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Caloric restriction and lifespan: a role for protein turnover?

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Abstract

Oxidative damage to cellular macromolecules has been postulated to be a major contributor to the ageing of diverse organisms. Oxidative damage can be limited by maintaining high anti-oxidant defenses and by clearing/repairing damage efficiently. Protein turnover is one of the main routes by which functional proteins are maintained and damaged proteins are removed. Protein turnover rates decline with age, which might contribute to the accumulation of damaged proteins in ageing cells. Interestingly, protein turnover rates are maintained at high levels in caloric restricted animals. Whether changes in protein turnover are a cause or a consequence of ageing is not clear, and this question has not been a focal point of modern ageing research. Here we survey work on protein turnover and ageing and suggest that powerful genetic models such as the nematode *Caenorhabditis elegans* are well suited for a thorough investigation of this long-standing question.

Keywords: Ageing; *Caenorhabditis elegans*; Oxidative stress; Protein turnover; Translational regulation

1. Damage to proteins occurs during ageing

One of the clear themes that has emerged from several lines of ageing research is that oxidative stress is a major factor in ageing and in cellular senescence (recently reviewed by Finkel and Holbrook, 2000). Mitochondrial and cytoplasmic production of hydrogen peroxide, superoxide free radicals and hydroxyl free radicals can cause substantial modifications of DNA, lipids and protein (Stadtman, 1992). That these alterations result in a cumulative macromolecular damage that contributes to senescent decline is the foundation of the free radical theory of ageing originally outlined by Harman in 1957 (Harman, 1988). Key findings in support of this hypothesis include that generation of reactive oxygen species (ROS) is highly correlated with longevity in many species (Ku et al., 1993), that transgenic lines including increased dosage of anti-oxidants proteins such as glutathione reductase, Cu^{2+}–Zn^{2+} SOD and catalase can have extended lifespan (Mockett et al., 1999; Orr and Sohal, 1994; Sun and Tower, 1999; Parkes et al., 1998), and that an SOD/catalase mimetic compound can extend lifespan in the nematode *Caenorhabditis elegans* (Melov et al., 2000). Further support is provided from studies of

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the nematode *C. elegans* dauer larvae and *age-1* mutant strains, both of which show increased lifespan and have increased levels of SOD that could protect from macromolecular damage, thus contributing to lifespan extension (Larsen, 1993; Vanfleteren and De Vreese, 1995).

2. Protein modifications that accompany ageing

Several protein modifications accumulate in ageing (Rothstein, 1975, 1979, 1989; Stadtman, 1995). The most widely studied oxidative stress-induced modification is carbonyl addition. Carbonyl formation can occur through a variety of mechanisms including direct oxidation of certain amino acid side chains and oxidation-induced peptide cleavage. Such modifications normally mark enzymes for degradation by cytosolic neutral alkaline proteases or the proteosome. Recent work in *Escherichia coli* suggests that carbonyls can also be added to misfolded proteins in the absence of oxidative damage (Dukan et al., 2000). Whatever the origin, the pool of modified enzymes increases in size during ageing and in various pathological states (Rothstein, 1989; Stadtman, 1992).

Although all proteins are potential targets for carbonylation, the instability of free radicals suggests they should primarily attack macromolecules close to the site at which they are generated. Mitochondria are the major cellular source of free radicals with production occurring during electron transport in complex I (NADH dehydrogenase) and complex III (coenzyme Q). Interestingly, mitochondrial aconitase, an enzyme in the citric acid cycle, is a prevalent target for carbonyl modification during ageing in the housefly with loss of specific activity paralleling the extent of damage and shortened lifespan under hyperoxia (Yan et al., 1997). However, not all reported major targets for carbonylation are mitochondrial proteins. For example, in rat kidney the age-related increase in the protein carbonyl content includes prevalent changes in serum albumin (Goto et al., 1999). In nematodes, vitellogenin, an abundant egg-yolk protein that is expressed late into lifespan is one of the major carbonylated proteins (Goto et al., 1999). Thus, diverse proteins may be subjected to carbonyl addition, with most easily detected being highly abundant proteins.

In addition to generating oxidizing free radicals, metabolism can produce other byproducts including glyoxal and methylglyoxal, both of which can contribute to advanced glycation end-product formation that may contribute to the ageing phenotype (Golubev, 1996). Proteins can also suffer additional modifications during physiological processes such as racemization, isomerization and deamination (reviewed by Martin et al., 1996; Stadtman, 1988; Fujii et al., 1999; Cloos and Fledelius, 2000). Such modifications can have mild to devastating effects in protein function. Accumulation of these modified forms of proteins could contribute to senescent decline.

