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LITERATURE HIGHLIGHTS

Sideways glance: does dietary restriction promote longevity, though impairing fecundity? Not necessarily, if the diet has a correct nutrient balance

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A wide body of evidence indicates that genetic and environmental interventions affect lifespan. Nutrition is a key regulator of tissue growth and dietary restriction (DR), the reduction in dietary intake without malnutrition is one of the several interventions that have been shown to increase longevity in invertebrate and vertebrate organisms [1–4]. In fact, DR extends lifespan in invertebrate model organisms such as the budding yeast *S.cerevisiae* [5], the nematode worm *C. elegans* [6], the fruit fly *Drosophila* [7], as well as laboratory rodents, where its effects were first observed. [8]. During DR, lifespan reaches a peak as nutrition is lowered, but with further reduction in nutrients, starvation causes a decline in the lifespan. The invertebrate model organisms are relatively short lived and easy to maintain and manipulate under laboratory conditions; they are therefore an important resource for analysing the mechanisms by which interventions such as DR increase longevity. At least some mechanisms of ageing show a remarkable degree of evolutionary conservation; an understanding of ageing mechanisms in invertebrate model organisms can therefore be very useful in identifying homologous mechanisms in mammals.

During metazoan evolution, the control of cell growth has evolved from a simple cell autonomous response to nutrient levels to a complex network of intercellular growth factor-mediated signals. Despite these layers of control, individual cells of higher eukaryotes have retained the ability to sense and respond directly to levels of nutrients such as amino acids and others [9]. This primal mode of regulation may serve as a checkpoint to gauge the appropriateness of intercellular growth signals, and coordination of these regulatory inputs is likely to be essential for normal cell growth, proliferation and survival.

"Cell" has published in the issue of October 2, 2009, a paper entitled: "4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*" by Zid and coworkers [10], where interesting observations are reported on the mechanisms of lifespan extension and the slowing of age-related diseases upon DR in *D. melanogaster*. The conservation of signalling pathways and the short lifespan render this organism an excellent model for the investigation. In this paper, the authors suggested possible mechanisms which may prolong lifespan during times of nutrient limitations. It has become apparent in recent years that nutrient-sensing growth pathways are important regulators of lifespan. In order to assess their nutritional status, cells measure the availability of energy and of amino acids. The target of rapamycin (TOR) pathway, a key nutrient-sensing pathway conserved from yeast to humans, integrates nutrient and environmental signals to mediate growth and metabolism. TOR was discovered in 1991 by Hall et al. [11]. The authors identified two genes in yeast, TOR1 and TOR2, by virtue of mutants that conferred resistance to the antifungal drug rapamycin. Subsequent studies led to the identification of a single TOR in other organisms, including mammals [12]. TOR protein kinases promote cell growth and proliferation in response to nutrients and growth factors. The biochemical purification of TOR-associated proteins has revealed that TOR kinase is present in two complexes, with distinct sets of binding partners. Both TOR complexes have important roles in growth control, but they act in different ways. TOR complex 1 (TORC1) is the major rapamycin-sensitive form of TOR and is the primary mediator of energy and amino acid sensing for growth control. TOR complex 2 (TORC2) is insensitive to the inhibitory effects

