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Ageing, Neuronal Connectivity and Brain Disorders: An Unsolved Ripple Effect

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Abstract Cognitive decline associated with ageing and age-related disorders emerges as one of the greatest health challenges in the next decades. To date, the molecular mechanisms underlying the onset of neuronal physiological changes in the central nervous system remain unclear. Functional MRI and PET studies have indicated the decline in working memory performance in older adults. Similarly, age-related disorders, such as Alzheimer's disease, are associated with changes in the prefontral cortex and related neural circuitry, which underlines the decline of integrative function between different brain regions. This is mainly attributed to the loss of synaptic connectivity, which is a feature commonly observed in neurodegenerative disorders. In humans, the morphological and functional changes in neurons, such as reduction of spine numbers and synaptic dysfunction, precede the first signs of cognitive decline and likely contribute to pathology progression. Thus, a new scenario emerges in which apparently unrelated diseases present common features, such as the remodelling of neuronal circuitries promoted by ageing. For many years, ageing was considered a process of slow deterioration triggered by accidental environmental factors. Conversely, it is now evident that ageing is a biological process tightly controlled by evolutionary highly conserved signalling pathways. Importantly, genetic mutations that enhance longevity significantly delay the loss of synaptic connectivity and, therefore, the onset of age-related brain disorders. Accordingly, tweaking ageing might be an attractive approach to prevent cognitive decline caused by age-related synaptic dysfunction.

Keywords Ageing · Cognitive decline · MicroRNA · Mitochondria · Neurodegenerative disorders · Dendritic spine

We would like to dedicate this review to Prof Rita Levi-Montalcini in recognition of her fundamental contribution to science.

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Introduction

High fecundity and somatic maintenance are mutually exclusive. With abundant food, the natural selection promotes high progeny number, whereas during shortage of food supply, offspring fare poorly and as a result the organism allocates more nutrients to somatic maintenance. This strongly favours survival and a fully functional reproductive system until resources are again available. Interestingly, limitation of food intake without starvation can extend the life span of a wide range of animals,

including non-human primates [1]. Similarly, genetic mutations that affect the activity of nutrient sensors can shift the physiology of an organism from a reproductive to a maintenance state, which is echoed by a lower incidence of cancer, cardiovascular and neurodegenerative disorders [2]. Thus, although harsh conditions promote ageing by increasing the accumulation of damaged biological macromolecules, animals are able to initiate a wide range of defensive molecular pathways that extend survival and prevent the onset of age-related disorders.

The Plasticity of Ageing

The marked increase of aberrant macromolecules and defective organelles is considered a lethal intracellular toxin affecting the survival of an organism. The accumulation of these flawed cellular components is a feature and a consequence of ageing, a molecular process that is now known to be influenced by specific pathways conserved across species. Most of these programmes converge at nutrient and stress sensors, which can maximize the response amplitude by engaging directly gene transcription or indirectly by affecting cellular metabolism (Fig. 1). In the adult rhesus monkey, moderate caloric restriction delays ageing and prevents the loss of grey matter in cortical, putamen, caudate and insula regions [1]. Studies in yeast, invertebrates and mammals support the notion that the cellular response to food regimen requires the "target of rapamycin" TOR [3]. In eukaryotic cells, TOR serves as a checkpoint: it assesses the quality and quantity of nutrients, ensures that the cellular growth rate occurs in accordance with available resources and, eventually, favours somatic maintenance [4]. In this context, TOR signaling relays towards the regulation of downstream targets, including forkhead transcriptional factor PHA-4/FoxA and hypoxia-inducing factor HIF-1, which contribute to the caloric