3. Aberrant or modified proteins accumulate during ageing

Why do modified inactive proteins accumulate in ageing? A combination of increased oxidative damage in old cells and a dramatic decline in protein synthesis and degradation appear to be the major factors (Fig. 1). As damaged mitochondria deleted for DNA accumulate, dysfunctional mitochondria may contribute to elevated levels of ROS (Brand, 2000; Melov et al., 1994, 1995a,b; Melov, 2000). Also, an age-related decline in anti-oxidant defense proteins such as SOD and catalase has been reported in several species (Rosenberger, 1991; Larsen, 1993; Mo et al., 1995; Oh-Ishi et al., 1995).

A decline in protein synthesis and degradation is a well-characterized feature of many animal systems, ranging from nematodes closely related to *C. elegans* (Sharma et al., 1979; Reznick and Gershon, 1979; Prasanna and Lane, 1979) to mammals (Rattan and Clark, 1996). This decline in protein turnover has been suggested to be a major contributor to the accumulation of modified proteins that accompanies ageing (Rattan and Clark, 1996; Stadtman, 1988, 1992).

4. Effects of ageing on protein synthesis

4.1. Protein synthesis is a highly complex process

Translation of an mRNA molecule into a protein product is an ordered and conserved process that involves three major events—initiation, elongation and chain termination. Numerous protein factors participate in each translation step (Fig. 2; for a recent review see Ibba and Soll, 1999). During initiation, the initiation complex that will scan for and recognize the initiator codon forms by association of the mRNA template with the small ribosomal subunit, numerous initiation factors and the methionine-charged methionyl-tRNA. During elongation, the actual synthesis of the peptide chain takes place on a fully assembled ribosome that reads through mRNA and uses amino acid-charged tRNAs to catalyze polypeptide extension. Chain termination concludes mRNA translation when a stop codon is encountered.

4.2. The fidelity of protein synthesis is maintained during ageing

All aspects of protein synthesis are tightly regulated and are executed with exquisite accuracy, which ensures the highest possible fidelity for the produced protein products. Although at one time the idea that mutationally induced production of aberrant proteins is a consequence of age (error catastrophe hypothesis; Goel and Ycas, 1976), several studies indicate that the fidelity of protein synthesis does not markedly decline with age. For example, a probe for age-related decline in the fidelity of tyrosine-tRNA charge by rat liver tyrosyl-tRNA synthetase failed to reveal a significant

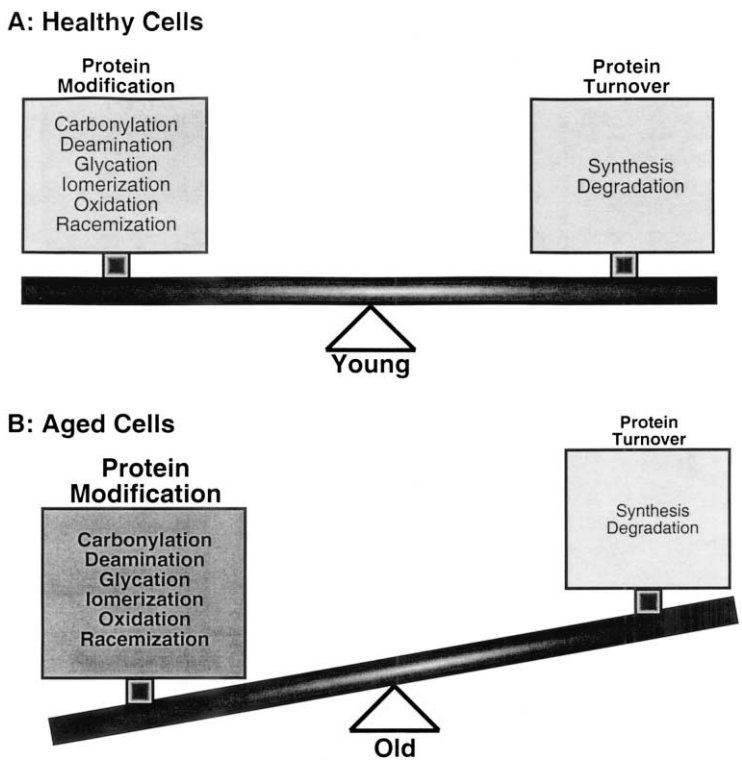

Fig. 1. Protein modification and turnover. The delicate balance between detrimental protein modification and protein turnover that exists early in life is tipped in favor of deleterious protein modification during late stages of life. Protein turnover cannot keep up with ever increasing accumulation of damaged proteins. The rate at which a protein pool is refreshed at any given point in time is determined by the rate of protein synthesis and protein degradation at that particular point.

