Review

The role of mitochondria in the life of the nematode, *Caenorhabditis elegans*

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Abstract

Mitochondria are essential organelles involved in energy metabolism via oxidative phosphorylation. They play a vital role in diverse biological processes such as aging and apoptosis. In humans, defects in the mitochondrial respiratory chain (MRC) are responsible for or associated with a bewildering variety of diseases. The nematode *Caenorhabditis elegans* is a simple animal and a powerful genetic and developmental model system. In this review, we discuss how the nematode model system has contributed to our understanding of mitochondrial dynamics, of the genetics and inheritance of the mitochondrial genome, and of the consequences of nuclear and mitochondrial DNA (mtDNA) mutations. Mitochondrial respiration is vital to energy metabolism but also to other aspects of multicellular life such as aging and development. We anticipate that further significant contributions to our understanding of mitochondrial function in animal biology are forthcoming with the *C. elegans* model system.

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1. Introduction

1.1. Mitochondria

Observations of intracellular structures that likely correspond to mitochondria date back to the mid-19th century [1]. In 1890, Altmann [2] described a cytoplasmic structure that resembles bacteria and exists as an “elementary organism” living within the cell. It is now widely accepted that the ancestors of mitochondria are eubacterial endosymbionts that became established within a host cell [3]. The symbiotic relationship led to the loss or transfer of genes from the bacterium to the host genome during the course of evolution [4].

Mitochondria are a double-membrane organelle. The inner membrane, which forms convoluted and folded structures called cristae, encloses the matrix. The inner membrane is impermeable to ions and most metabolites and is very protein-rich, containing numerous transporters that mediate metabolic communication with other cellular compartments. The outer membrane, which is permeable to most molecules of less than 10 kDa, is notable for its roles in organelle biogenesis and structure.

1.2. Mitochondrial DNA (mtDNA)

mtDNA was discovered in the early 1960s [5]. To date, over 250 metazoan mitochondrial genomes have been sequenced. These are extremely compact; introns are absent and only one noncoding region called the displacement loop or D-loop is present. The D-loop region contains one of the origins of replication and two promoters for mtDNA transcription [6]. mtDNAs reside in the mitochondrial matrix attached to the inner membrane and are maternally inherited [7]. All mammalian mtDNAs are circular double-stranded DNA molecules of 15 to 18 kb in length encoding only 13 proteins, all subunits of the mitochondrial respiratory chain (MRC). Two ribosomal RNAs (12S rRNA and 16S rRNA) and 22 transfer RNAs necessary for mitochondrial translation are also encoded. The remainder of the approximately 1000 mitochondrial proteins are nuclear-encoded and are imported into the organelle.
1.3. The MRC

The primary function of mitochondria is to generate ATP via oxidative phosphorylation, the major source of cellular energy for most eucaryotic cells. The MRC, located in the inner membrane, is composed of four electron transporting protein complexes: complex I (NADH–ubiquinone oxidoreductase), complex II (succinate–ubiquinone oxidoreductase), complex III (ubiquinol–cytochrome c oxidoreductase), and complex IV (cytochrome c oxidase) (Fig. 1). The oxidation of the substrates, NADH or succinate, coupled to the reduction of oxygen is accompanied by the generation of an electrochemical proton gradient across the inner membrane. A fifth protein, complex V (ATP synthase or the F0F1-ATPase) uses this proton gradient to drive ATP synthesis. Coenzyme Q or ubiquinone (Q) is an essential, lipophilic carrier needed to shuttle electrons between complexes I or II to complex III. Cytochrome c (cyt c) is a water-soluble heme-containing protein that mediates electron passage between complexes III and IV (Fig. 1).

1.4. Other roles of mitochondria

By the 1970s, substantial progress had been achieved in understanding the biochemical roles of mitochondria. In addition to being the site of respiration and oxidative phosphorylation, mitochondria are also involved in several other important functions such as heme, lipid, and amino acid biosynthesis, the Krebs cycle, the urea cycle, fatty acid oxidation, and ion homeostasis [8]. However, the contributions of mitochondria to organismal development and inheritance have been only recently appreciated. The first mitochondrial disease was described in 1962 [9] but it would take another 26 years for the identification of a mutation in a mitochondrial disease [10,11]. Since then, the field of mitochondrial medicine has experienced rapid growth and hundreds of mutations affecting nuclear- or mtDNA-encoded MRC genes have now been identified. The clinical manifestations of mitochondrial diseases can be diverse and bewildering [12]. Mitochondria have been associated with aging [12,13], with neurodegenerative conditions such as Alzheimer’s [14], Parkinson’s [15], and Huntington’s diseases [16], and with cancer, such as hereditary paraganglioma [17–19]. Mitochondria are also intimately involved in apoptosis or programmed cell death, but other reviews have addressed this area [20–22].

1.5. Caenorhabditis elegans and mitochondria

C. elegans is a small, free-living nematode found in the soil. In the 1960s, Brenner [23] selected it as a simple model for his intensive studies of the genetics and development of the nervous system. We now understand more about the biology of C. elegans than of many other eucaryotes. Its life cycle is complex but short, taking about 3 days to complete at 25 °C [24]. A single self-fertilized adult hermaphrodite is capable of producing 300 progeny. Each embryo will develop, hatch, and proceed through four larval stages (L1–L4) before becoming an adult [24]. Under food deprivation or stress, an alternative developmental path, called the dauer larva, can be launched. Dauer larvae can survive for months in this stage and, in response to more favorable growth conditions, can exit dauer to resume maturation to the adult [25]. The adult C. elegans hermaphrodite is anatomically simple with only 959 somatic cells and yet contains multiple, highly differentiated tissue types such as neurons, muscles, intestine, and epidermis [26]. The nematode’s transparency has allowed a complete microscopic description of its invariant cell lineage [27,28].