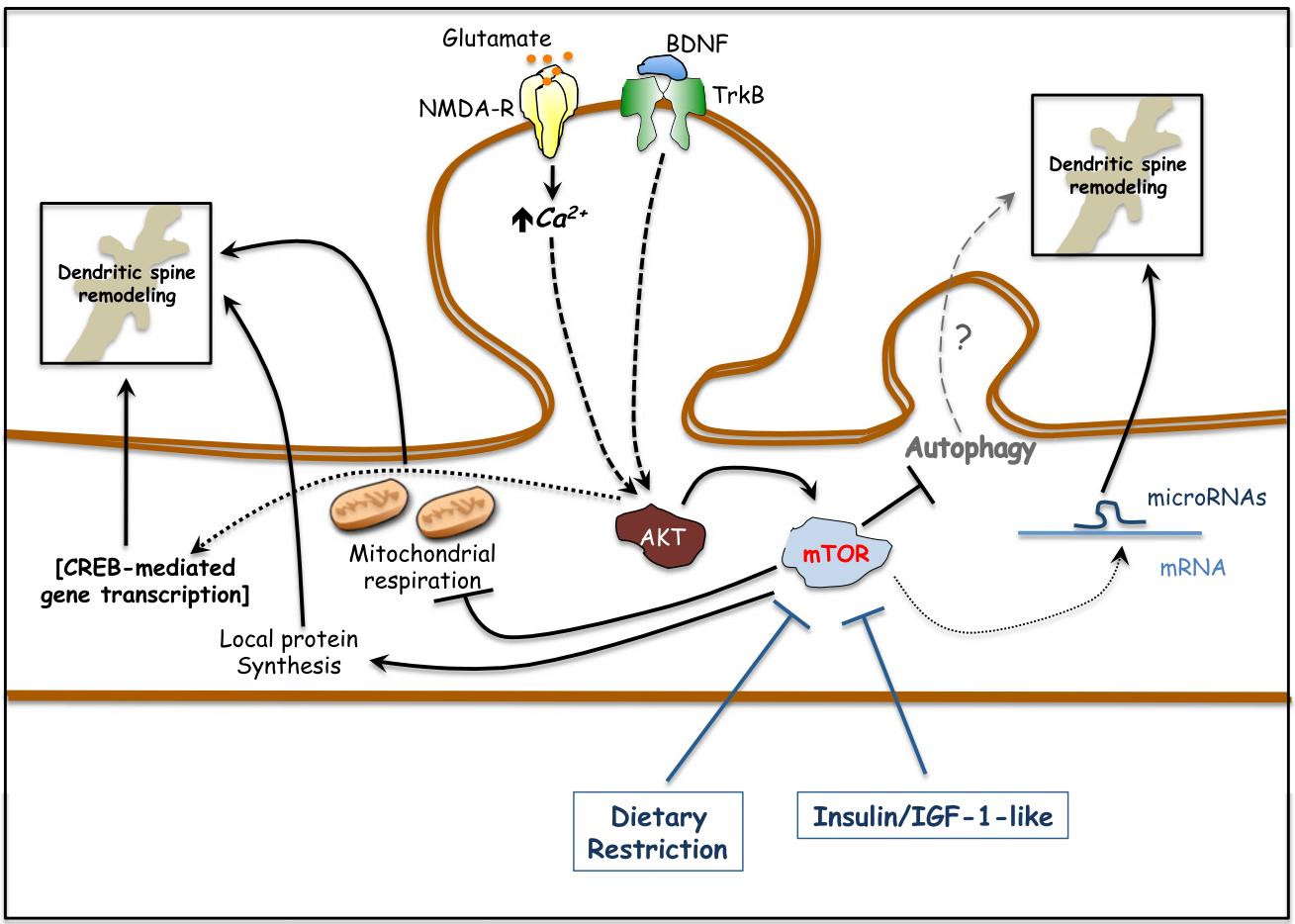

Fig. 1 Signalling pathways that promote longevity can influence dendritic spine remodelling. Excitatory neurotransmitters as well as neurotrophins regulate the activation of mTOR, which is the central sensor of nutrient availability. mTOR controls several processes, such as autophagy, mitochondrial respiration, local protein synthesis and, in some way, microRNA expression. Most of these pathways promote dendritic spine remodelling and, therefore, synaptic connectivity and can act synergistically with the activity-dependent dendritic arborization promoted by CREB-dependent transcription