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of rapamycin, and it phosphorylates substrates distinct from those phosphorylated by TORC1. TORC2 is emerging to have a modulatory role in signalling events, such as insulin signalling. The mammalian target of rapamycin [13] (mTOR, also known as FRAP or RAFT1) has been implicated in such coordination. mTOR is a large serine/threonine kinase (289 kDa) of the phosphoinositide 3-kinase (PI3 K)-related family, conserved throughout the evolution. Inactivation of mTOR by rapamycin results in G1 arrest accompanied by a dephosphorylation of two mTOR's targets, $p70^{S6K}$ and 4E-BP. These proteins regulate ribosome biogenesis and cap-dependent translation, respectively. Their phosphorylation is required to achieve the high levels of protein synthesis necessary for cell cycle entry. 4E-BP (initiation factor IF4E binding protein), a protein phosphorylated by TOR, regulates mRNA translation and growth in flies and in mammals [14]. When TOR pathway is inhibited, the hypophosphorylated form of 4E-BP acts as a translational repressor by binding the protein synthesis initiation factor IF4E, blocking the activity of the IF4F complex. The eukaryotic initiation factor 4F (eIF4F) complex mediates growth-dependent protein synthesis. This activity is accomplished by regulating the association of the mRNA cap binding protein eIF4E with the scaffold protein eIF4G, both components of the eIF4F complex. eIF4G helps the assembling of eIF4F complex by bridging the poly(A) binding proteins (PABPs) with eIF4E [15]. This leads to the circularization of mRNAs with a synergistic effect on the rate of translation [16]. Transcripts with extensive secondary structure in their 5′ untranslated regions (UTRs) are very sensitive to the activity of the eIF4F cap binding complex. In cultured mammalian cells, the overexpression of eIF4E causes fibroblasts transformation and cell size increase that can be reversed by increasing the abundance of 4E-BP. eIF4E is critical for growth in *Drosophila*, and its upregulation has been observed in some cancers [17]. Finally, its overexpression has been shown to accelerate the senescence in mammalian cells [18]. It has been shown [19, 20] that inhibition of ribosomal genes extend lifespan of *C. elegans* and *S. cerevisiae*. These findings, and several others in converging to the same direction, suggest an important role of mRNA translation in modulating the ageing process.

Even though the regulation of gene expression at the level of genomewide transcription has led to advances in the field of ageing, the studies on mRNA translation utilized by Zid and coworkers [10] provided new insights into the ageing processes that are modulated by DR in *D. melanogaster*. The authors separated mRNAs bound to varying number of ribosomes by density gradient centrifugation and made the novel observation that, under DR, some mRNAs are differentially loaded onto ribosomes compared to rich nutrient conditions and mitochondrial electron transport components were one specific class of genes upregulated at translational level upon DR. The inhibition of the expression of mitochondrial electron transport chain (ETC) subunits diminished the DR-dependent lifespan extension, suggesting a key role for the enhancement of mitochondrial function upon DR in lifespan extension. The modulation of mitochondrial function was dependent on d4E-BP and was associated with the structural properties of the 5′UTR. Furthermore, the DR-dependent extension in lifespan required d4E-BP which was also found to be sufficient to extend lifespan.

In order to obtain DR in *D. melanogaster*, the authors reduced the concentration of yeast extract in the fly diet keeping constant sucrose concentration. This treatment was sufficient to extend lifespan. They then investigated the changes in mRNA translation and observed a decrease in protein synthesis in this experimental condition. The relative translation rate of an mRNA can be inferred from the number of ribosomes it recruits, as initiation is the rate-limiting step for the translation of most mRNAs. The study of the polysomal profiles showed that, under DR, an overall reduction in the number of ribosomes bound to mRNAs and a decrease in ^{35}S-methionine incorporation into proteins were present.

In DR, even though the overall proteins synthesized are decreased, a number of nuclear encoded mitochondrial genes, including those in Complex I and IV of the ETC, showed increased ribosomal loading and enhanced overall activity in this dietary condition. Interestingly, the authors found that various mitochondrial genes possess shorter 5′UTRs, with lower GC content and less secondary structure than the average cell proteins. These characteristics came out to be important for their enhanced mRNA translation. The translational repressor e4E-BP, the eukaryotic translation initiation factor 4E binding protein, was upregulated upon DR and mediated DR-dependent changes in mitochondrial activity and lifespan extension. The increase in mitochondrial ETC activity was important for the lifespan extension upon DR. This was investigated by reduction in individual mitochondrial ETC Complex I and Complex IV subunits, using RNAi. The RNAi knockdown was sufficient to lower Complex I and IV mRNA levels under different yeast concentrations and to counter the lifespan increase induced by DR. These data demonstrate the importance of the mitochondrial ETC function in the lifespan extension upon DR in *Drosophila* and presumably also in all the other organisms.