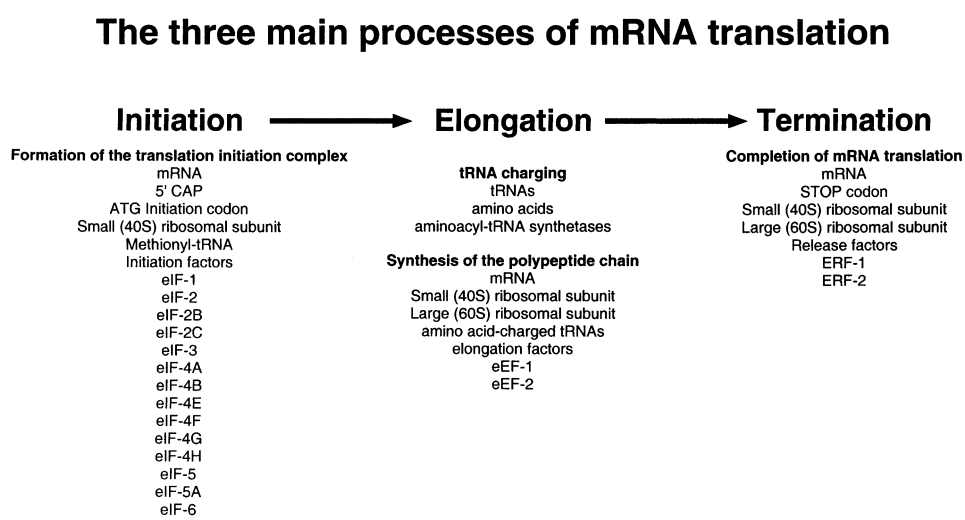

Fig. 2. mRNA translation. The three steps in the translation of an mRNA: initiation (A), elongation (B) and termination (C). The key translation factors involved in each step are indicated.

difference (Takahashi and Goto, 1988) that might result in misincorporation of tyrosine. In cultured human fibroblasts, there is no correlation between translational error frequency, donor age, or maximal lifespan in vitro (Harley et al., 1980; Goldstein et al., 1985). Investigations of age-correlated errors in specific proteins such as hemoglobin also failed to support that aberrant proteins are synthesized in older cells (reviewed by Morrow and Garner, 1979). Taken together, such studies suggest that damaged proteins, rather than genetically mis-specified or -produced aberrant proteins, are the primary candidate factors in ageing.

4.3. Protein synthesis rates decline with ageing

It is well documented that protein synthesis rates decline with age in a variety of organisms (Makrides, 1983; Van Remmen et al., 1995). For example, effects of ageing on protein synthesis have been measured as a lower capacity of rat microsomal preparations to produce proteins (Moldave et al., 1979; Webster and Webster, 1982; Vargas and Castaneda, 1983).

The levels of actively translating polysomes (large polysomes) have been found to decline with age in several organisms. For example, when the content and size distribution of membrane-bound and free polyribosomes in mouse liver was measured during ageing, old animals tended to show an increase in small polysomes and a decrease in large polysomes. This is consistent with a reduction in the rate of translation (Makrides and Goldthwaite, 1984). Supporting evidence in adult *Drosophila melanogaster* shows that polyribosome levels exhibit a marked, age-related decrease (Webster et al., 1981; Webster and Webster, 1983). In *Physarum polycephalum*, a reduction in the efficiency of utilization of mRNA for translation as the age of the mRNA population increased was demonstrated by measuring the proportion of poly(A)+ mRNA present in the polysomal fraction as compared with post-polysomal material (Brown and Hardman, 1981). Biochemical data (Rattan and Clark, 1996), as well as micro-array expression assay data (Lee et al., 1999), correlate lowered protein synthesis rates with senescent decline (Table 1). The totality of the evidence suggests a decline in protein synthesis with increased age.

Interestingly, mitochondrial protein synthesis activity also declines markedly with age. The rate of mitochondrial protein synthesis decreased approximately 35% in 24-month-old rats compared to the 6-month-old rats (Hudson et al., 1998).

4.4. Molecular mediators of the age-related protein synthesis decline

The activities of specific translation factors have been reported to decrease with age and could be the major effectors of the decline of protein synthesis rates.

4.4.1. Translation initiation factors

The activity of brain elF-2, as well as that of other eukaryotic initiation factors that contribute to the binding of initiator aminoacyl-tRNA to ribosomes (Fig. 2), have been reported to decrease with age and to decline in parallel to the decrease in total protein synthesis in rat brain (Vargas and Castaneda, 1983).