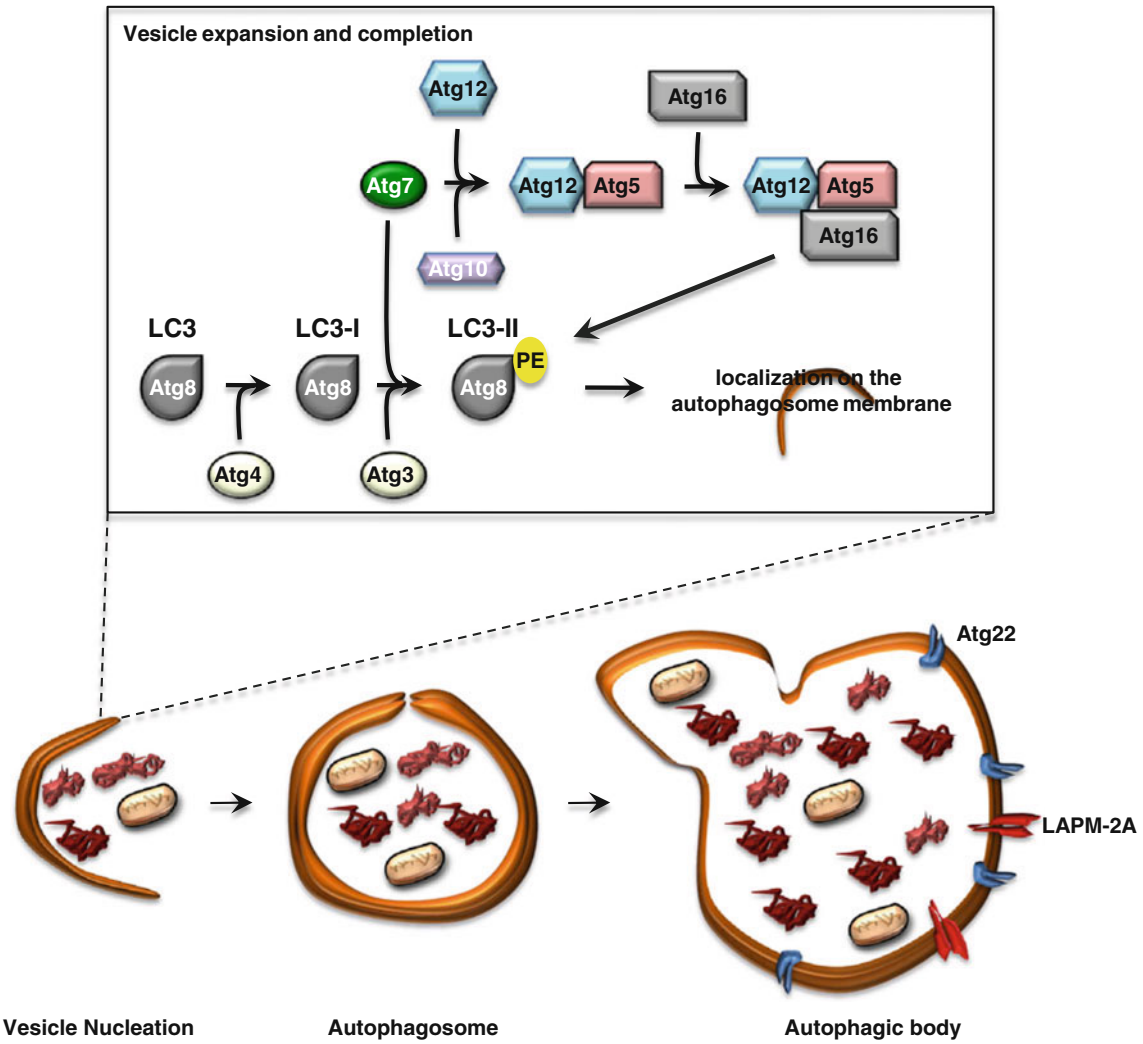

Fig. 2 Schematic representation of autophagy. Autophagy is a catabolic process evolutionary conserved that plays a role in the clearance of intracellular damaged macromolecules and organelles. Vesicle expansion and completion are a multistep process controlled by two ubiquitin-like proteins, Atg7 and Atg12. Posttranslational modification of Atg8 results in the recruitment to the pre-autophagosomal structure membrane