A number of human disease genes have homologs in the nematode, and the nematode system has already yielded important insights into the function of some of these [29,30]. The availability of the complete mtDNA and

Fig. 1. The mitochondrial respiratory chain. The five complexes of the MRC (I–V) are localized to the mitochondrial inner membrane. Complexes I, III, and IV translocate H+ to the cytosolic side (top) of the inner membrane. Complex V uses the proton gradient to synthesize ATP in the matrix (bottom).
nuclear DNA sequences facilitates the rapid investigation of gene function using forward and reverse genetic approaches [31,32]. The construction of the physical map and its alignment to the genetic map eases the molecular analysis of mutations (http://www.wormbase.org, WS93 release, December 20, 2002). Specific loss-of-function phenocopies that offer a quick and easy way to study the relationship between genotype and phenotype can be produced experimentally by a phenomenon known as RNA interference, or RNAi [33]. The introduction of cloned genomic DNA fragments by microinjection into mutants to rescue their phenotype is also a powerful genetic tool [34].

The structure, metabolism, and bioenergetics of the nematode MRC are very similar to the mammalian counterpart [35]. Many pathways of intermediary metabolism, including the Krebs cycle, are also conserved in C. elegans [36,37]. In 1992, Okimoto et al. [31] sequenced the nematode mtDNA, which turned out to be slightly smaller than its human counterpart, missing the ATP8 gene and having a different gene organization and transcriptional pattern. The 12 mtDNA-encoded MRC polypeptides are highly conserved among eucaryotes (Table 1). A survey of the C. elegans nuclear genome reveals that the majority of mammalian nuclear-encoded MRC genes are also highly conserved (Table 1).

The conservation of mitochondrial function and the many technological and anatomical advantages offered by the nematode will contribute to our understanding of mitochondria in the life of complex organisms. In the following sections, we discuss a number of recent findings that highlight the importance of mitochondrial function in the nematode (Fig. 2).

2. Mitochondrial dynamics and genetics

2.1. Mitochondrial distribution and division

Mitochondria can be very dynamic structures capable of forming reticular or tubular networks [38]. They can colocalize and interact with certain cytoskeletal components [39], thus providing the first clues to the mechanisms of their distribution and movement. These interactions may serve to localize mitochondria to regions where there is an active demand for energy such as the synapses of neurons [40,41].

The distribution and positioning of mitochondria in the nematode is also regulated. In 1982, a mutation in the anc-1 (anchorage of nuclei abnormal) gene of C. elegans was found to cause mis-anchoring of nuclei and mitochondria in the hypodermis, allowing these organelles to float freely in the cytoplasm of syncytial cells [42]. The mitochondria were mostly spherical as opposed to tubular in shape and often clustered. Recently, the anc-1 gene was cloned and was found to encode a protein consisting of several large coiled regions, a nuclear envelope localization domain, and an actin-binding domain [43]. ANC-1 may thus connect nuclei and mitochondria to the actin cytoskeleton and the nuclear envelope. It remains to be determined whether the anchoring machinery is shared by different organelles or whether the disruption of nuclear positioning indirectly affects the distribution of mitochondria.

Mitochondrial division is required to regulate organelle numbers during cell division, cell differentiation, or in response to environmental changes. A role for mitochondrial fusion is more elusive. Labrousse et al. [44] identified the C. elegans dynamin-related protein, DRP-1, which affects both mitochondrial distribution and division. DRP-1 belongs to the dynamin family of proteins and has a characteristic N-terminal GTPase domain, a middle domain, and a C-terminal assembly domain that mediates protein–protein interactions [45]. RNAi of C. elegans drp-1 causes embryonic lethality and abnormal morphology and distribution of mitochondria, consistent with a role for drp-1 in mitochondrial segregation [44]. Using various dominant negative drp-1 mutations, it was shown that the protein is involved in the scission of the mitochondrial outer membrane while the inner membrane is unaffected, suggesting that mitochondrial division comprises two independent events. Overexpression of wild-type DRP-1 enhances the rate of division and mitochondrial fragmentation. Time-lapse photography reveals that DRP-1 is localized in spots where mitochondrial severing occurs and disappears after division, consistent with a role in division. DRP-1 may participate directly in the fission process or it may do so by recruiting other molecules of a scission machinery. DRP-1 may also serve to tether dividing mitochondria to specific locations within a cell by interacting with components of the cytoskeleton. It will be interesting to determine whether DRP-1 activity is adjusted to accommodate variable rates of cell division during growth.

2.2. Mitochondria and mtDNA copy number

The abundance of mitochondria in a cell as well as its mtDNA content are generally believed to be related to the energy requirements of that cell [46,47], but the precise relationships between these parameters remain obscure. Yet, the numbers of mitochondria and mitochondrial genomes in a cell are regulated [47] and can vary substantially between tissues.

Recently, we performed a comprehensive investigation of the developmental regulation of mitochondrial genome copy number in C. elegans [48]. The mtDNA copy number of a wild-type embryo is ~2.5 × 10^5 and remains unchanged through the early stages of development and into the L3 stage. It increases fivefold to 1.3 × 10^6 in the L4 stage and a further sixfold to 7.8 × 10^6 in the adult stage. The copy number increases coincide with sexual maturation; spermatogenesis begins at the early L4 stage while oogenesis begins in the young adult.

mtDNA content is closely associated with reproduction. Mutations in the germ line proliferation gene, glp-1, result in an almost complete absence of germ line cells while somatic
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development is normal [49]. The GLP-1 protein is related to the Notch family of transmembrane receptor proteins and regulates the mitotic division of germ cells. Germ cells are few in number in a young larvae but proliferation becomes significant after the L3 stage. The mtDNA copy number of an L4-stage glp-1 animal is about $7.0 \times 10^4$, which is about half of a wild-type L4 [48]. The glp-1 animal does not show a further copy number increase upon developing to the adult. We have concluded that the increase in mtDNA copy number observed in the wild type during development from the L3 to the adult stages has two components: a somatic component, which is glp-1 independent, occurs between the L3
and L4 stages (\(\sim 7.0 \times 10^4\) in the glp-1 L4), and a glp-1 dependent, germ line-associated component occurs between the L3 and adult stages (the difference between \(\sim 7.0 \times 10^4\) and \(1.3 \times 10^5\) occurs during development to L4 and the increase from \(1.3 \times 10^5\) to \(7.8 \times 10^5\) occurs during development to the adult).

