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Review

Aging of the brain, neurotrophin signaling, and Alzheimer's disease: Is IGF1-R the common culprit?

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Abstract

The last decade has revealed that the lifespan of an organism can be modulated by the signaling pathway that acts downstream of the insulin/insulin-like growth factor 1 receptors (IR/IGF1-R), indicating that there is a "program" that drives the process of aging. New results have now linked the same pathway to the neurogenic capacities of the aging brain, to neurotrophin signaling, and to the molecular pathogenesis of Alzheimer's disease. Therefore, a common signaling cascade now seems to link aging to age-associated pathologies of the brain, suggesting that pharmacologic approaches aimed at the modulation of this pathway can serve to delay the onset of age-associated disorders and improve the quality of life. Work from a wide range of fields performed with different approaches has already identified some of the signaling molecules that act downstream of IGF1-R, and has revealed that a delicate checkpoint exists to balance excessive growth/"immortality" and reduced growth/"senescence" of a cell. Future research will determine how far the connection goes and how much of it we can influence.

Keywords: Aging; Alzheimer's disease; IGF1-R; Neurotrophin receptors; Amyloid β-peptide

1. Introduction

In 1906 the German physician Alois Alzheimer described the case of a severe cognitive decline in a 51-year-old woman that resembled previously described forms of senile "dementia". He also attempted (and this was very unusual for his time, the beginning of the 20th century) to link the clinical/mental manifestations of the patient to a very detailed pathological analysis of the brain performed by his close collaborator, the Italian Gaetano Perusini (an exciting report on the early story of Alzheimer's disease is offered in Bick (1999)). The description of the disease included extracellular dense deposits (amyloid plaques) and twisted bands of fibers inside the neurons (neurofibrillary tangles). Today, this degenerative brain disorder bears his name, Alzheimer's disease (AD), and the observation of amyloid (or senile) plaques and neurofibrillary tangles at autopsy is still required to obtain a definitive diagnosis of AD.

During the last century we have experienced a sharp increase in average lifespan, which has led to a marked increase in the number of individuals reaching the seventh and eight decade of their life, resulting in a parallel increase in the prevalence and incidence of age-related disorders. In 2000, the number of individuals affected by AD in the world was estimated to be approximately 11.5 million, with approximately 4.5 million in the United States and the rest almost equally split between Europe and Southeast Asia. Because of the increased lifespan of the population, this number is expected to rise to approximately 14 million in the United States and 50 million worldwide by 2050. The distribution of AD patients among the different age-groups shows a sharp increase with aging. In fact, in 2000, 5% of the patients were between the ages of 65 and 74 years, 18% were between the ages of 75 and 84 years, and 45% were 85 years of age and older (Hebert et al., 2003; Thal et al., 2004). Therefore, the peculiar and unusual form of neurodegeneration described

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by Alzheimer has become the leading cause of dementia in the world and one of the most common age-related diseases (Costantini et al., 2005a; Hebert et al., 2003; Morris, 1999).

This review will try to tie together the findings implicating the insulin-like growth factor 1 receptor (IGF1-R) in the regulation of lifespan to those that connect the same receptor to some of the events associated with normal aging of the brain and the Alzheimer form of neurodegeneration. Its main objective is to summarize these exciting new developments, and to bridge events thought to be totally unrelated. Hopefully, this approach will stimulate further interest of the scientific community in the molecular and biochemical events that influence the normal process of aging of the brain.

2. AD pathology

The pathological and histological hallmarks of AD include amyloid plaques, neurofibrillary tangles, and amyloid angiopathy accompanied by diffuse loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain. The dominant component of the plaque core is the amyloid β-peptide (Aβ) organized in fibrils of approximately 7–10 nm intermixed with non-fibrillar forms of this peptide. The most characteristic form of the amyloid plaque, the "neuritic plaque", is characterized by a dense core of aggregated fibrillar Aβ, surrounded by dystrophic dendrites and axons, activated microglia, and reactive astrocytes (reviewed in Selkoe, 1999, 2004). In addition, diffuse deposits of Aβ (probably a pre-fibrillary form of the aggregated peptide) are also found without any surrounding dystrophic neurites, astrocytes, or microglia; these plaques appear diffuse (therefore, called "diffuse plaques") and can be found in limbic and association cortices, as well as in the cerebellum (where the classical neuritic plaques are always absent) (reviewed in Selkoe, 1999, 2004).