4.4.2. Translation elongation factors

A few specific elongation factors have been implicated in the age-related decline in protein synthesis. In *Drosophila melanogaster*, peptide chain elongation rate decreases markedly with age. Of the three reactions (binding, translocation and release) involved in peptide chain elongation, it is the binding of aminoacyl-tRNA to ribosomes that is most diminished with age, and the decrease parallels that of the decrease in peptide chain elongation and overall protein synthesis (Webster and Webster, 1982). Effects may be principally due to lowered activity of EF-1, which facilitates the GTP-dependent binding of aminoacyl-tRNA to the A-site of the ribosome (Webster and Webster, 1983). The selective disappearance of translatable poly(A) + RNA for EF-1 precedes a decline in EF-1 levels and might constitute the molecular basis for the decline in overall protein synthesis in ageing *D. melanogaster* (Webster and Webster, 1984). Interestingly, it was initially reported that transgenic *D. melanogaster* flies carrying an additional copy of the EF-1 alpha gene under control of a heat-inducible promoter have an extended lifespan (Shepherd et al., 1989). However, subsequent studies (Shikama et al., 1994) failed to verify these results, thus uncoupling lifespan extension from EF-1 overexpression in *Drosophila*.

EF-1 activity has also been implicated in the age-related changes in protein synthesis in mammals. In liver and brain of 30-month-old rats EF-1 activity has been measured to be 30–40% lower than in 3-month-olds (Moldave et al., 1979). The activity of brain EF-1 was found to decrease exponentially with age and to decline in parallel to the age-dependent decrease in total protein synthesis in both mice and rats. EF-1 derived from young brains functioned as a rate-limiting component in polypeptide synthesis in previously saturated adult systems. The data sug-

Table 1
Ageing-related increases (denoted by +) and decreases (denoted by −) in gene expression in the mouse gastrocnemius muscle

Change in Age (fold)	Gene	Function	Reversal
+3.6	Ypt 1/Ras-related GTP binding protein	Protein trafficking	C
+3.5	Heat shock 27 kDa Protein	Chaperone	C
+3.4	Heat shock 71 kDa protein	Chaperone	C
+2.2	Protease do precursor	Protease	C
−2.9	EF-1-gamma	Protein synthesis	63%
−2.6	20S Proteasome subunit	Protein turnover	44%
−2.2	Ubiquitin thiolesterase	Protein turnover	C
−2.1	HSP70	Chaperone	N
−2.1	26S Proteasome component TBP1	Protein turnover	C
−2.1	Unp ubiquitin specific protease	Protein turnover	N
−2.0	Rhodanese	Mitochondrial protein folding	C
−1.7	Proteasome Z subunit	Protein turnover	C

The influence of caloric restriction (CR) on the increased expression with age of specific genes was either complete (C; greater than or equal to 90%), none (N) or partial (greater than or equal to 20%, percent effect indicated). Data in the table are taken from Lee et al., 1999

gest that brain EF-1 has a critical modulatory effect on total brain protein synthesis (Vargas and Castaneda, 1981).

The activity of elongation factor 2 (EF-2), which promotes the GTP-dependent translocation of the nascent protein chain from A-site A to P-site of the ribosome, was also found to undergo age-related changes, in mouse and rat liver (Takahashi et al., 1985). This observation further supports the idea that a decrease in translational activity in old animals is due, in part, to alterations of protein components of the translation machinery.

5. Effects of ageing on protein degradation

The term 'protein turnover' describes both protein synthesis and degradation, which appear tightly coordinated. The essential structural and catalytic roles of proteins in the cell are the basis of a long-standing interest in the effects of ageing on protein turnover (reviewed by Ward, 2000). A general decline in gene expression, translation and transcription has been observed to occur with increasing age in a wide variety of organisms and tissues, yet surprisingly, the levels of most enzymes and proteins remains relatively constant with increasing age. Thus one would predict that a decline in gene expression should be accompanied by an age-related decline in protein degradation. Indeed, this appears to be the case. Biochemical studies (Rattan and Clark, 1996), and micro-array expression assays (Lee et al., 1999), correlate lowered protein degradation capacities with senescent decline (Table 1).