The majority of the mtDNA copy number increase associated with the germ line is related to the production of oocytes [48]. The feminization or fem genes are needed during spermatogenesis in both males and hermaphrodites. A loss-of-function fem-1 mutation blocks sperm production in the hermaphrodite while oocyte production remains unaffected [50]. In contrast, a gain-of-function fem-3 mutation blocks oocyte production and results in excess sperm in the hermaphrodite germline [51]. The mtDNA copy numbers in a fem-1 L4 (\(1.4 \times 10^5\)) and in a fem-1 adult (\(8.0 \times 10^5\)) are comparable to the wild type situation. The mtDNA copy numbers of fem-3 mutants show a markedly different profile; the fem-3 L4 (\(1.7 \times 10^5\)) resembles the wild type, but its mtDNA content increases little in the adult (\(1.9 \times 10^5\)), suggesting that the majority of the organellar genomes are associated with the production of oocytes. In fact, the mtDNA copy numbers of the fem-1 oocyte (\(1.8 \times 10^4\)), the wild type embryo (\(2.5 \times 10^4\)), and the first three larval stages are similar. The mtDNA inherited through the oocyte supports the early developmental steps. Sperm production contributes little to the mtDNA content of the germ line.

The mtDNA content of *C. elegans* cells is considerably lower than the estimated values of \(10^3\) to \(10^4\) in most higher eucaryotic cells. *C. elegans* has an invariant cell lineage; the L1 and L4 larvae have about 560 and 1000 somatic cell nuclei, respectively. The \(\sim 2.5 \times 10^4\) copies of mtDNA in the wild-type L1 and the \(\sim 7.0 \times 10^4\) copies in the germ line deficient L4 correspond to averages of \(\sim 45\) to \(\sim 70\) mtDNA per somatic cell. It remains to be determined whether different somatic cells or tissues have distinct mtDNA copy numbers. For instance, tissues that are highly oxidative, such as muscles and neurons, might be expected to carry more mitochondria and mtDNAs. We have estimated that undifferentiated germ line cells and sperm have \(\sim 250\) and \(30–40\) copies of mtDNA, respectively. Does the low organellar genome content of the nematode reflect a diminished role for mitochondrial energy generation because of the hypoxic environment of the soil where it is normally found?

How the number of mitochondria changes during the course of nematode development has not been investigated. In normal somatic tissues, the number of mitochondria, the number of mtDNA copies, and the respiratory capacity of a cell correlate loosely with one another [47,52,53]. The correlation no longer holds in transformed cells [54] or in cells with mitochondrial dysfunction [55–57]. The increases in mtDNA copy numbers during nematode maturation are suggestive of a parallel increase in the number of organelles, although this has not been documented. Interestingly, increases in mitochondrial number and in the frequency of mitochondrial division upon shifting the temperature from 15 to 25 °C have been observed [44]. The increased number of organelles may represent an adaptation to higher metabolic activities. Consistent with this idea, oxygen consumption rates [58] and ubiquinone (Q9) contents of young adults [59] are doubled with such a temperature shift. It remains to be determined whether mtDNA content also changes with temperature.

**2.3. mtDNA inheritance, transmission, and maintenance**

Mitochondrial genetics differs from nuclear genetics in three aspects. First, nuclear genes follow a Mendelian pattern of inheritance, whereas mitochondrial genes are maternally inherited [60]. Second, the nuclear genome is
usually haploid or diploid while the mitochondrial genome is polyploid. Normally, only one form of mtDNA is present; this is a state called homoplasmy. Occasionally, two or more forms of mtDNA coexist within a cell in a state termed heteroplasmy, which is often associated with mtDNA mutations [61]. Third, unlike a diploid nuclear gene that can assume three states (homozygous wild type, heterozygous, homozygous mutant), mtDNA heteroplasy does not vary by discrete steps. The proportion of mutant mtDNAs can vary, increasing or decreasing by mitotic segregation as cells divide [62]. The factors controlling heteroplasmy are poorly understood but are under investigation [63].

Several observations suggest that the presence of mtDNA mutations is related to the aging process and to oxidative stress. The first deletions in the nematode mitochondrial genome were detected in an aging, wild-type population [64,65]. The deletions arose spontaneously and their frequency increased with age. Similarly, a wide array of mtDNA rearrangements had been detected in the skeletal muscle of elderly people [66]. Interestingly, the C. elegans age-1 (aging abnormal) mutant, which is more resistant to oxidative stress and lives twice as long as the wild type, exhibits a small but significantly lower rate of mtDNA deletions [65]. It is not certain whether these mtDNA deletions reduce the carrier’s fitness or whether they can be transmitted to the next generation.

A direct estimate of the nematode mtDNA mutation rate was obtained using a series of mutation accumulation lines maintained by single-progeny descent for over 200 generations [67]. There were 16 base substitution and 10 insertion–deletion mutations detected in over 770,000 bp of sequenced DNA, corresponding to a measured rate of mutation approximately 100-fold higher than previous indirect estimates. Four of the insertion–deletion mutations were predicted to introduce drastic changes to coding sequences; all four are homoplasmic or nearly so for the mutated genomes and have modest but significant reductions in fitness.

mtDNA inheritance and maintenance are most poorly understood for the heteroplasmic condition. The maternally inherited uaDf5 mutation in the C. elegans mtDNA is a large-scale deletion that removes a total of 11 genes, including genes for four MRC subunits and seven tRNA genes [68]. The mutation has been maintained in a stable heteroplasmy for over 100 generations in the absence of selection without the appearance of homoplasmic wild-type or mutant animals. While individual animals have between ~20% and ~80% uaDf5 mtDNA, the population average is ~60% and does not change as the animals develop. We have not observed any phenotype associated with the deletion. The stability of the heteroplasmic condition suggests that the mutated and wild-type mtDNAs do not segregate from each other. Stable heteroplasmy has also been observed in cybrids [69] and in a Drosophila subobscura mtDNA deletion mutant [70]. The D. subobscura mutation, also a large-scale deletion, shares many features with the uaDf5 line including a stable heteroplasmic state ranging from 50% to 80% mutant mtDNA, the absence of homoplasmic segregants, and the lack of a phenotype. The stable heteroplasm seen in worms and in D. subobscura is in contrast to the unstable heteroplasm seen in Holstein cows [71] and in crosses between D. mauritiana and D. melanogaster [72] where the segregation of mtDNA genotypes can occur within a single generation. Unstable heteroplasmies may result from inter-mitochondrial heteroplasmy (the different forms of mtDNA are present in distinct organelles), whereas stable heteroplasmies may be intra-mitochondrial (the mtDNAs are intermixed within an organelle).