Aβ is a 39–43 amino acid hydrophobic peptide proteolytically released from a much larger precursor, the amyloid precursor protein (APP) (Fig. 1). The generation of Aβ from APP requires the sequential recruitment of two enzymatic activities: β-secretase, also called BACE1 (for beta-site APP cleaving enzyme) (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999), and γ-secretase, a multimeric protein complex containing presenilin, nicastrin, Aph-1, Pen-2, and CD147 (Francis et al., 2002; Goutte et al., 2002; Yu et al., 2000; Zhou et al., 2005). The β cleavage is

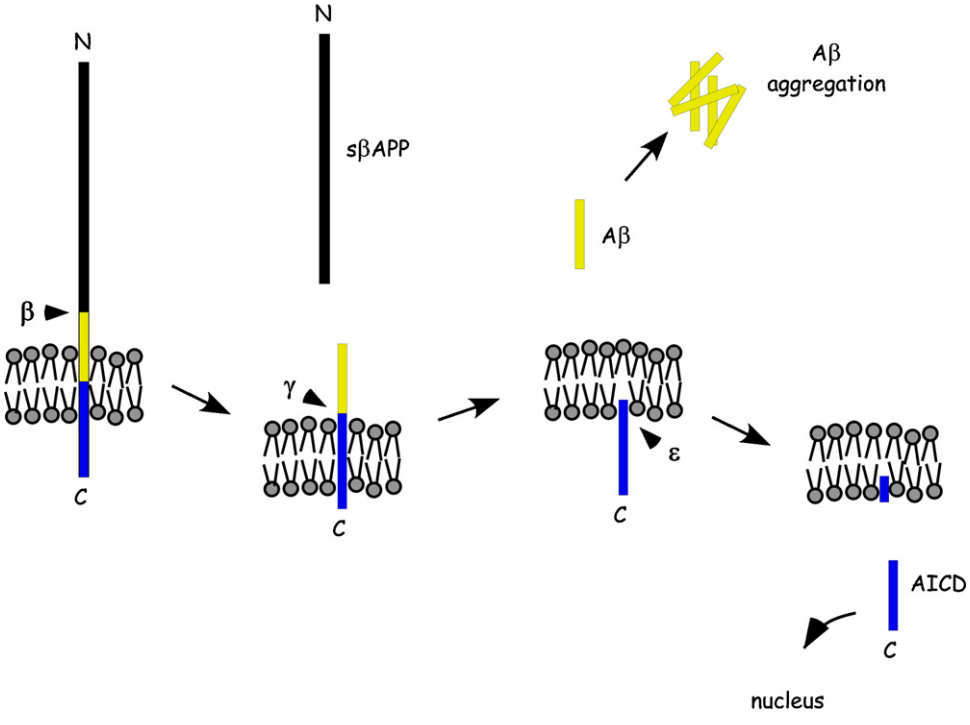

Fig. 1. Schematic view of Aβ generation from APP. APP is a type 1 membrane protein with a large extracellular domain, a single membrane-spanning domain, and a short cytoplasmic tail. The Aβ region of APP (in yellow) includes the first 12–14 amino acids of the membrane domain. The initial enzymatic step for the generation of Aβ requires proteolysis of APP at β site (amino acid 1 of the Aβ region). This event liberates a large N-terminal fragment (sβAPP) that is rapidly secreted into the extracellular *milieu* and a small C-terminal fragment (β-APP-CTF) of 99 amino acids (Costantini et al., 2005a; Puglielli et al., 2003b; Selkoe, 1999). The removal of sβAPP most likely induces a conformational change and/or a shift of the β-APP-CTF from the lipid bilayer allowing subsequent cleavage by the γ-secretase multimeric complex. The shift from the bilayer might be necessary to allow the cleavage of the peptide bond, which requires a molecule of water. However, the structure of the active γ secretase complex, which has recently been resolved by electron microscopy, shows a central aqueous chamber with two exit ports (Lazarov et al., 2006; Ogura et al., 2006), suggesting that the cleavage could occur without involving a shift of the β-APP-CTF outside of the lipid bilayer. Once generated, the Aβ peptides aggregate in the brain in the form of plaques. Further cleavage of β-APP-CTF at the *ε* site liberates the signaling active APP intracellular domain (AICD). In addition to the above β/γ pathway, APP can also be cleaved at the α site (between amino acids 16–17 of the Aβ region), precluding the generation of Aβ (Costantini et al., 2005a; Puglielli et al., 2003b; Selkoe, 1999).

the rate-limiting step and occurs before γ cleavage. It liberates a large N-terminal fragment of the protein (sβAPP) that is released in the extracellular *milieu*, and a small (∼12 kDa) membrane-anchored fragment called β-APP-CTF (or C99). The release of the large N-terminal domain allows subsequent γ cleavage, and liberation of Aβ and the signaling active intracellular domain of APP (AICD) (Fig. 1). γ cleavage does not seem to be sequence specific and normally occurs either at position 40 or 42 of the Aβ region generating $A\beta_{40}$ and $A\beta_{42}$, respectively. Release of Aβ in the extracellular *milieu* is followed by oligomerization and aggregation in the form of amyloid plaques. The molecular mechanisms involved in the secretion, aggregation, and toxicity of Aβ are still in part unknown (reviewed in Selkoe, 1999; Walsh and Selkoe, 2004).