These results imply that translational regulation of nuclear-encoded mitochondrial gene expression by 4E-BP plays an important role in lifespan extension upon DR. It has been suggested recently that an age-related decline in metabolic function as evidenced by reduced expression of genes in the electron transport chain may be a common

feature among species as diverse as flies, worms and mammals [21, 22]. It is therefore significant the observation that DR which is known to slow the aging in several species, increases the translation of mitochondrial electron transport chain genes via the downstream TOR effector 4E-BP. This could have a protective effect by maintaining the function of the electron transport chain and hence ATP production with age. The results also suggest that d4E-BP modulates mRNA translation to induce a metabolic shift towards increased mitochondrial capacity which may prolong lifespan during times of nutrient limitation and translational regulation of nuclear-encoded mitochondrial gene expression by 4E-BP plays an important role in lifespan extension upon DR.

Interestingly, simultaneously to the paper of Zid et al. on October 2, 2009, another paper was published on Science entitled "Ribosomal protein S6 kinase signalling regulates mammalian life span". Dominique J. Withers and coworkers [23], from the Institute of Healthy Ageing, Department of Medicine of University College of London found that the deletion of ribosomal S6 protein kinase 1 (S6K1), a component of the nutrient-responsive mTOR signalling pathway, prolonged life of about 80 days, i.e. 9% more than control mice, with females surviving 20% longer. The animals were also less likely to develop certain pathological signs of ageing, such as bone, immune and motor dysfunction and loss of insulin sensitivity. S6K1 deletion induced gene-expression patterns very similar to those observed in mice undergoing long-term caloric restriction or with pharmacological activation of AMP-activated protein kinase (AMPK), suggesting that manipulating S6K1 signalling could be a good strategy to find drugs mimicking the positive effects of DR. S6K1 transduces anabolic signals to regulate cell size, growth and metabolism through various mechanisms that include effects on the translational machinery and on cellular energy levels through the activity of AMPK. These results are in agreement with those described previously [10]. In fact, inactivation of mTOR with rapamycin results in G1 arrest and in a dephosphorylation of two mTOR's targets, 4E-BP investigated by Zid and coworkers [10] and $p70^{S6K}$ studied by Withers and coworkers [23]. The phosphorylation of both proteins is necessary to achieve a high level of protein synthesis essential for cell cycle entry. This effect would be responsible for influencing lifespan and age-related pathologies. However, it has also been reported that dietary restriction together with an extension of lifespan is associated to a reduction in fecundity in diverse organisms [24]. The widely accepted interpretation was that DR induces an adaptive reallocation of nutrients from reproduction to essential functions for survival [25] and therefore long life under DR and high fecundity is mutually exclusive through competition for the same limiting nutrients. Very recently, this assumption has been tested and disproved by Grandison et al. in their paper entitled "Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*" [26]. The authors reported that this idea is almost certainly wrong and identified the nutrients producing the responses of lifespan and fecundity, respectively, to DR in *Drosophila*. These authors found that dietary amino acids are responsible for lifespan shortening and increasing reproduction, but both longevity and fecundity can be maximized when intake of these nutrients is finely tuned. The authors have identified the specific nutrients that modulate lifespan and reproduction by manipulating all the components of a defined diet. They fed female flies a restricted diet that extend lifespan at the expense of fecundity and then tried to restore the short-life and high-fecundity characteristics of fully fed flies by adding back specific nutrients. The addition of carbohydrates, lipids or vitamins had no effects. Differently, the addition of amino acids shortened lifespan and reverted egg production to the level observed under full feeding. The authors then asked the question of whether all amino acids contributed equally to this effect. The answer was that all non-essential amino acids (NEAAs) only marginally shortened lifespan and did not affect fecundity whereas the addition of all essential amino acids (EAAs) decreased lifespan and increased egg production, as much as combining all amino acids or full feeding. The authors then manipulated individually each EAA and found that methionine supplementation alone was able to restore fecundity at the level of full feeding, but without reducing lifespan and no single EAA shortened lifespan to the fully fed state. Yet, adding all EAAs except methionine failed to reduce lifespan, suggesting that methionine together with one or several EAAs is responsible for the lifespan-shortening effect of full feeding. This result is in agreement with the results of the work showing that methionine restriction increases fly lifespan [27]. To explain the effect, one possibility is that methionine restriction counters oxidative damage increasing the levels of glutathione [27]. Another explanation might be that methionine restriction extends lifespan by reducing signalling through a major regulator of longevity in many species [25, 27–29], the IIS–insulin/insulin-like growth factor IGF-pathway. In fact, methionine-deficient mice show lowered levels of IGF-1 pathway [25]. Moreover, when Grandison et al. [26] added back EAAs to the diet, they observed a strong decrease in lifespan in normal flies, but only a very small decrease in mutant flies lacking the insulin-like receptor that mediates the IIS activity. These results demonstrate that the IIS pathway mediates key effects of amino acids on ageing and reproduction. Addition of methionine did not promote fecundity in these mutants. During development and growth, IIS interacts with TOR signalling [10, 29], through