The intergenerational stability of the uaDf5 heteroplasmy appears to be controlled by two opposing forces, one that increases the proportion of uaDf5 mtDNA when it is low, and a second, decreasing it when proportions are high [68]. At intermediate proportions, the forces are balanced and the uaDf5 levels in the offspring reflect the maternal state. Single, self-fertilized hermaphrodites with intermediate levels of uaDf5 mtDNA (~50–60%) have broods that form random, non-skewed distributions centered on the parental mean. However, hermaphrodites with more extreme proportions of uaDf5 mtDNA (~20% or ~80%) have broods with skewed distributions; the proportions of uaDf5 mtDNA in the offspring shift towards intermediate levels. The same forces may also account for the maintenance of a stable heteroplasm in the D. subobscura mtDNA deletion mutant.

What are the forces involved in maintaining a stable heteroplasmy and when do they act? It has been suggested deletion-containing mtDNA molecules may have a replicative advantage over the wild-type mtDNA molecules because they are shorter. In the case of the uaDf5 deletion, there does not appear to be a replicative advantage, since the proportion of uaDf5 is constant at ~60% through development from the L1 to the adult. This includes times of intense mtDNA replication from L3 to adult [48]. This is in contrast to the D. subobscura situation where an increase in heteroplasmy appears to correlate closely with the time of active mtDNA replication [73]. Thus, a replicative advantage due to size, if it exists, may be species- or even tissue-specific. Alternatively, a respiratory advantage may account for both forces in maintaining a stable heteroplasmatic state. The wild-type DNA may harbor an undetected mutation that would severely affect respiratory function. If this were the case, the uaDf5 mtDNA must be maintained to complement the undetected lethal mutation in the ‘wild-type’ genome; both genomes are needed to produce a fully functional MRC and homoplasmic with either genome may be severely compromising or lethal. Sequencing the ‘wild-type’ mitochondrial genome should address this possibility. Respiratory selection against homoplasm must operate prior to embryogenesis, possibly through the elimination of germ cells or oocytes with very high or very low uaDf5 contents, since there is no observed phenotype, including inviable embryos, associated with the uaDf5 mutation. Elimination of cells via
apoptosis has been observed in the C. elegans hermaphro-
dite germ line [74].

Heteroplasmic uaDf5 animals, although without a pheno-
type, have adjusted their mitochondrial genome contents.
Both male and female animals carrying the uaDf5 deletion
have approximately twice the number of mitochondrial
genomes as their wild-type counterparts [68]. The up-
regulation of the mtDNA copy number may in part com-
penstate for the effects of the mutation by enhancing the
production of functional mitochondrial transcripts and/or
encode in the replication or expression of the mtDNA. In contrast to
cytoplasmic cybrids derived from Kearns–Sayre syndrome
cells, which harbor large deletions in their mtDNA, that
maintain a constant mass of mtDNA [75]. A parallel
increase in mtDNA content has also been reported in the
heteroplasmic D. subobscura deletion strain [55], although
this up-regulation was not observed in a backcrossed strain with a similar level of heteroplasmy [76]. These observa-
tions suggest that mtDNA copy number regulation may
differ between organisms. Understanding the signals that
regulate mtDNA copy number and the mechanisms by
which regulation is achieved remain important challenges.

3. Mitochondrial diseases

3.1. mtDNA mutations

Surprisingly, all mutations in the nematode mitochondrial
genome have had little or no effect on the carriers (see
examples in Section 2.3). Animal mtDNAs are extremely
compact with few noncoding regions and, thus, most
mutations affect a coding sequence or a region involved in the
replication or expression of the mtDNA. In contrast to the
nematode situations, over 100 pathogenic point mutations
and 200 insertions, deletions, or rearrangements have been
identified in the human mitochondrial genome (MITO-
www.mitomap.org, 2002). Perhaps simple eucaryotes, like
the worm or the fly, can better tolerate deleterious mtDNA
mutations or have mechanisms to prevent their transmission.
It is also puzzling why mtDNA mutations have not been
reported from the large numbers of genetic screens for
mutations that affect motility, reproduction, development,
or other functions [77]. Clearly, the genetics of the mito-
chondrial genome in C. elegans still contains much to be
explored.

3.2. Nuclear mutations

For the majority of human mitochondrial diseases of
nuclear origin, the precise molecular defect is not known.
The first defect in a nuclear-encoded MRC gene, reported in
1995, was a point mutation in the SDHA gene encoding the
large or flavoprotein subunit (A subunit) of succinate
hydrogenase or complex II, which results in Leigh disease
[78]. Several pathogenic mutations in complexes I, II, and
IV have since been characterized [79]. Like mtDNA muta-
tions, the phenotypes of nuclear-encoded mutations are
diverse and tissue-specific.

With the availability of the complete genome sequence, it is
now possible to use a reverse genetic approach called
target-selected mutagenesis to identify mutations in genes of
choice [80]. The nuo-1 gene, which encodes the 51-kDa
active site subunit of the NADH-ubiquinone oxidoreductase
or complex I and contains FMN or flavin mononucleotide
and iron–sulfur cluster cofactors, is the first MRC gene to
have been targeted in this way [81]. The recovered mutation
nuo-1(ua1) is a deletion allele that has the first three of six
exons removed. It is likely a null mutation and is homozy-
gous lethal. Heterozygous mutant offspring of a hetero-
ygous hermaphrodite hatches and develops through two larval stages before arresting at the L3 stage prior to sexual
maturation. Gonad development is more severely affected
and is arrested at the earlier L2 stage. Mutant animals
exhibit a number of behavioral defects indicative of mus-
cular and/or neuronal defects, including reduced mobility,
impair feeding, and a slower defecation cycle. Mutations
in the human nuo-1 homolog, NDUFV1 (NADH–ubiqui-
none oxidoreductase flavoprotein subunit), result in myo-
clonic epilepsy, hypotonia, ataxia, Leigh syndrome,
leukodystrophy, and other neurological conditions [82,83].
In those cases, all probable null NDUFV1 mutations coexist
with point mutations that are likely hypomorphic, suggest-
ing that the gene is also essential in humans. Similarly, the
C. elegans atp-2 gene, which encodes the β-subunit of the
ATP synthase, has also been targeted for gene disruption
[81]. The atp-2(ua2) mutation deletes the first exon and part
of the second, completely removing the coding region for the
amino-terminal mitochondrial targeting sequence. Not
surprisingly, the atp-2(ua2) mutation is also lethal, produc-
ing a phenotype that is almost indistinguishable from the
nuo-1(ua1) mutation. The coq-3(qm188) mutation is a de-
letion in a methyltransferase-encoding gene of coenzyme Q
biosynthesis [84]. Heterozygous hermaphrodites (coq-3(+))
produce 1/4 homozygous coq-3 progeny that reach adult-
hood but are usually sterile. Those that are fertile have
extremely low brood sizes and their progeny die as L1
larvae. Interestingly, homozygous coq-3 mutants com-
plemented with a coq-3(+) transgenic array are normal, but loss
of the array produces an L2-stage arrest. In all three nuclear
mutants (nuo-1, atp-2, coq-3), a maternal effect suggests
that the mother deposits a product, perhaps mRNA, into the
oocyte, which supports development until zygotic expres-
sion can supply further needs.

