig. 2.11b, c). McGee et al. [50] reported a significant loss of intestinal nuclei with age and showed a possible involvement of the apoptotic pathway in nuclei loss. Intestinal cells also show a progressive decline in the integrity of their nuclei with age. In some cases, individual nuclei appear shrunken with darker staining, or possibly undergo autophagy [50]. Poorly regulated gene expression in the old intestine has been suggested to be

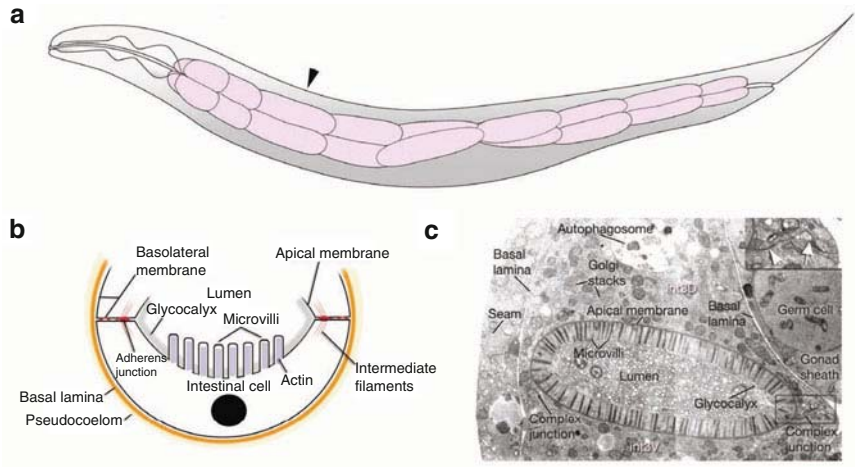

Fig. 2.10 Anatomy of the adult *C. elegans* intestine. (**a**) The intestine is positioned on the left side of the body anterior to the vulva and on the right side of the body posterior to it. At its anterior end, the intestine is connected to the pharynx via the pharyngeal valve. The most posterior portion is squeezed by the stomatointestinal muscle (not shown), near where the intestine connects to the rectum and anus. *Arrowhead* indicates the position of the TEM cross-section shown in (**c**) (Adapted with permission from [58]). (**b**) Key structural elements of the healthy intestinal cytoskeleton. At its basal pole the intestine is covered by a basal lamina (*orange*), separating it from the pseudocoelom. Pairs of intestinal cells meet to form a lumen between them, with the two cells firmly linked by adherens junctions at their apical borders. Gap junctions and septate-like junctions form a complex junction just beneath the adherens junctions on the basolateral membranes where the two intestinal cells meet. Intermediate filaments help to anchor a terminal web of fibres running just beneath the microvilli that face the lumen itself. An actin-based cytoskeleton fills each villus; the actin fibrils anchor into the terminal web at one end, and to an electron dense cap at the tip of the villus. A thick glycocalyx covers the outer surface of the microvilli. At adulthood, most intestinal cells contain two very large nuclei (*black circle*). The lumen of the young adult intestine usually is filled by debris from partially digested bacteria, but few if any intact bacteria (Image source: [WormAtlas]). Graphic adapted from Wood et al. [60]. (**c**) Electron micrograph showing the key features of the young adult intestine. The intestinal cytoplasm is filled with a complex mixture of organelles, including mitochondria, Golgi apparatus, RER, yolk-filled granules, and occasional large autophagosomes (Image source: [WormAtlas])

deleterious to lifespan. The intestine produces yolk that nourishes embryos, but in the old-age absence of oocytes, yolk is still produced and accumulates throughout animal [8, 21, 50]. Inappropriate yolk accumulation appears to be detrimental and limits lifespan [51].