5.1. Decline in proteosome-mediated protein degradation

In whole brain homogenates of old mice the amount of ubiquitin (Ub)–protein conjugates increases, suggesting inefficient protein degradation or increased damage with age (Ohtsuka et al., 1995). Recent studies have demonstrated that the activity of the cytosolic proteasomal system declines dramatically during the proliferative senescence of human fibroblasts. The decline in the activity of the proteasomal system occurs in both post-mitotic ageing and proliferative senescence and results in an increased half-life of oxidized proteins (Sitte et al., 2000a). Interestingly, the proteasome is directly inhibited by lipofuscin/ceroid. Accumulation of oxidized proteins (and lipids) such as lipofuscin/ceroid may thus exacerbate the accumulation of damaged proteins (Sitte et al., 2000b).

5.2. Decline in chaperone function

Changes in chaperone expression and function in the ageing process may indicate a possible involvement in the development of longevity and cellular senescence (reviewed by Soti and Csermely, 2000). Molecular chaperones are abundant, well-conserved proteins responsible for the maintenance of the conformational homeostasis of cellular proteins and RNA. Environmental stress creates a proteotoxic insult to the cell, which leads to chaperone (heat shock protein, stress protein) induction. The protective role of chaperones is a key factor for cell survival and in repairing cellular damage. The fact that long-lived nematode and fly mutants exhibit enhanced stress responses, including heat shock protein induction (Lithgow et al., 1995; Murakami and Johnson, 1998; Walker et al., 1998; Yang and Wilson, 2000) is consistent with a model in which chaperones play critical roles in maintaining cellular health. The decline in chaperone capacity can affect health of the cell. Recently, an interesting study suggested drug-induced synthesis of aberrant *E. coil* proteins led to increased carbonyl modification (Dukan et al., 2000). Thus, the importance of folding-assisting chaperones in the maintenance of a healthy protein pool may have been underestimated.

6. Caloric restriction and protein synthesis

6.1. Caloric restriction can delay deleterious consequences of ageing in many organisms

Caloric restriction, a significant reduction in calorie intake without essential nutrient depriva-

tion, can slow the intrinsic rate of ageing in yeast, nematodes, flies, rodents and probably primates (Merry and Holehan, 1979; Holehan and Merry, 1986; Sohal and Weindruch, 1996). The fascinating effects of dietary restriction include maintenance of most physiological processes in a youthful state and a delay in the occurrence and/or progression of age-associated disease. Little is actually understood about the mechanism by which reduced caloric intake is translated into longevity.

In rodents, the anti-ageing action of dietary restriction is dependent upon the reduced intake of calories, rather than reduction of the body fat content or metabolic rate (Greenberg and Boozer, 2000; Masoro, 2000). If the life-prolonging stimulus is reduced caloric intake, what are the molecules that 'sense' this signal and convert it to the many physiological changes in calorie-restricted cells? To date, the only molecular understanding of caloric restriction effects have been elaborated in the singe-celled yeast, *S. cerevisiae* (Guarente, 2000). In this system, caloric restriction is thought to be conferred by limiting media glucose levels or by genetic mutation of components of the cyclic AMP-dependent protein kinase A pathway. Ras function has also been implicated in replicative lifespan extension in yeast (Jazwinski, 1999, 2000a,b). Caloric restriction effects on yeast replicative capacity requires the activity of the SIR2 histone deacetylase and NPT1, a gene required for production of NAD, the oxidized form of nicotinamide adenine dinucleotide. NAD availability plays an important role in broad aspects of metabolic regulation (Tavernarakis et al., 1996; Lin et al., 2000; reviewed by Guarente, 2000). Whether NAD and components of the cyclic AMP-dependent kinase pathway contribute to mammalian caloric restriction lifespan extension mechanisms remains to be tested but since the caloric restriction phenomenon appears conserved, the molecules that mediate this response are of clear interest for intervention with the deleterious consequences of ageing.

6.2. Caloric restriction is correlated with reduced oxidative damage

Dietary restriction protects rats and mice of all ages against the damaging actions of acute stressors (reviewed by Masoro, 1998). Microarray expression studies also support that caloric restriction selectively attenuates the age-associated induction of genes encoding inflammatory and stress responses (Lee et al., 2000). How is lower cellular stress accomplished? The free radical theory of ageing predicts that caloric restriction should reduce oxidant damage. Indeed, caloric restriction has been reported to markedly reduce the accumulation of oxidatively damaged proteins (Youngman et al., 1992). In mammals, the oxidative processes centered in the liver are a major source of free radicals. Liver catalase has the dominant role in the intracellular detoxification of hydrogen peroxide. In male rodents, catalase gene transcription decreases with age and caloric restriction obviates this effect. Limitation of caloric intake increases the efficiency of catalase mRNA translation in the liver of old female mice (Dhahbi et al., 1998). These findings suggest that the beneficial effects of dietary restriction upon lifespan could depend upon its ability to acutely reduce steady-state levels of oxidative stress by reducing protein oxidation (Dubey et al., 1996).