Neurofibrillary tangles are observed almost exclusively in the cytoplasm of neurons. They appear as paired, helically twisted protein filaments and are made of highly stable polymers of cytoplasmic proteins called tau. Tau proteins constitute a group of alternatively spliced proteins found in the cytoplasm that possess either three or four microtubule-binding domains and that can assemble with tubulin, thus helping the formation of cross-bridges between adjacent microtubules. Tau proteins can be phosphorylated in multiple sites and the degree of phosphorylation inversely correlates with binding to microtubules. Therefore, highly phosphorylated tau proteins dissociate from microtubules and polymerize into the above described filaments (reviewed in Lee et al., 2001b). In addition to AD, the abnormal accumulation of filamentous tau is observed in frontotemporal forms of dementia, which include progressive supranuclear palsy, corticobasal degeneration, and Pick's disease. Finally, multiple mutations in the *tau* gene have been found associated with frontotemporal dementia with parkinsonism, therefore indicating that tau themselves can produce neurodegenerative disorders (reviewed in Lee et al., 2001b). The possible role of tau proteins in the pathogenesis of AD, and their potential interaction with Aβ is still a matter of discussion. Studies in transgenic animals indicate that human Aβ – but not tau – is sufficient to cause a mouse neuropathology that resembles human AD (reviewed in Gotz et al., 2004). However, studies in both AD patients (Delacourte et al., 2002) and mouse models (reviewed in Gotz et al., 2004) seem also to suggest that Aβ and tau can synergistically interact, fostering their respective aggregation and the neuronal loss. These arguments seem to find further validation in the fact that suppression of tau can improve memory function in an AD mouse model (SantaCruz et al., 2005).

3. Insulin/IGF1 receptors: a signaling pathway for the general program of aging

Insulin and insulin-like growth factor 1 (IGF1) have similar tertiary structures and substantial amino acid identity. IGF1 is mostly secreted into the blood by the liver where its synthesis is regulated by the pituitary growth hormone (GH). However, many other tissues, including the brain, are also able to synthesize IGF1 locally where it is not under the control of GH (Costantini et al., 2006; Lupu et al., 2001; Sun et al., 2005). The proof that brain IGF1 is not under the control of circulating GH comes essentially from $Ghr^{-/-}$ and Ames dwarf (Prop 1^{df}) mice. The former animals have a selective disruption of the GH receptor and cannot secrete IGF1 in response to GH (Lupu et al., 2001), whereas the latter have a defect in the production and secretion of GH by the anterior pituitary (Sun et al., 2005). Both animals have undetectable levels of IGF1 in the serum, but completely normal levels of IGF1 in the brain (Lupu et al., 2001; Sun et al., 2005). In addition, Ames dwarf mice (Sun et al., 2005) also show normal activation of IGF1-R signaling in different areas of the brain. This is further supported by the fact that primary neurons secrete IGF1 in the conditioned media in the absence of any hormonal stimulation (Costantini et al., 2006). In contrast to IGF1, insulin is almost exclusively synthesized and secreted into the plasma by pancreatic beta cells.

Although plasmatic IGF1 decreases during aging, brain IGF1 shows either no change or a slight increase during aging (Carro and Torres-Aleman, 2004). In addition, the plasmatic levels of its cognate ligand, insulin, increase during aging, most likely because of progressive peripheral resistance. Insulin resistance is also a risk-factor for AD (Carro and Torres-Aleman, 2004; Galasko, 2003) and has been implicated with several aspects of AD neuropathology (Carro and Torres-Aleman, 2004), including production/secretion of Aβ (Carro and Torres-Aleman, 2004; Craft et al., 2003; Galasko, 2003; Watson et al., 2003).