2.3.7.2 Bacterial Infection

In ageing animals, large clumps of undigested bacteria are often found in the intestinal lumen (Fig. 2.11c), likely the result of reduced pumping and grinding efficiency in these older animals [10, 50]. In rare cases, bacteria invade the lumen of the

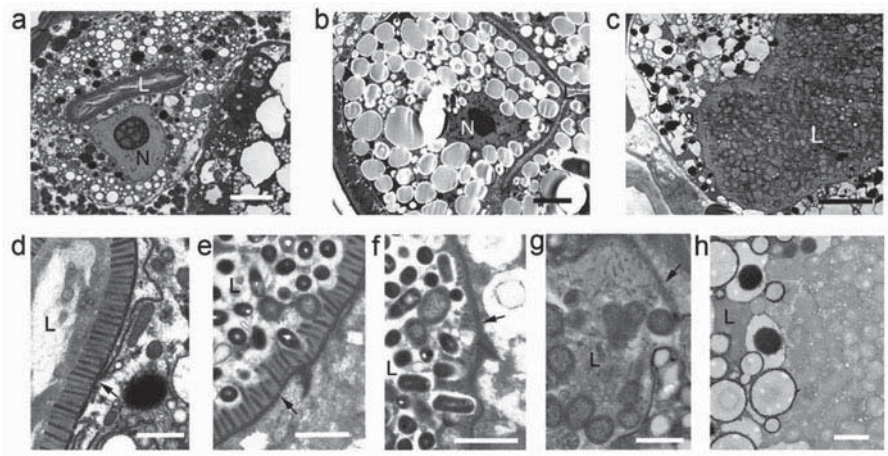

Fig. 2.11 Anatomical decline of the intestine during ageing (**a–c**) Low power transverse TEM views of aged intestine show large changes in the cytoplasm and lumen (compare to Fig. 2.10c). These declines are stochastic, as individual animals show markedly different rates of change. (**a**) In a day 7 adult intestinal cell, the nucleus (*N*) has lost heterochromatin and contains an enlarged, vacuolated nucleolus. The cytoplasm is highly vacuolated, with a profound reduction in ground substance, or RER, although many mitochondria remain intact. The apical zones are studded with microvilli but the lumen (*L*) is almost empty (Image source: [Hall] N826 5353). Bar, 5 μm. (**b**) A day 15 adult intestinal cell in which the cytoplasm is choked by lipid storage droplets. *L* lumen, *N* nucleus (Image source: [Hall] N812 F815). Bar, 5 μm. (**c**) A day 15 adult intestinal cell in which the cytoplasmic contents have become highly eccentric, with substantial degradation of all remaining organelles, and no intact ground substance. The lumen has intact microvilli but is swollen with intact bacteria. The intestine may not be competent for digestion, but provides a barrier against further bacterial invasion (Image source: [Hall] N807 G583). Bar, 5 μm. (**d–h**) Higher power transverse TEM views displaying major defects in the adult day 7 intestinal microvilli in adulthood. Progressive loss of several barriers to bacterial invasion of the cytoplasm are evident. (**d**) Healthy microvilli facing a lumen filled mostly with soluble items or a few bacterial fragments. *Arrow* indicates a region where the terminal web may be separating from the base of the microvilli (Image source: [Hall] N826 4239). Bar, 1 μm. (**e**) Microvilli are no longer uniform in length, and intact bacteria can be seen in the lumen, some of which are attached to individual villi, possibly beginning to degrade them. *Arrow* indicates the terminal web (Image source: [Hall] N821 4872). Bar, 1 μm. (**f**) In this region, most microvilli are gone, although the terminal web (*arrow*) remains thickened and electron dense. The lumen contains many intact bacteria (Image source: [Hall] N821 4855). Bar, 1 μm. (**g**) The microvillar border and the terminal web (*arrow*) separating the lumen from the intestinal cytoplasm are less electron dense and possibly incomplete, allowing bacteria to invade the intestinal cell cytoplasm (Image source: [Hall] N831 W006). Bar, 1 μm. (**h**) The bacteria -filled lumen (*L*) (*right*) and the intestinal cytoplasm (*left*) seem to be in direct contact along an ill-defined interface, with no obvious structure to divide them (Image source: [Hall] N833 W090). Bar, 1 μm