The nuo-1(ua1), atp-2(ua2), and coq-3(qm188) muta-
tions indicate that a functional MRC is essential for viability
and larval development past the L3 stage. By extension,
other conditions or mutations that impair MRC function
may also lead to L3 stage larval arrest. Other phenotypes are discussed later (refer to Sections 3.3 and 4). The ~200 proteins involved in MRC biogenesis, which include mtDNA and nuclear DNA-encoded MRC subunits, components of the mitochondrial import apparatus, chaperones and assembly factors, and the mitochondrial replication, transcription, and translation machinery, may also cause lethality when their genes are mutated. Two further lines of evidence support a larval arrest phenotype for such mutations. First, clk-1 (biological clock abnormal) mutations, which disrupt the biosynthesis of ubiquinone, have a conditional lethal phenotype. When grown on a ubiquinone-deficient strain of E. coli, clk-1 mutants suffer an L2 stage developmental arrest [85]. The L2 arrest, as judged by gonad development, is remarkably similar to the nuo-1 and atp-2 induced arrests. Second, when MRC biogenesis is inhibited with ethidium bromide, an inhibitor of mtDNA replication [48], or with doxycycline or chloramphenicol, inhibitors of mtDNA translation [81], a quantitative and homogeneous developmental arrest as L3 larvae also results. An L2 or L3 stage larval arrest may therefore be a common outcome for mutations that produce MRC defects, suggesting that a genetic screen using this endpoint may be a fruitful way of isolating additional MRC gene mutations.

Why do worms with an impaired MRC arrest at the L2 or L3 stage? We believe that maturation to the L4 stage is associated with substantially increased energy demands. Three observations support this notion. First, aerobic metabolism, as measured by oxygen consumption, peaks at the L3 and L4 stages [86]. This is consistent with an observed decrease in activity of the glyoxylate cycle during the L1 stage and a shift towards respiration in the later larval stages [37]. Second, the mtDNA copy number increases fivefold between the L3 and the L4 stages [48]. Finally, the ATP-2 protein levels also increase significantly with development to the L4 stage (W. Tsang and B. Lemire, unpublished data). The increases in mtDNA copy number and in ATP-2 protein contents presumably reflect active synthesis of and demand for MRC components. The homogeneous and quantitative developmental arrest that results when MRC biogenesis is blocked suggests that the L3-to-L4 transition involves an energy-sensing mechanism that invokes a developmental checkpoint when an energy deficit arises. An energy sensor could respond to one or more metabolites, whose concentrations communicate information about the status of mitochondrial energy production.

Recently, a number of proteins capable of sensing the concentrations of metabolites that may reflect the mitochondrial energy status have been identified. The corepressor CtbP or carboxyl-terminal binding protein is a transcriptional regulator containing an NAD+/NADH binding motif important for development and cell cycle regulation [87]. CtbP binding to its partner proteins is dramatically sensitive to the levels of NADH, allowing it to serve as a redox sensor. SIRT3, a homolog of silent information regulator two or SIR2, is a conserved mitochondrial protein with a NAD+-dependent protein deacetylase activity that may sense the organellar or cellular redox state [88]. Unfortunately, the SIRT3 targets have yet to be identified, but members of the SIR2 family of proteins are NAD+-dependent histone deacetylases involved in chromosome stability, gene silencing, and cell aging [89,90]. Provocatively, a Salmonella enterica SIR2 homolog was recently shown to regulate metabolism by deacetylating a posttranslationally modified acetyl-coenzyme A synthetase, which is needed for growth on propionate and acetate [91]. The mammalian target of rapamycin (mTOR), which functions to regulate ribosome biosynthesis and cell growth, is a protein kinase that directly senses the intracellular concentration of ATP [92]. Interestingly, mutations of the mTOR homolog in C. elegans are lethal and result in an L3 developmental arrest [93]. The nematode mTOR homolog may well be the developmental or energy sensor that controls the transition from the L3 stage to the L4 stage. Thus, a developmental checkpoint is possibly regulated by a sensor protein that responds to the levels of ATP and/or the ratio of NADH/NAD⁺.

In addition to proteins, other molecules may act in energy sensing. Ubiquinone, in addition to its role as an electron carrier, functions as a signaling molecule. In E. coli, ubiquinone is a redox signal for the Arc regulatory system [94]. Observations with C. elegans clk-1 and coq-3 mutants suggest a non-mitochondrial site of action for ubiquinone in animal development. The C. elegans nuclear hormone receptor DAF-12 (dauer larva formation abnormal) is important for the developmental decision between reproductive growth and the alternate L3 stage called the dauer [95]. The DAF-12 ligand, a steroid hormone, is produced in response to environmental signals, including food availability, and may thus link development and energy production [96].

Other mutations in nuclear-encoded C. elegans genes (gas-1, mev-1, isp-1) have been identified in genetic screens for particular phenotypes. Mutations in these genes do not result in larval arrest and are discussed in Section 4.

3.3. RNAi and maternal contribution

RNAi is a process by which double-stranded RNA (dsRNA) interferes with gene expression. RNAi was first reported in C. elegans in 1998 and many features of the RNAi effect have since been described [33]. The dsRNA can be introduced by microinjection [33], by feeding the worms with bacteria engineered to produce dsRNA [97], by soaking the worms with a dsRNA solution [98], or by expressing constructs with inverted repeats that form double-stranded regions [99]. The RNAi effect is usually very specific and phenocopies the null allele of the targeted locus [100,101].