6.3. Caloric restriction and protein synthesis

The influence of chronic dietary intervention on protein turnover has been studied in rats. Between weaning and senescence muscles exhibit progressive decreases in their fractional rates of growth, protein synthesis and protein breakdown (Goldspink et al., 1987). Somewhat counter to expectation, elevated protein synthesis and turnover rates are a response to caloric restriction conditions (Lewis et al., 1985; Holehan and Merry, 1986; Merry and Holehan, 1991; Ward and Richardson, 1991; Jazwinski, 2000a). A recent study employing primary rat hepatocyte cultures confirm earlier reports that protein synthesis and degradation rates decline with age in liver, and this decline is retarded by caloric restriction (Lambert and Merry, 2000).

Microchip analysis of gene expression changes in mouse skeletal muscle that accompany caloric restriction indicates a significant molecular change in 'ageing-delayed' muscle is a shift to increased macromolecular biosynthesis and protein turnover (Lee et al., 1999). This observation is

Table 2
Caloric restriction-related increases (denoted by +) and decreases (denoted by −) in gene expression in the mouse gastrocnemius muscle

Change in CR (fold)	Gene	Function
+2.3	26S Protease subunit TBP-1	26S proteasome component
+2.2	Elongation factor 1-gamma	Protein synthesis
+2.1	Signal recognition particle receptor alpha subunit	Protein synthesis
+2.1	Proteasome activator PA28 alpha subunit	Protein turnover
+2.0	mCyP-S1 (cyclophilin)	Protein folding
+1.9	Translocon-associated protein delta	Protein translocation
+1.8	60S ribosomal protein L23	Protein synthesis
+1.8	Proteasome Z subunit	Protein turnover
−2.0	Aminopeptidase	Protein turnover
−1.8	PHAS-II	Translation inhibitor
−1.8	P31	Protein turnover
−1.6	SUI1	Translation initiation factor
−1.6	Seryl-tRNA synthetase	Protein synthesis

The genes listed were not influenced by age. Data in the Table are taken from Lee et al., 1999

consistent with the hypothesis that one lifespan-extending component of caloric restriction could be the elevation of protein turnover rates and maintained pools of healthy proteins free of oxidant damage (Table 2).

7. *C. elegans* is well suited to an investigation of the roles of protein turnover on ageing and caloric restriction

Decreases in protein turnover are associated with senescent decline and the caloric restricted state, which confers longevity, increases protein turnover. Taken together: (1) data implicating aberrant protein modification as a major factor in ageing; (2) the strong correlation of diminished protein turnover and ageing; and (3) lowered generation of oxidation products and increased protein turnover under conditions of caloric restriction, suggest a potential role of protein turnover in ageing and life-extending protocols. More specifically, work with powerful genetic models that can be easily engineered such as *C. elegans* should enable direct testing of the hypothesis that modulation of protein turnover rates are critical in lifespan and are a required component of caloric restriction.

C. elegans is a powerful organism for experimental investigation of the ageing process. This small (approximately 1 mm) free-living hermaphroditic nematode completes a reproductive life cycle in 2.5 days at 25 °C, progressing from a fertilized embryo through 4 larval stages to become an egg-laying adult, and lives for about 2 weeks (Fig. 3). Under adverse conditions such as starvation, over-crowding or high temperature, larvae can enter an alternative life stage called the dauer (enduring) larva, during which animals move but do not feed. The dauer larva is a 'non-ageing' organism that for weeks or even months (Klass and Hirsh, 1976). When a dauer larva encounters favorable environmental conditions, it re-enters the life cycle at the fourth larval stage, progresses into adulthood to reproduce and then completes the final week or so of its lifespan.

C. elegans development and anatomy are exceptionally well characterized. The complete sequence of cell divisions that occur as the fertilized egg develops into the 959-celled adult has been recorded (Sulston and Horvitz, 1977; Sulston et al., 1983). Serial section electron microscopy has produced a description of the shape and pattern of connection of each of its 302 neurons, so that the full 'wiring diagram' of the animal is known (White et al., 1976). *C. elegans* has a strong foundation in classical genetics and genes can be positioned on the genetic map using standard mapping techniques. The vast majority of *C. elegans* strains studied in research labs are derived from the same parental strain, N2. Thus, as best as is possible, *C. elegans* research involves genetically identical populations not subject to inbreeding depression (but also see Gems and Riddle, 2000). The molecular biology of *C. elegans* organism is quite sophisticated, and is greatly facilitated