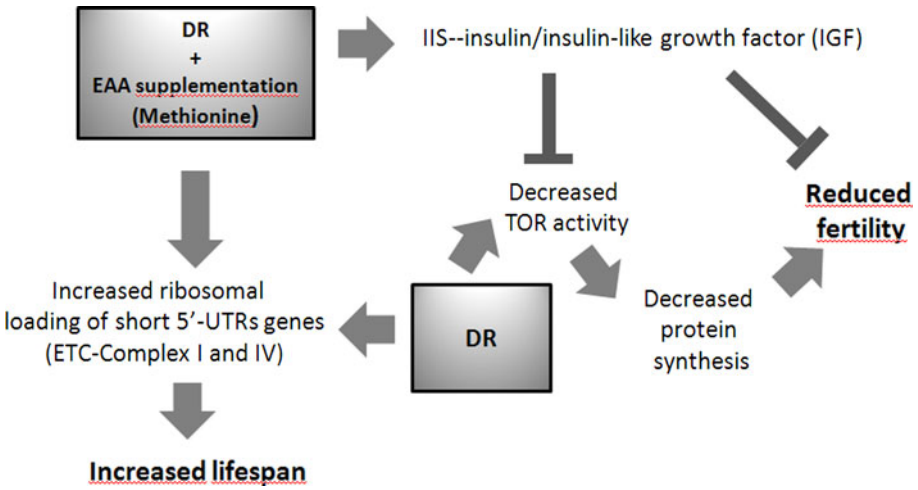

Fig. 1 Dietary restriction (DR) is associated with a decreased activity of the target of rapamycin (TOR) pathway which down regulates the level of protein synthesis, leading to a reduced fertility. DR induces an increase in the ribosomal loading of genes encoding for proteins participating to the electron transfer chain (ETC.) having shorter 5′UTRs, with lower GC content and lesser secondary structure than the average cell proteins. The increased expression of Complex I and Complex IV proteins results in an enhanced ETC functions and increased lifespan. Essential amino acid (EAA) supplementation, and in particular by methionine, in the presence of DR is associated with an of IIS–insulin/insulin-like growth factor (IGF) expression which counters the down-regulation of TOR activity and the negative effects on fertility while lifespan extension is maintained

which EAAs could act to affect lifespan. The repressor protein 4E-BP, which lies downstream of TOR, is required for lifespan extension in flies when their diet is restricted [10]. Furthermore, dietary amino acids stimulate insulin secretion in the brain through a TOR-dependent mechanism in the *Drosophila* fat body. The connections between dietary amino acids, IIS and TOR should be further investigated. In flies, amino acids that are not used for reproduction could shorten lifespan through metabolic costs associated with their removal. Nutrient imbalance in the diet could also account for the responses of lifespan and fecundity to dietary restriction in other organisms, including mammals, if specific nutrients in their diet are also limiting for full physiological function (Fig. 1).

Protein quality is implicated in human health, because the ratio of amino acids in the diet can affect traits important for ageing, for example, glucose homeostasis, cell signalling and bone health. However, currently accepted methods for measuring protein quality do not take into the due account the diverse, and not fully understood, roles for EAAs beyond the need for growth or nitrogen balance maintenance. As understanding of protein's action expands beyond its role in maintaining body mass and satisfying metabolic demands for biosynthetic pathways, the concept of protein quality must expand too to incorporate the newly emerging actions of protein and of individual AAs.

In conclusion, the mechanisms that influence lifespan are conserved over the large evolutionary distances between invertebrate and mammals [30, 31], and the results reported by Grandison and coworkers [26] imply that also in mammals, the benefits of DR for health and lifespan may be obtained without impairing fecundity and without DR itself, by a suitable balance of nutrients in the diet.

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