The RNAi technology has been applied to a number of MRC genes and has uncovered a strong maternal effect. When wild-type nematodes are fed with E. coli engineered
to produce *nuo-1* dsRNA or *atp-2* dsRNA, approximately 10% and 65% of the progeny arrest as embryos after gastrulation but prior to tissue differentiation, respectively [81]. Recent analyses of genes on chromosomes I and III in systematic RNAi screens have revealed that embryonic lethality is associated with many MRC genes [102,103]. The *nuo-1*(RNAi) or *atp-2*(RNAi) phenotypes are more severe than the corresponding null alleles, suggesting that *nuo-1*(+) or *atp-2*(+) genes in the heterozygous hermaphrodite contribute to the survival of the respective homozygous mutant offspring. A maternal contribution of mRNA or protein supports development in the homozygous offspring up to the L3 stage. This maternal contribution of metabolic potential to the embryo, made evident with RNAi technology, appears to be a conserved event for mitochondrial genes. Mutations in the *C. elegans dif-1* (differentiation abnormal) gene have a maternal-effect, embryonic lethal phenotype [104]. The DIF-1 protein belongs to the family of mitochondrial carrier proteins, has 56% identity to the human carnitine-acylcarnitine translocase, and is thought to have a role in embryonic energy metabolism. *dif-1* homozygotes derived from a heterozygous mother are completely wild-type. However, the progeny of a homozygous *dif-1* hermaphrodite are unable to complete the early steps of embryogenesis. Likewise, the *clk-1* mutation exhibits a maternal effect; the slow development and the long-life phenotypes are only seen in the *F₂* generation and beyond [105]. Maternal effects for mitochondrial genes have also been observed in mice. The losses of TFAM (transcription factor A, mitochondrial) [106], cytochrome c [107], or CLK-1 [108] in mice all result in embryonic lethality, although the earliest steps of development, which are presumably supported by maternal products, are apparently normal.

3.4. Tissue specificity

The tissue-specificity of mitochondrial diseases has been attributed to at least two factors: the level of dependence of a tissue for MRC-generated ATP, and differences in the levels of heteroplasmacy when mtDNA mutations are present [109]. Perhaps the best studied examples of tissue-specific mitochondrial effects are with *Tfam*-deficient mice [106,110–113], but discussion of these results is beyond the scope of this review.

The tissue-specificity of an MRC mutation has also been addressed in the nematode system by utilizing a technique called mosaic analysis [114,115]. In mosaic animals, some cells are genotypically wild-type, while others are genotypically mutant due to the infrequent, spontaneous loss of a complementing transgenic array introduced by transformation. The array contains the complementing gene as well as a gene for a reporter molecule such as the green fluorescent protein [116]. When the transgenic array is present in a mutant cell, the cell is fluorescent and is genotypically wild-type. In the absence of fluorescence, the cell is assumed to have lost the transgenic array and be genotypically mutant.

The mosaic expression of the *atp-2* gene has been investigated [117]. The *atp-2*(ua2) mutation results in an arrest at the L3 stage of development, but a complementing transgenic array present in all cells renders the animals genetically and phenotypically wild-type. Frequently, the complete loss of the array occurs and the animals become identical to *atp-2*(ua2) mutants. However, rare mosaic animals with a spontaneous loss of the transgenic array from certain cell lineages early in development can be isolated by their incomplete fluorescence patterns. *atp-2* mosaic animals with array losses in some of their neuronal, pharyngeal, or hypodermal cells, or in all their intestinal cells, can occasionally develop past the L3 stage [117]. Mosaics in which some or all of the body muscle cells have not inherited the transgenic array invariably result in L3 developmental arrest. Although the array is required by all tissues for optimal development, transgene function in body muscle is closely correlated to larval development from the L3 to the L4 stage. Muscle may rely heavily on the MRC for energy generation or it may play a pivotal role in developmental regulation.

Animal development appears to be an all-or-none phenomenon. In any single *atp-2* mosaic, either all or none of the cells or tissues develop past L3, even though a mixture of genotypically wild-type and mutant cells is present [117]. The *atp-2* gene functions in a cell nonautonomous manner; a one-to-one correspondence between a cell’s genotype and its phenotype is absent. Cell nonautonomy suggests that development beyond the L3 stage is controlled by a global mechanism in which all cells somehow reach a consensus decision to continue development or not.

4. Aging and life span

4.1. Short life span

Several genetic studies indicate that MRC function is intimately linked to life span determination in *C. elegans*. Mitochondria are the major endogenous source of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals [118,119]. Superoxide anions are constantly generated at low levels by the MRC and can be converted to hydrogen peroxide by the mitochondrial manganese superoxide dismutase. The hydrogen peroxide can in turn be detoxified to water by glutathione peroxidase. Reduced metal ions can catalyze the conversion of hydrogen peroxide to the hydroxyl radical, the most reactive and damaging of all ROS. The “free radical theory of aging” proposed by Harman [120] states that increased respiration will lead to increased ROS production and damage to cellular lipids, proteins, and nucleic acids. Oxidative damage inflicted by ROS is believed to be a determinant of premature aging.

The *mev-1*(kn1) mutation, a missense allele in the *cyt-1* gene encoding the *cytochrome b* subunit of complex II, was
originally isolated in a mutant screen for hypersensitivity to methyl viologen (paraquat), a compound that increases ROS production [121]. Hyperoxia is toxic to the mutant; its life span decreases rapidly with increasing oxygen concentrations [122]. Complex II activity is severely reduced, the production of lactate and superoxide anions are elevated, and glutathione content is decreased [123]. mev-1 mutants accumulate markers indicative of an accelerated rate of aging such as protein carbonyl derivatives [124,125] and are hypermutable, especially at higher oxygen concentrations [126]. The mev-1 phenotype has been primarily attributed to the increased generation of ROS at complex II, either through the release of electrons directly to oxygen or through the premature release of ubisemiquinone radicals [123]. Curiously, ROS scavengers such as EUK-8 and EUK-134, compounds that exhibit both catalase-like and superoxide dismutase-like activities, can dramatically increase the life span of wild-type worms under certain culture conditions [127]. They can also restore the short-lived mev-1 mutants to a normal life span, demonstrating that endogenous mitochondrial ROS production in this mutant strain is the major cause of accelerated aging [127]. However, the dramatic results achieved with the scavengers have been questioned [128]. The role of complex II in mitochondrial energetics and in oxygen sensing has recently received increased attention with the identification of numerous complex II mutations leading to the development of paragangliomas, tumours in the head and neck region [129].