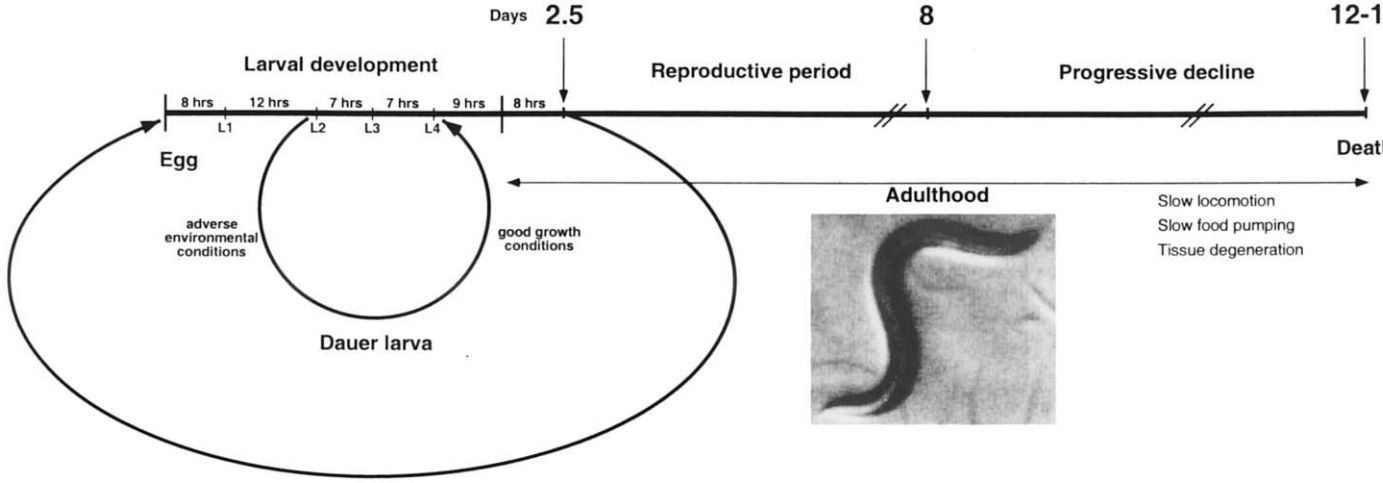

Fig. 3. *C. elegans* life cycle. Following hatching, worms progress through four larval stages before reaching adulthood (Brenner, 1974). The duration of each
25 °C is shown in hours. Adult nematodes lay eggs for about 5 days. The average lifespan of animals is about 15 days at 20 °C.

by a sequenced genome (The *C. elegans* Sequencing Consortium, 1998). Investigators can take advantage of genome data to perform 'reverse genetics'—directly knocking out genes (Liu et al., 1999) or transiently knocking down gene expression by a novel method of generating mutant phenocopies called double-stranded RNA mediated interference (RNAi) (Fire, 1999). In the RNAi protocol, introduced double-stranded RNA corresponding to specific genes can target the homologous endogenous transcript for degradation. In addition, transgenic animals can be created rapidly and with ease (Mello et al., 1991) so that candidate genes involved in ageing can be tested for cell-specific and temporal expression, high copy number expression effects, etc.

8. Ageing in *C. elegans*

The *C. elegans* lifespan has a mean value of approximately 13 days at 25.5 °C (Larsen et al., 1995). Caloric restriction, as administered by *E. coil* rationing or by axenic medium, can markedly extend *C. elegans* lifespan (Vanfleteren and Braeckman, 1999; our unpublished observations). As they age, nematodes feed (as can be measured by pharyngeal pumping), move and defecate more slowly than their younger counterparts (Klass, 1977; Bolanowski et al., 1981; Duhon and Johnson, 1995). Death is usually assayed by a failure to respond to touch with an eyelash hair, failure to move, and/or failure to pump in food.

Old nematodes loose their ability to tolerate various stresses. Old animals are more sensitive to oxidative stress either from higher concentrations of oxygen (Honda et al., 1993) or exposure to oxidizing agents (Larsen, 1993; Darr and Fridovich, 1995), than are their younger counterparts. Similarly, old animals are more sensitive to thermal stress (Lithgow et al., 1995). Since older nematodes lose their ability to increase their levels of superoxide dismutase (SOD) upon exposure to oxidative stress (Darr and Fridovich, 1995), oxidative damage might accumulate during ageing. Indeed, studies with the long-lived mutant *age-1* (see below) found that these mutants have elevated levels of SOD as well as catalase as they grow older, suggesting that the activity of anti-oxidant enzymes may help increase survival times (Larsen, 1993; Vanfleteren, 1993; reviewed by Gems, 1999).