restriction response by promoting gene expression. Furthermore, inhibition of TOR leads to a wide range of changes, such as blocking of protein translation, increase of mitochondrial respiration and activation of autophagy, with the latter taking place during conditions of stress such as nutrient restriction. Macroautophagy, hereafter simply referred to as autophagy (Fig. 2), is a tightly regulated biological process in which intracellular organelles and macromolecules are enclosed within a double-membrane structure and degraded by lysosomal enzymes, with the breakdown products subsequently recycled. Autophagic machinery is highly conserved across evolution, and its role during nutrient deprivation was firstly described in yeast [5, 6] and confirmed in invertebrates and mammals. In *Caenorhabditis elegans*, autophagy is essential for dauer formation and life span extension in the insulin/IGF-1 receptor loss-of-function mutant *daf-2* [7]. Similarly, in the feeding-defective *eat-2* mutant, limited food intake inhibits TOR and leads to the up-regulation of autophagic genes, which are required for life span extension [8]. Notably, the loss of constitutive basal autophagy leads to an increased number of defective organelles and accumulation of cytoplasmic inclusions, which ultimately cause neuronal degeneration in animal models [9]. Pharmacologically, inhibition of TOR by rapamycin extends the life span of rodents [10] and can moreover abolish amyloidosis-associated cognitive decline [11] or other genetic neurodegenerative disorders [12]. Activation of TOR by ketamine, a powerful nonselective NMDA receptor antagonist, has an antidepressant effect and leads to an increased formation of active dendritic spine synapses [13], which suggests TOR signaling as a modulator of spine formation. Whether TOR

affects spine remodelling exclusively via protein synthesis or other TOR-controlled pathways is still under debate as genetic evidences have shown that altered autophagy can promote synaptic growth [14]. In summary, by integrating signaling information from growth factors, nutrients, intracellular energy status and environmental cues, TOR controls cellular metabolism, promotes longevity and has a broad positive impact on ageing and on neuronal function [15].

Similarly to caloric restriction, other pathways have been widely documented to be beneficial on life span and to reduce age-related disorders. The most well-known signaling pathway shown to influence longevity is mediated by the insulin/IGF-1 receptor. In *C. elegans*, reduced activity of the hormone receptor *daf-2* inhibits the downstream phosphatidylinositol-3-kinase AGE-1/PI3K and ultimately results in the nuclear localization of non-phosphorylated DAF-16/FOXO. In response to multiple environmental and tissue signals, the combined activities of DAF-16/FOXO isoforms regulate the expression of hundreds of genes involved in defensive responses, thereby extending the life span of mutant animals [16]. In worms, different tissues have different capabilities for responding to environmental and hormonal regulations. Indeed, expression of DAF-16/FOXO in the nervous system partially promotes longevity, whereas its expression in the intestine is sufficient to extend the life span substantially [17]. In contrast, in mammals, reduction of insulin receptor substrate 2 specifically in the nervous system is sufficient to extend mouse life span [18]. Similarly, heterozygous knockout mice for the insulin-like growth factor type 1 receptor present greater stress resistance and live much longer than wild-type animals [19]. Human population studies from different cohorts throughout the world have identified variants of enzymes involved in insulin/IGF-1 signaling pathway, such as IGF-1 [20] receptor and FOXO 3 [21]. Remarkably, reduced IGF-1 signaling protects animal models from amyloid deposition and amyloid-associated behavioural defects [22]. Indeed, heterozygous *Igf-1r* mice carrying the human Swedish mutant APP and the mutant presenilin 1 are protected from Aβ-associated neuronal and synaptic loss. This is mediated by an increased hyperaggregation of Aβ-plaques and, therefore, by a decrease of less soluble, highly toxic oligomers. Reduction of IGF signaling prevents astrocytosis and neuroinflammation; more importantly, it protects from synaptic loss, with a significant improvement in cognitive and locomotion tasks [22]. Collectively, this suggests that ageing and the onset of neurodegenerative pathologies have common molecular mechanisms and can be modified by targeting the same molecular pathways.