uterus or spermatheca or cross into cell cytoplasm along the alimentary canal, infecting the marginal cells of the pharynx [50]. Enlargement of the bacterial clumps over time suggests bacteria are able to divide inside the *C. elegans* body. Bacterial cells are occasionally found within the microvilli bed and may contribute to their destruction in patches (Fig. 2.11e–h). Studies showed that while the most decrepit

of the ageing animals tended to show more severe villar degeneration, sometimes healthy microvilli were found in areas with significant intestinal distortion [50]. Conversely, some relatively healthy animals show early shortened villi phenotypes in their intestinal cells. After much searching by TEM, we have still not found cases where bacteria have succeeded in penetrating into the intestinal cytoplasm in aged adults, although they must occur eventually. Even where all villi have been degraded, the terminal web still represents a barrier to entry (Fig. 2.11f–h).

2.3.8 Excretory System

The *C. elegans* excretory system carries out several functions, including concentrating and expelling metabolic waste, regulating internal osmolarity, and expulsion of exsheathment fluid after moults and hormone secretion [52]. The four cell types that make up the excretory system are: (1) a large, H-shaped excretory canal cell extending canals bilaterally along the length of the animal, (2) a pulsatile excretory duct cell, (3) a pore cell, and (4) two fused gland cells [52]. The normal organization and appearance of this system in the young adult has been well illustrated in WormAtlas.

2.3.8.1 Ageing of the Excretory System

The excretory system cells are heterogeneously affected during ageing. Most commonly, the canal cells appear swollen or become cystic in appearance (Fig. 2.12). Side branches may form in the canal cell lumen. The excretory gland cells may become enlarged in older animals (not shown). The duct and pore cells must remain intact and somewhat functional or the animal should quickly die from a fluid imbalance [53]. The sudden death of some ageing animals, often typified by a "straight-rod" death posture, may be attributed to failure of the excretory system.

2.3.9 Pseudocoelom and Coelomocytes

The *C. elegans* body lacks specialized vasculature and blood cells, but nutrients and debris can move throughout the body via the pseudocoelom, the contents of which are distributed by internal pressure changes during locomotion. The pseudocoelom occupies the interstitial spaces of the main body cavity, between the apical intestinal borders and the cells lining the cuticle. Since the pseudocoelom is a fluid-filled space, it lacks structural elements, except for the mesh-like basal lamina. The only cells that specifically occupy the pseudocoelom are the coelomocytes. These six cells move in limited fashion within the body cavity, removing detritus and foreign materials from the pseudocoelom by phagocytosis (cf. [54]).

2.3.9.1 Ageing in the Pseudocoelomic Space

As the cells bordering the pseudocoelomic cavity change with age, they infringe into the pseudocoelomic space or withdraw from it. This causes the pseudocoelomic cavity to become progressively distorted as the result of changes at its borders. Shrinkage causes some cells to shed their basal laminae, which fold into loops and whorls that can be found floating within the pseudocoelom (Fig. 2.5c, d). The