9. Genetics of ageing in *C. elegans*

Until recently, *C. elegans* was the only multicellular organism in which single gene mutations that dramatically extend lifespan had been identified. To date there are more than 50 known *C. elegans* genes which, when mutant, can extend lifespan. Some of the best studied of these are the *age*/*daf* genes that affect an insulin-like signaling pathway required for dauer formation (Friedman and Johnson, 1988a,b; Kenyon et al., 1993), the 'clock' *(clk)* mutants in which development and rhythmic behaviors of the nematode are slowed (Lakowski and Hekimi, 1998; Hekimi et al., 1998), and *eat* mutants defective in pharyngeal pumping thought to experience caloric restriction effects (Hekimi et al., 1998; reviewed by Guarente and Kenyon, 2000). Since some double mutants between members of *age*/*daf* and *clk* exhibit considerably more lifespan extension than either single mutant, it has been suggested that *age*/*daf* and *clk* genes participate in two different genetic mechanisms by which lifespan is influenced. Similarly, double mutants of certain *age*/*daf* genes and *eat* genes life longer, suggesting that caloric restriction might act via a different pathway than the *age*/*daf* insulin signaling pathway.

Interestingly, all tested long lived mutations of *C. elegans* appear to confer resistance to environmental stress, including oxidative stress, high temperature, and exposure to ultraviolet radiation (reviewed by Vanfleteren and Braeckman, 1999; Van Voorhies and Ward, 2000). Another feature that seems common to some tested long-lived mutants to date is that metabolic rates of such *C. elegans* mutants are reduced compared with that of wild-type nematodes (Van Voorhies and Ward, 1999). This is mostly true for long-lived *clk-1* mutants but long-lived *age-1* mutants

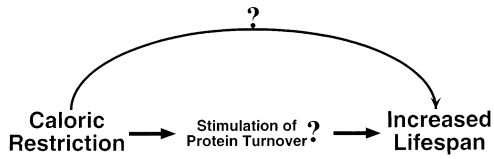

Fig. 4. Caloric restriction, protein synthesis and ageing. The effects of caloric restriction at the organismal level are pleiotropic. Several physiological changes that are triggered by low caloric intake contribute to lifespan extension. Elevation of protein turnover rates appears to be one of the major players.

also show observable reduction of metabolic rates. The *daf-16* genetic suppressor mutations that restore normal longevity to *age-1* long-lived mutants also restore normal metabolic rates (Vanfleteren and De Vreese, 1995; Vanfleteren et al., 1998; reviewed by Gems, 1999).

There are no striking changes in the profile of *C. elegans* proteins that can be visualized using two-dimensional polyacrylamide gel electrophoresis (Johnson and McCaffrey, 1985; Vanfleteren and De Vreese, 1994), indicating that at a gross level the profile of abundant proteins remains approximately similar later in life. Obviously, only significant changes in major proteins can be detected by 2D electrophoresis analysis. Today, studies of gene expression changes at the transcript level can be determined by microchip array analysis—a study well worth performing (Hill et al., 2000). It also remains to be seen whether protein turnover contributes to lifespan extension offered by known gerontogene mutations in the nematode. However, the activities of 3 lysosomal proteases were found to be markedly lower in older animals. The aspartyl protease cathepsin D declines about 10-fold from day 3 (early adulthood) to day 11 (near the mean lifespan). The specific activity of the thiol protease cathepsin Ce1 declines about 2.5-fold over the same period, and the specific activity of the thiol protease cathepsin Ce2 declines about eight-fold (Sarkis et al., 1988). The data are consistent with the hypothesis that reduced protease activity in older animals may cause a decline in the rate of protein turnover with age.

10. Future directions

Changes in the efficiency of protein turnover are hypothesized to contribute to cellular accumulation of damaged proteins that accompanies old age. Age-related protein turnover changes have been reported in several organisms ranging from yeast to humans and can thus be considered a conserved component of a 'public' ageing mechanism (Martin et al., 1996). Moreover, regulated protein turnover has been correlated with, and could be instrumental in, the beneficial effects of caloric restriction (Fig. 4). Given newly developed experimental approaches and well-defined genetic systems in which ageing has been studied, work in model organisms such as nematodes and flies should now enable the links between protein turnover, caloric restriction and ageing to be readdressed in new experimental detail. In particular, experimental approaches currently available in the *C. elegans* system are well suited to facilitate the identification of specific biochemical steps underlying alterations of protein turnover during ageing. The molecules enacting these steps could constitute attractive pharmacological targets for therapeutic reversal of senescent decline.

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