Insulin and IGF1 bind to high-affinity tyrosine kinase receptors, the insulin receptor (IR) and the IGF1 receptor (IGF1-R), which are widely expressed throughout the body, including the brain (Bondy and Cheng, 2004). Co-expression has been reported in many brain areas, including cortex and hippocampus (Bondy and Cheng, 2004; Werther et al., 1987, 1990). IR and IGF1-R are also expressed on brain capillaries and mediate the high-efficiency translocation of insulin and IGF1 into the brain across the blood–brain-barrier (BBB) (Reinhardt and Bondy, 1994). Cross-binding of insulin to IGF1-R at the level of the BBB has been reported, but IGF1 translocation occurs with higher efficiency (Reinhardt and Bondy, 1994). IGF1 can mimic insulin-like signaling in the brain (Bondy and Cheng, 2004), suggesting a partial overlapping of IR and IGF1-R signaling abilities. Even though cross-reaction can occur, each receptor binds to its own ligand with a 100–1000-fold higher affinity than that of the heterologous peptide. In agreement with these biochemical observations, IR and IGF1-R have certain shared functions, but also very distinct biological roles, and activate completely different sets of genes (Dupont et al., 2003; Jones and Clemmons, 1995).

IR and IGF1-R share a high degree of identity in their primary and tertiary structure. The functional active receptor contains two α and two β subunits. The two disulfide linked

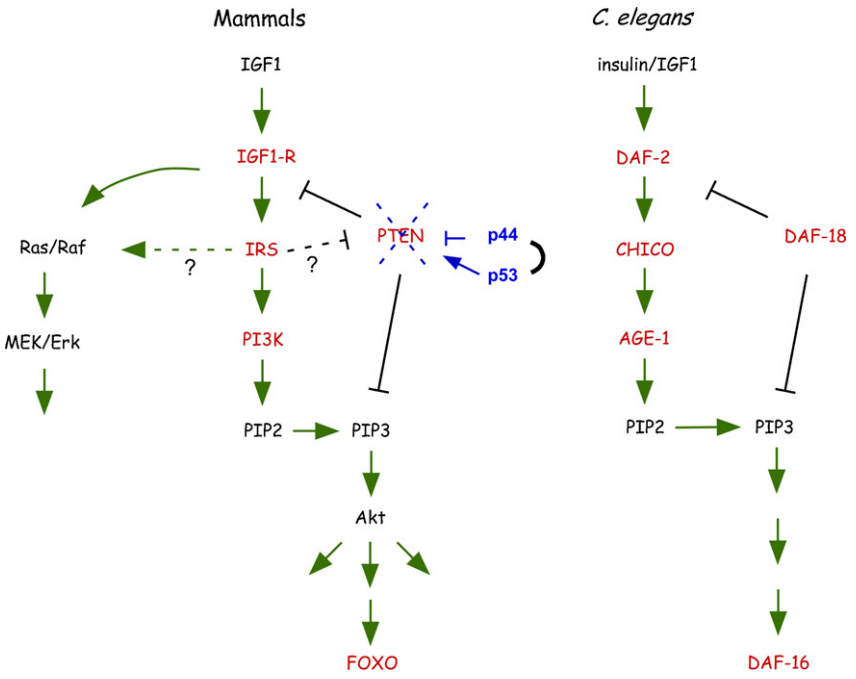

Fig. 2. Schematic diagram of IGF1-R signaling in mammals and *Caenorhabditis elegans*. The description of the signaling pathway is in the text. The common elements in mammals and worms are shown in red.

α subunits constitute the extracellular ligand-binding domain of the functional IR and IGF1-R. The signal transducing and largely intracellular β subunits share a high degree of amino acid identity in the tyrosine kinase domain (∼85%). IGF1-R has a slightly longer carboxyl-terminal domain with an additional tyrosine phosphorylation site, which represents the only apparent structural difference between IR and IGF1-R. This domain shows only a ∼45% identity between the two receptors (Ullrich et al., 1986). As a consequence of this high level of homology/identity, hybrid receptors, comprised of an insulin αβ-hemireceptor and an IGF1 αβ-hemireceptor, can form in tissues and cells expressing both IR and IGF1-R (Federici et al., 1997a,b). Whether hybrid receptors play specific roles in mediating insulin and/or IGF1 signaling is still unknown.