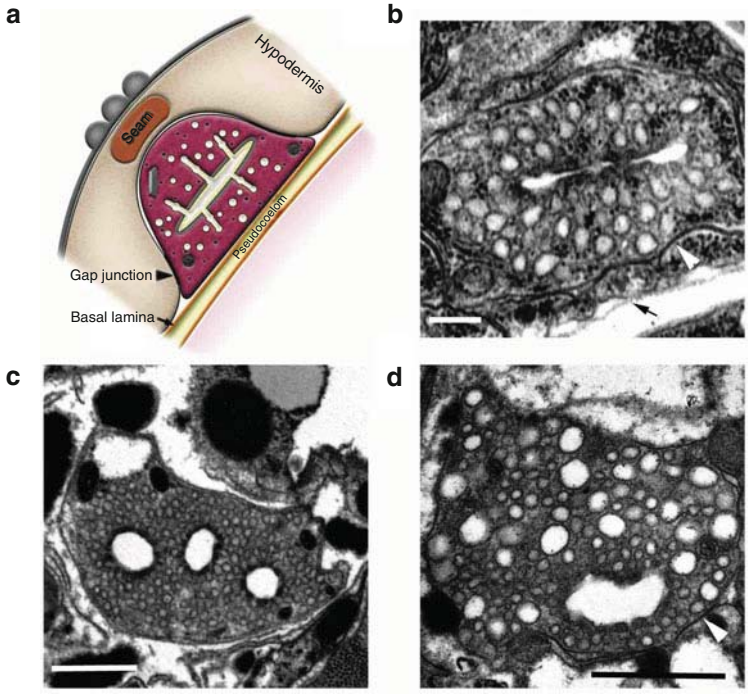

Fig. 2.12 Excretory canal cell structural changes during ageing. (**a**) Schematic cross-sectional view of one excretory canal cell arm. Canal cells extend lengthwise bilaterally in the body wall from head to tail, closely apposed to the hypodermis and cuticle alae over most of its length. Abundant large gap junctions link the canal arms to the hypodermis, presumably to allow exchange of small molecules and perhaps fluid. The canal cell has a single central lumen and many smaller canaliculi that connect to the lumen. Any other cytoplasmic organelles tend to be excluded by the lumen and canaliculi, coming to rest at the periphery of the excretory canal. The basal lamina of the hypodermis is shared with that of the excretory canal cell where they face the pseudocoelom (Image source: [WormAtlas]). (**b**) TEM cross-section of a canal cell in a young adult showing uniformly-sized canaliculi surrounding a central lumen. Here the canaliculi seem disconnected from their neighbours. In other sections, the canaliculi may appear as short chains of pearls, linking to each other and to the lumen (Image source: [Hall] N506 Z805). Bar, 5 μm. (**c, d**) Canal cells in two different 15-day old adults, showing development of multiple lumens (**c**), and/or a smaller lumen and large vacuoles (**d**), which might be enlarged canaliculi or endosomes. The canal has not shrunken in size so much as the hypodermis, and is sometimes left to float on its own within the pseudocoelom due to recession of the hypodermis (**c**). *White arrowheads* indicate gap junctions; *black arrow* indicates basal lamina (Image sources: [Hall] (**c**) N813 G501; (**d**) N805 G490). Bars, 1 μm

progressive shrinkage of many tissues causes the volume of the pseudocoelom to increase markedly in older adults. This volume change is most apparent adjacent to the shrunken distal gonad and intestine, but can also be seen along many muscles. The ageing proximal gonad expands in size at the ovaries, where the pseudocoelom is still squeezed to a minimum.

The pseudocoelomic contents also change during ageing. Intestinal yolk and lipids continue to be produced in adults and can be exported into the pseudocoelom, leading to massive buildup over time [8]. Moreover, the pseudocoelom becomes a repository for cellular detritus that accumulates during ageing, possibly due to declining coelomocyte and hypodermal activity in older adults (Fig. 2.5c, d).

2.3.10 Germline

The hermaphrodite reproductive system produces mature gametes and also provides the structure and environment for fertilization, early embryonic development and egg-laying. The *C. elegans* reproductive system consists of three major regions: (1) the somatic gonad, including the distal tip cell (DTC), gonadal sheath, spermatheca (sp), spermathecal-uterine (sp-ut) valve, and uterus; (2) the germline with mitotic and undifferentiated cells in the distal region that become meiotic and specialized as they progress through the proximal arm; and (3) the egg-laying apparatus, consisting of the vulva, uterine and vulval muscles and specialized neurons (Fig. 2.13a, b). In hermaphrodites, sperm production occurs in larval stages only since at the adult moult, germline precursors switch to forming oocytes. However, males produce sperm continuously throughout adulthood.