The gas-1(fc21) (general anesthetic sensitive) mutation, originally isolated in a screen for volatile anesthetic hypersensitivity, affects the 49-kDa subunit of complex I and profoundly impairs complex I activity [130]. Like the mev-1 mutant, gas-1 animals are also hypersensitive to free radical damage and hyperoxia, and have a shortened life span [126,130]. In contrast to the mev-1 mutant, the gas-1 mutant is not hypermutable and does not overproduce superoxide anions [126]. GAS-1 is most abundant in mitochondria of developing embryos, without affecting cell number, and reduced ATP levels, respectively, extended life span, reduced the size of the adult with arrested gonad development. This phenotype suggests that the compromised mitochondria cause a metabolic shift from respiration towards the glyoxylate cycle as seen in dauer larvae [36,37].

4.2. Long life span

MRC mutations can also promote longevity. A mutation in the isp-1 gene encoding the iron–sulfur protein of complex III slows embryonic development and doubles the maximum life span [132]. isp-1 mutants have a reduced rate of oxygen consumption, indicating that electron transport along the MRC is compromised. A spontaneous partial suppressor of the slow rate of embryonic development in isp-1 animals was isolated and determined to be a point mutation in the mitochondrial encoded cytochrome b gene. The ctb-1(qm189) suppressor mutation is maternally inherited, exists in a stable homoplasmic state, and has no independent phenotype but increases respiration and partially suppresses the slow behavioral and reproductive features without affecting the extended life span of isp-1 animals [132]. The ctb-1 mutation appears to enhance the low rate of electron transport of the isp-1 mutant. The isp-1, ctb-1 double mutant displays increased resistance to paraquat-induced oxidative stress. Mutations in the daf-2 gene, which affects the dauer pathway, result in an elevated expression of superoxide dismutase and an increased life span. daf-2 mutations do not further increase the life span of the isp-1 mutant, suggesting the increases in life span operate through a similar pathway or mechanism in both mutations.

A probable null mutation (lrs-2(mg312)) in the gene encoding the mitochondrial leucyl-tRNA synthetase markedly extends life span (twofold at 20 °C) [133]. This synthetase is needed to charge mitochondrial tRNAs for the translation of the 12 mtDNA-encoded MRC genes. [133]. Mutants have disorganized, swollen, and sometimes fused mitochondria, possibly as a result of impaired oxidative phosphorylation. lrs-2 mutants do not arrest development but rather develop more slowly into L4-sized, sterile adults with arrested gonad development. This phenotype suggests that the mg312 allele does not impair oxidative phosphorylation to the same extent as other MRC mutations such as nuo-l(uil1) and atp-2(uil2). The mg312 allele may not be a null or other synthetases can partially compensate for the lost activity of the lrs-2 gene product. lrs-2 mutants are hypersensitive to paraquat, suggesting that the extended life span is not due to lower mitochondrial ROS production or to increased defenses against ROS. Rather, the authors speculate that the compromised mitochondria cause a metabolic shift from respiration towards the glyoxylate cycle as seen in dauer larvae [36,37].

In a systematic RNAi screen for gene inactivations that increase life span, genes important for mitochondrial function formed the most prominent group, accounting for 15% of all longevity genes [133]. These genes encoded subunits of the MRC, mitochondrial carrier proteins, a subunit of the mitochondrial ribosome, and a protein involved in the biogenesis of the MRC [133]. The RNAi treatments lowered ATP levels, reduced respiration, and altered organellar morphologies. For other genes encoding mitochondrial functions, lethality (L2, L3, or L4 arrest) or sterility was observed, suggesting that life span extension might result from more moderate losses of respiratory function [133]. A second systematic RNAi screen also emphasizes the connection between MRC function and aging [134]. RNAi-mediated inactivation of the nuo-2, cyc-1 (cytochrome c reductase), cco-1 (cytochrome c oxidase), and atp-3 genes encoding subunits of complexes I, III, IV, and V, respectively, extended life span, reduced the size of the adult without affecting cell number, and reduced ATP levels. Interestingly, exposure to antimycin A, a complex III inhibitor which should reduce rates of respiration, also
promotes longevity [134]. Unexpectedly, life span extension only occurred when RNAi treatments were employed during worm development prior to reaching the adult stage. Furthermore, when *cco-1* RNAi treatment imposed throughout development is removed during adulthood, *cco-1* mRNA content increases to almost normal levels, but this does not lead to a concurrent restoration of ATP levels. Respiratory chain activity established earlier during development permanently limits ATP production, even when the original impairment imposed by RNAi treatment is removed. Thus, *C. elegans* may possess a regulatory system that sets the rate of respiration during development and maintains this rate throughout the animal’s life, thus affecting growth rate, body size, and life span [134].

Mutations in the *clk-1* gene, which encodes a mitochondrial protein with a high degree of similarity to yeast Coq7p, disrupt the biosynthesis of ubiquinone and result in significant life span extension [105,135]. Timing is also deregulated in *clk-1* mutants; they exhibit a lengthening of embryonic and post-embryonic development, an extended cell cycle period, a longer defecation cycle, and reduced mobility, pharyngeal pumping rates, and brood sizes. Overexpression of CLK-1 in a wild type background accelerates aging [136], suggesting that besides ubiquinone synthesis, the protein regulates developmental and behavioral processes. In contrast to *C. elegans* clk-1 mutants, which are healthy, yeast *COQ7* mutants are respiration-deficient [137,138]. How do *clk-1* mutants grow and respire with apparently normal rates of respiration? The answer lies in the culture conditions; the nematodes are routinely grown on normal rates of respiration? The answer lies in the culture conditions; the nematodes are routinely grown on a diet of *E. coli*. Ablation of the somatic reproductive system [134]. Ablation of the somatic reproducible.