Some aspects of ageing remain fascinating, albeit still elusive. It is clear that nutrient and stressor sensors have multiple targets. One of the main outcomes of TOR and insulin/IGF-1 signaling pathways is the stimulation of mitochondrial respiration rates. However, this seems contradictory to other recent publications in which mild inhibition of respiration can promote longevity in a wide range of animal models. For instance, a mutation in worms and mice that alters ubiquinone biosynthesis [23, 24] and indirectly affects mitochondrial oxidative phosphorylation or the partial down-regulation by RNAi of the respiratory chain components substantially extends the organism's life span [25]. Similarly, pharmacological induction of mitochondrial uncoupling decreases tissue oxidative damage and enhances longevity in mice [26]. These phenomena may occur by a nuclear transcriptional response to mitochondrial defects, which leads to the adoption of alternative metabolic pathways in order to respond to energetic demands. Conversely, age-dependent decline of mitochondrial activity makes neurons vulnerable to environmental and metabolic stress, and it has been largely associated with the onset of Parkinson's and Alzheimer's diseases. Mice that present increased mitochondrial DNA mutations and therefore impairment of the electron transport chain have a shorter life span and display neuronal loss [27, 28]. Likewise, flies and mice defective for the zinc carboxypeptidase [29, 30], a protein that controls the level of mitochondrial enzymes, or mice with reduced expression of the mitochondrial intermembrane apoptosis-inducing factor [31], which is involved in the stabilization of the electron transport chain, display a reduced life span and an extensive neuronal degeneration. Clearly, mitochondria uncoupling is a double-edged sword: too much is detrimental and leads to neuronal vulnerability, whereas a mildly lower activity stimulates alternative metabolic pathways, increases gene-mediated stress resistance and foremost promotes longevity. Particularly interesting in this regard is the beneficial effect of resveratrol on mitochondrial function in rodents [32]. Resveratrol is a natural polyphenolic compound that significantly enhances sirtuin activity and affinity for NAD^+ and acetylated substrate. The increased activity of SIRT1, a highly conserved NAD-dependent histone deacetylase, promotes adaptation to caloric restriction, renders animals resistant to diet-induced obesity and confers insulin resistance by a mechanism that involves PGC-1α and, indirectly, mitochondrial proliferation. As expected, activation of sirtuins delays the ageing process in metazoans and yeast by engaging the same molecular pathways stimulated by caloric restriction [33, 34]. Nevertheless, the extension of life span by resveratrol and sirtuins occurs not only because of the increase of mitochondrial proliferation and activity. In yeast, Sir2 controls genome stability: it can silence chromatin by preserving intact telomeric chromatin and by changing the acetylation state of histones [35, 36]. Similarly, recent findings have shown that members of the H3K4 trimethylation complex can also regulate the life

span of multicellular organisms. In this context, deficiency of the methyltransferase tritorax complex decreases histone methylation in *C. elegans* gonads and extends the life span of the animals, whereas down-regulation of demethylase causes an excess of H3K4 trimethylation and compromises worm survival [37]. Although it is unclear how histone methylation in the germline can control somatic longevity, these findings, together with those on sirtuins, raise the possibility that ageing can be controlled by epigenetic modification of chromatin states.