2.3.10.1 Ageing of the Germline

Hermaphrodites produce viable embryos for about 1 week following the L4-to-adult moult. As hermaphrodites grow older, progeny production declines sharply due primarily to sperm depletion. Unfertilized oocytes accumulate in the uterus and cause noticeable swelling in the hermaphrodite's midbody (Fig. 2.13c). The oocytes undergo nuclear endoreduplication and produce large masses of chromatin surrounded by complex cytoplasm. In some regions, the borders between oocytes disappear as they merge into syncytial masses. Within these syncytial zones, enlarged nuclei aggregate and form chromatin-filled nuclear masses, which may be separated from one another by intact nuclear membranes (Fig. 2.14c.d) [55, 56]. Cellular debris from degrading oocytes eventually blocks the vulval opening to the exterior and also impairs the egg-laying muscles (Figs. 2.13c and 2.14b). In very old hermaphrodites, germline tumours begin to comprise separate sectors containing endoreduplicating oocytes, degenerating cells and nuclei, masses of chromatin, and a few trapped embryos in various stages of morphogenesis (Hall, unpublished data).

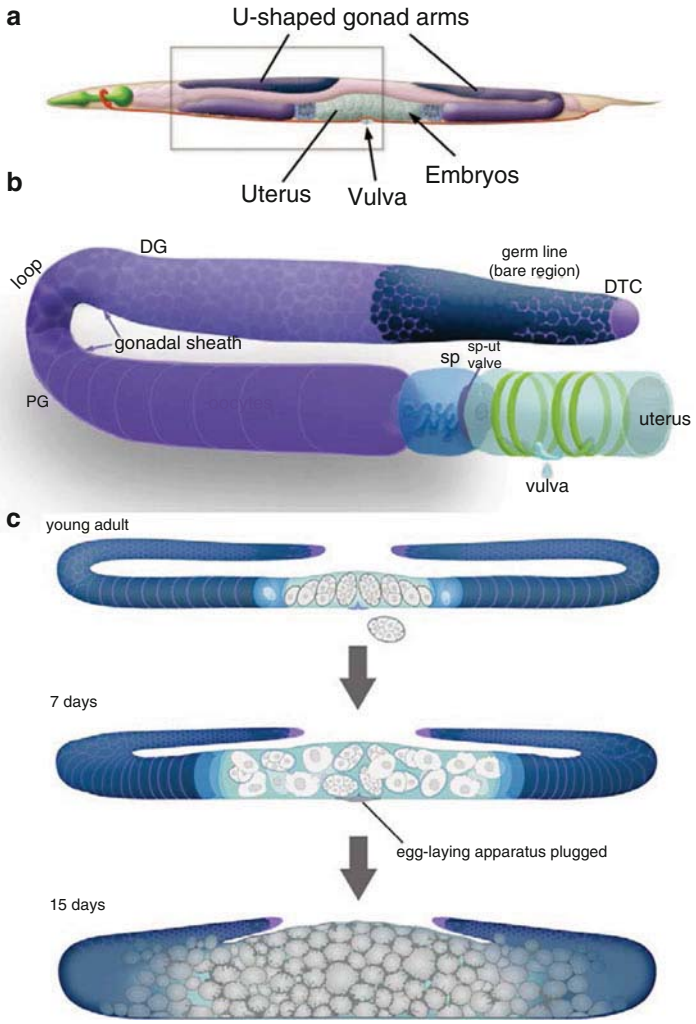

Fig. 2.13 Ageing-related changes in the germline and gonad. (**a**) Adult hermaphrodite, lateral view, left side, showing the location of the reproductive system within an intact animal. The reproductive system has twofold symmetry and consists of two U-shaped gonad arms joined to a common uterus. The reproductive system opens to the environment via the vulva, located in the ventral midbody. The distal portion of each gonad arm lies dorsally, with a cluster of immature germ cells surrounding a central rachis, to which each germ cell is linked via an open syncytial connection. The proximal portion of each gonad arm lies ventrally, where single large oocytes are surrounded by thin somatic sheath cells (Image source: [WormAtlas]). (**b**) One half of the reproductive system, enlarged and separated from other body parts (see *rectangle* in **a**). *DTC* Distal tip cell, *DG* distal gonad, *PG* proximal gonad, *sp* spermatheca, *sp-ut* spermathecal-uterine