Mitochondria from *clk-1* animals do not contain detectable levels of Q8, the main form of ubiquinone in wild-type *C. elegans*, but instead accumulate demethoxy-Q (DMQ9), an intermediate in Q synthesis [59,139]. The precise role of DMQ9 in mitochondrial function and in development is controversial. DMQ9 cannot replace Q8 during development; *clk-1* animals remain arrested as L2 larvae unless supplied with a Q-replete diet [59]. Surprisingly, *clk-1* mitochondria respire at near normal rates [136,139] and these findings have been used to postulate a nonrespiratory developmental role for Q. The levels of another quinone species, rhodoquinone (RQ9), are also elevated in *clk-1* mutants [59,85] and the normal respiration rates may reflect the use of RQ9 or diet-derived Q8 [59]. RQ9 may also be important in anaerobic respiration. In parasitic helminths such as *Ascaris suum*, RQ9 functions as an electron carrier between complex I and fumarate reductase, allowing fumarate to serve as the terminal electron acceptor instead of oxygen [140,141]. Fumarate reductase and succinate dehydrogenase or complex II are closely related enzymes that catalyze the transfer of electrons between succinate/fumarate and quinones/quinols, although in opposite directions. In *C. elegans*, there are three loci encoding flavoprotein subunits; two are closely related to succinate dehydrogenases, while the third is more closely related to fumarate reductases (Table 1). The presence of RQ9 in *clk-1* mitochondria raises the possibility that *clk-1* mutants may respire with fumarate as terminal acceptor [142].

How do *clk-1* mutations and the resultant alterations in quinone compositions lead to the observed *clk-1* phenotype, which includes the increase in life span? The biochemical data suggest that decreased energy production may not be responsible for all aspects of the *clk* phenotype since *clk-1* animals have ATP levels comparable to the wild type [136,143]. However, the validity of using ATP levels as an indirect measure of metabolism has been questioned [144]. Genetic suppressors that accelerate the slow defecation cycle of *clk-1* mutants can do so without affecting other aspects of the *clk* phenotype [145,146]. The existence of these suppressors makes it unlikely that the *clk-1* slow defecation cycle is due to altered quinone levels; it seems improbable that the suppressors could restore Q8 synthesis only to the set of cells that controls the timing of defecation. These observations further support the hypothesis that CLK-1 or the downstream product of its action, Q, has an additional role beyond quinone synthesis and energy production and that the accumulated DMQ9 intermediate in *clk-1* mutants is unable to substitute for Q in its other physiological functions, which may include regulatory roles [94,146,147]. Curiously, an eat-2 (*eating abnormal*) mutation, which disrupts pharyngeal function and results in a low caloric intake, can increase life span through the same pathway(s) in which *clk-1* works; a *clk-1*, eat-2 double mutant does not result in any further extension of life span [148]. Therefore, *clk-1* mutations, like caloric restriction, may promote longevity by lowering metabolic rates and/or the ROS production of mitochondria. In contrast to the eat-2 situation, the effects of *clk-1* and *daf-2* mutations on life span are additive, suggesting that *clk-1* and *daf-2* increase longevity via two different mechanisms; the *daf-2* longevity is attributed to increased stress resistance [149].

The *clk-1* mutants suggest that longevity is linked to a decreased level of Q8. Larsen and Clarke [142] tested the hypothesis that withdrawal of dietary Q8, the form present in *E. coli*, would lead to a prolonged life span and observed a 60% increase in the life span of wild-type worms. The longevity of *daf-2* mutants could also be further increased on the Q-less diet. They attributed the longer life span of Q withdrawal and of *daf-2* to parallel pathways that converge in the mitochondrion to reduce the generation of ROS or increase defenses against them [142]. The electrochemical properties of DMQ9 may also contribute to life span extension through a reduced production of ROS in *clk-1* mutants [139]. Interestingly, the longevity, but not the slow phenotypes, of *clk-1* mutants is dependent on the presence of the reproductive system [134]. Ablation of the somatic mitochondria.
gonad precursor cells suppresses the life span extension caused by the clk-1 mutation [134].

In addition to isp-1 and clk-1, mutations in three other genes (gro-1, nuo-1, atp-2) can also lead to an extended life span. The phenotype of gro-1 (growth rate abnormal) mutants shares a number of common features with that of clk-1 including a maternal effect, an increased life span, a brood size reduction, and deregulated developmental and behavioral rates [150]. GRO-1 is an enzyme called isopentenylpyrophosphate: tRNA transferase, which functions to modify a subset of mitochondrial tRNAs and thus could affect translation of mtDNA encoded genes. gro-1 and clk-1 interact genetically and thus may act through same genetic pathway as caloric restriction [149]. Interestingly, nuo-1 and atp-2 mutants can also live significantly longer and have reduced mobility, reduced pharyngeal pumping rate, and a longer defecation cycle [81]. Although these mutants shares a number of common features with that of clk-1, mutations in three other genes (gro-1, atp-1, atp-2) can also lead to an extended longevity may work via the same mechanisms as isp-1, clk-1, or gro-1.

5. Conclusion

Mitochondria are fascinating organelles long recognized for their role in metabolism but more recently acclaimed for their pivotal involvement in diseases, apoptosis, organismal development, and life span determination. Animal models are invaluable for understanding and elucidating the molecular bases of the pathophysiologies stemming from mitochondrial dysfunction. The nematode, C. elegans, has a particularly prominent place in aging research, but also more recently in elucidating the still elusive mechanism of mitochondrial communication with the cellular control center in the nucleus. Genes that control mitochondrial attachment, division, and morphology have now been identified in C. elegans. Elaboration of the factors and processes involved in mitochondrial biogenesis, maintenance, and genetics is still an emerging field but the tools and opportunities furnished by the nematode system promise rapid progress.

Mitochondria influence and possibly control the rate of aging. Increased ROS production associated with mitochondrial dysfunction can precipitate premature aging and shorten life span. In contrast, caloric restriction and increased stress resistance can prolong life span. Integrating the contributions of ROS production, defense mechanisms against ROS, respiration rates, and ATP concentrations, which are all focussed in the mitochondrion, to organismal health and life span poses a considerable but exciting challenge. We believe that the nematode system will continue to generate significant advances in our understanding of the eucaryotic cellular powerhouse.

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References