Dendritic Spines Alterations During Ageing and Brain Pathology

Increased difficulties with learning and memory tasks are cognitive dysfunctions mainly associated with ageing and age-related disorders. Although some evidences suggest a widespread neuronal loss as a cause of the cognitive frailty, current work indicates a more subtle and restricted reorganization of neuronal circuits. Independent studies observed regressive changes of neuronal morphology in the hippocampus and neocortex of aged human and non-human primate brains. In general, in the prefrontal cortex of old humans [38] and monkeys [39], there is a decrease between 25% and 46% in spines and spine densities in the apical and basal dendrites of pyramidal neurons compared to young subjects. Similarly, recent morphometric analyses have confirmed that aged animals present a lower density of dendritic spines caused by the loss of "thin", highly motile structures. This is associated with an age-related shift towards spines with head volume significantly greater compared to young organisms [40]. These age-related morphological changes are associated with a significantly lower expression of glutamate-ionotropic receptor subunits, which is mirrored by a significant decrease of synaptic activity and neuronal excitability. Anatomically, dendritic spines are tiny specialized membrane protrusions with an approximately volume of 0.01–0.5 μm^3, typically with the bulbous head connected to the shaft of the neuronal dendrite by a thin neck. The spine heads are enriched with excitatory receptors, and together with other neurotrophin receptors, such as the TrkB receptor for BDNF, they ensure the maintenance of the structure. Functionally, dendritic spines are plastic and dynamic structure influenced by their electrophysiological activity. Importantly, they are the contact point between neurons for the formation of neural circuitry. During normal conditions, fast turnover of spines occurs as part of long-term learning and memory maintenance. Conversely, massive loss of functional dendritic spines is detrimental as it is highly associated with brain pathologies. Alteration of spine density has been described in some mental disorders such as the fragile X syndromes (FXS). In this syndrome, the expansion of a polymorphic CGC trinucleotide in the FMR1 gene causes the loss of function of the RNA-binding protein FMRP. FXS patients present an abnormal turnover of transient spines, which results in a larger pool of immature excitatory spines [41]. Recent studies have described the potential function of non-coding RNA in FXS. Specifically, miR-125b and miR-32 are associated with FMRP, taking part in the regulation of NMDA receptor subunit composition and, therefore, are also involved in synaptic plasticity [42]. Because of the peculiar properties of neurons and because mRNAs are selectively transported to distant sites, it is not surprising that small non-coding RNAs can regulate mRNA translation and de novo synthesis of synaptic proteins, thereby shaping dendritic spine morphology, number and activity. One of the first microRNAs described to act at the synapto-dendritic compartment was miR-134 [43]. The expression of miR-134 changes during neuronal development and negatively regulates dendritic spine volume through the local repression of Lim-domain-containing protein kinase 1 (Limk1) mRNA translation. More importantly, the inhibition of mRNA translation by miR-134 is controlled by BDNF stimulation, which triggers the activation of the TrKB/TOR signaling pathway and enhances synaptic protein expression of Limk1. This process controls dendritic spine size and potentially neuronal plasticity. Neuronal activity can therefore locally regulate protein synthesis via microRNA binding to untranslated regions of target mRNAs, with important implications concerning dendritic structural and physiological properties. As expected, in the last few years, microarray studies have identified additional microRNAs taking part in the regulation of mRNA translation at the dendritic spine level [44]. It is intriguing to notice that the expression of some microRNAs decrease during ageing [45]. Correspondingly, in *C. elegans*, deficiency of microRNA *lin-4* results in shorter life span, whereas decreased expression of its target, the putative transcription factor LIN-14, results in longer life span via a DAF-16/FOXO-independent manner [46]. During ageing, dendritic changes are accompanied by a shift in the expression of neurofilament proteins, which might contribute to the formation of neurofibrillary tangles and amyloidosis. Whether the loss of microRNA expression can progressively compromise dendritic spines and enhance cognitive impairment in human is speculative, although under debate. Certainly, the fact that some microRNAs can be down-regulated in Alzheimer's disease or play a role in Huntington's disease suggests that the gradual loss of localized fine genetic regulation might predispose individuals to physiological and neurological declines, setting them up for brain pathologies. We have recently found that during mild synaptic activation by nicotine, there are changes in microRNA expression, with strong effects on dendritic spine density and morphology (unpublished data). Future studies will help us understand

whether the loss of these microRNAs is accompanied by cognitive decline.

Concluding Remarks

Though the increase of longevity is a great achievement of our health and hygienic systems, it also represents a critical challenge to our existing social structure and a burden to our financial system because it leads to a larger number of people affected by age-related disorders, especially brain diseases. It is evident that the brain undergoes morphological changes, such as the reduction of neuronal arborization, the density of dendritic spines and the number of functional synapses. This leads to altered intrinsic excitability and spike-frequency adaptation that adversely contribute to cognitive impairment in old organisms. Over the past years, molecular biologists have identified many genetic mutations that can extend longevity and reduce the risk of pathologies. Here, we have highlighted apparently distinct molecular pathways, namely autophagy, mitochondria, insulin/IGF-1 signaling and microRNAs, all converging on neuronal connectivity and neuronal ageing. Understanding these pathways will help improve good ageing, preserve intact neuronal circuitry and, as importantly, prevent cognitive decline and age-related brain disorders.

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