valve. Germline tissues are shown in *dark blue*, somatic gonad in *purple*, uterine muscles in *green*, spermatheca in *blue*, uterus in *pale blue* (Image source: [WormAtlas]), (**c**) illustration showing progressive changes in the germline with age. In young adults, eggs are fertilized as they pass through the spermatheca. In middle-aged adults, egg-laying declines and fertilized and unfertilized embryos can collect in the uterus. In older adults, complex germline masses can eventually expand to fill much of the body cavity of the animal

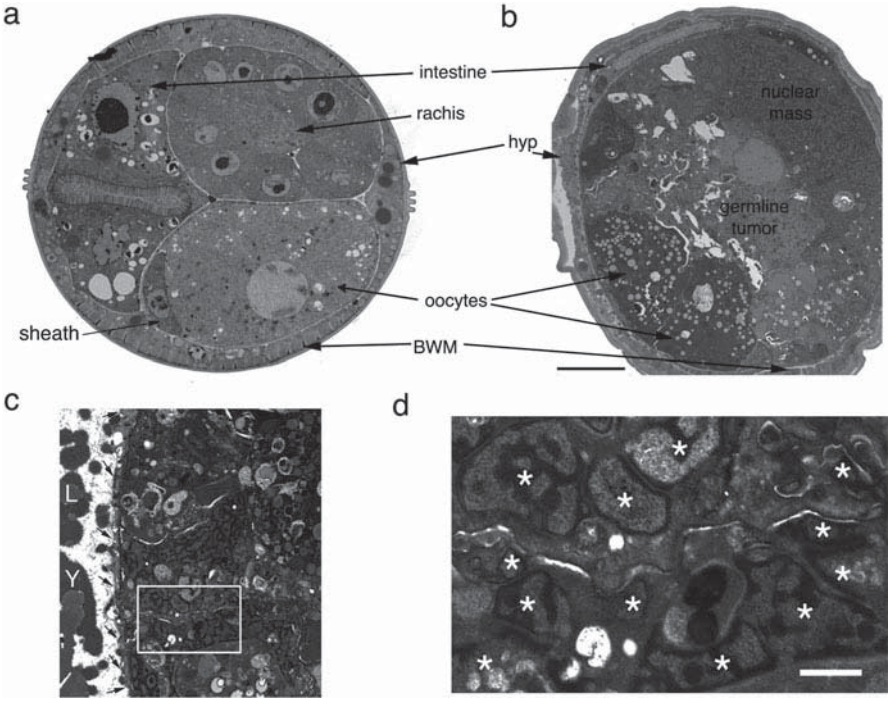

Fig. 2.14 Ageing adults develop germline masses of electron-dense acellular material. (**a**) TEM, transverse section, of a young adult hermaphrodite at low magnification. The distal portion of the gonad arm (dorsal) consists of thin gonadal sheath cells surrounding a syncytium of germ cells that are attached to a central cytoplasmic core (the rachis). The proximal region of the gonad (ventral) consists of a thicker gonadal sheath surrounding the oocyte. *BWM* body wall muscle, *hyp* hypodermis (Image source: N533 [Hall] F560). (**b**) A cross-section of the midbody in a 15 day adult in which germline tissue occupies more than 90 % of the total volume, with intestine, body wall muscle (BWM) and hypodermis (hyp) pushed to thin slivers at the periphery. Few normal oocytes remain, separated from the spermatheca by a large, complex germline tumour. The tumour includes a massive overgrowth of tightly compacted nuclear material which may be rigid enough to impair locomotion (Image source: [Hall] N816 H027). Bar, 10 μm. (**c**) Low power TEM image shows a portion of a germline tumour in a 15 day adult enclosed by a thin gonadal sheath cell (*black arrows*). Nearby pseudocoelom is filled with excess lipid (L) and yolk (Y). Within the tumour there are regions jammed with many nuclei and regions of complex cytoplasm, but no obvious maturing oocytes (Image source: [Hall] N801 E565). (**d**) *Boxed* region in (**c**) is shown at higher magnification. Each *asterisk* indicates a nucleus separated from other nuclei by membranes. Bar, 1 μm

The expanding germline tumour can eventually fill up to 90 % of the animal, compacting the intestine and other body tissues.

In fertile young adults, yolk is produced in the intestine and transported through the pseudocoelom to the germline, where it is absorbed by oocytes [46, 57]. As fertility declines during ageing, defective oocytes no longer absorb the yolk, which progressively accumulates as extracellular deposits in the pseudocoelom [8, 21, 50, 57]. There is apparently no negative feedback to intestinal yolk production, which

continues throughout adulthood. Virtually none of this yolk lies within the germline tumour itself, as the yolk cannot be transported into the gonad except by endocytosis into a viable primary oocyte [57]. Further discussion of the germline in the context of reproductive ageing can be found in Chap. 7.

2.4 End of Life Issues in *C. elegans*

Anatomical changes during ageing may constitute proximal causes of death in *C. elegans*, as for many other organisms. Weakened mechanical defences along the alimentary tract may allow bacterial cells to invade the body and once internalized, could proliferate unchecked due to coelomocyte ageing and inactivity. Indeed, environments supplemented with antimicrobial compounds can extend *C. elegans* lifespan [21]. However, the fact that antimicrobial protection does not confer immortality demonstrates that *C. elegans* adults also succumb to other causes of death.

Generalized physical deterioration may disrupt bodily functions to a lethal extent. Clearance of detritus and toxins appears to be impaired in older *C. elegans*, as evidenced by accumulation of debris in the pseudocoelomic space. Declining neuronal signalling, combined with muscle cell breakdown as ageing progresses, interfere with foraging and escape from environmental threats. In some hermaphrodites, gonad dysfunction leads to internal hatching of embryos, which is a lethal event for the mother.

2.5 Comparisons Between *C. elegans* and Human Ageing

Ageing in humans is characterized by frailty and declining mobility, features shared with ageing *C. elegans*. Both humans and *C. elegans* exhibit muscle deterioration and slower movement with ageing. The pathogenesis of human frailty remains an open question. Further studies of *C. elegans* muscle deterioration and locomotory decline during ageing could reveal new avenues for investigating these changes in people. Similarly, alterations in neuronal structure and function occur with ageing in these diverse species. Elucidating the factors that cause ageing-associated neuronal alterations in *C. elegans* may also lead to new insights regarding cognitive decline during human ageing. In adulthood, *C. elegans* is refractory to tumorigenesis due to the terminal differentiation of most cells. However, tumours can arise in the ageing germline, providing a possible window to study factors that contribute to the increased rate of certain cancers during human ageing.

2.6 Conclusions

Grounded in its simple, reproducible body plan and small size, working knowledge of tissue origin and maintenance of *C. elegans* anatomy is unparalleled in the animal world. Still, it is clear that our understanding of age-associated changes in this facile animal model remains primitive. Systematic, high-resolution, detailed studies of tissue changes over time could anchor critical investigations of tissue-specific ageing, while also providing information on stochastic occurrences of specific changes. A striking gap is our limited understanding of how physical changes within individuals relate to overall ageing of the animal or its behaviour. Current research has barely scratched the surface on the effort towards linking genetic or environmental influences, thought to change ageing quality, with features of tissue-specific decline.

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