Upon binding to their own ligand, IR and IGF1-R undergo auto-phosphorylation, associate with intracellular adaptor proteins called insulin/IGF1 receptor substrates (IRS) proteins, and activate either the PKB/Akt or the Ras/MEK/ERK signaling pathways in order to transduce IGF1 actions (Bondy and Cheng, 2004) (Fig. 2, left panel). Akt activation requires phosphorylation of phosphatidylinositol (3,4)biphosphate (also called PIP2) by a phosphatidylinositol 3-kinase (PI3K) in order to generate the second messenger phosphatidyilinositol (3,4,5)triphosphate (also called PIP3). This pathway is under the inhibitory control of the phosphate and tensin homologue deleted on chromosome ten (PTEN) anti-oncogene, which converts PIP3 back to PIP2 and down-regulates the expression levels of IGF1-R (Maehama and Dixon, 1999; Maier et al., 2004). An additional point of regulation is provided by the p44:p53 ratio. p44 is a naturally occurring short isoform of the transcription factor p53 (Scrable et al., 2005), and seems to act by stabilizing p53 tetramers (Campisi, 2004). Conditions that favor p44 *versus* p53 will down-regulate PTEN – thus releasing its inhibitory block – and activate the signaling cascade downstream of PIP3, and the "fail-safe" pathway downstream of Ras/Raf (Maier et al., 2004) (some of the above events are schematically described in the left panel of Fig. 2).

Compelling evidence indicates that the IR/IGF1-R signaling pathway plays a major role in controlling maximum lifespan and, at some extent, the incidence/prevalence of age-associated diseases (Brown-Borg, 2003; Kenyon, 2005; Longo and Finch, 2003). IGF1-R's role during aging has been demonstrated in all species analyzed so far, including yeast, *Caenorhabditis elegans*, *D. melanogaster*, and mammals (Brown-Borg, 2003; Kenyon, 2005; Longo and Finch, 2003). A partial block of the IGF1-R signaling pathway is also achieved by caloric restriction, which extends the maximum lifespan and delays many biological changes that are associated with aging (Sohal and Weindruch, 1996; Weindruch and Sohal, 1997).

The initial evidence linking the IGF1-R signaling pathway to the general process of aging came from *C. elegans*, where mutations in *age-1*, coding for PI3K (Fig. 2, right panel) resulted in extension of adult lifespan (Friedman and Johnson, 1988; Klass, 1983). This initial observation was later on linked directly to IGF1-R when mutations in *daf-2*, coding for the common insulin/IGF1 receptor, were found to double the maximum lifespan of the animal (Kenyon et al., 1993; Kimura et al., 1997). These effects were also observed with mutations of the *D. melanogaster* ortholog

of the common insulin/IGF1 receptor (Tatar et al., 2001). Similar approaches led also to the identification of *daf-16*, coding for a FOXO family transcription factor, *daf-18*, coding for PTEN, and *chico*, coding for an insulin/IGF1 receptor substrate (IRS)-like signaling protein (reviewed in Kenyon, 2005; Tissenbaum and Guarente, 2002).

Later on, *C. elegans* served to show that DAF-2 acts upstream of AGE-1, which in turn regulates DAF-16, therefore providing a clear molecular cascade that controls aging in the worm (Kenyon, 2005; Longo and Finch, 2003). DAF-16 is the apparent final downstream output of this pathway, as indicated by the fact that null mutations of *daf-16* suppress the phenotype produced by the double-mutant combination of *daf-2* and *age-1* (Lin et al., 1997; Ogg et al., 1997). In addition, the regulation of *daf-16* seems to involve lipophilic-hormone signaling between the reproductive system and the intestine of *C. elegans*, can be modulated by different outputs (including *kri-1* and *daf-9*), but can also be bypassed by a parallel and partially independent pathway mediated by *daf-12* (Berman and Kenyon, 2006; Gerisch et al., 2001; Hsin and Kenyon, 1999; Motola et al., 2006; Rottiers et al., 2006). Finally, the *daf-2*/*daf-16* pathway seems also to be – at least in part – under the control of the *lin-4*/*lin-14* system, which provides a regulatory function of IGF1-R signaling in the adult tissue of *C. elegans* (Antebi, 2005; Boehm and Slack, 2005). Therefore, a complex cross-talk between different genes (and tissues) is required to achieve fine tuning of a program that determines the lifespan and aging process of an organism. A more detailed overview of the many genes found to modulate lifespan in different model organisms can be found in Refs. (Beckstead and Thummel, 2006; Kenyon, 2005; Longo and Finch, 2003; Tissenbaum and Guarente, 2002). It is worth stressing the fact that the longevity induced by *daf-2* mutations requires the functional activity of AAK-2, the catalytic subunit of the AMP-activated protein kinase (AMPK), which regulates many metabolic and transcriptional events (Apfeld et al., 2004). In *D. melanogster*, AMPK exerts an inhibitory control on the biosynthesis of fatty acids and isoprenoids, and on the hydrolysis of diet-derived cholesterol esters. Disruption of AMPK in *D. melanogaster* produces the *loe* phenotype, which is characterized by a vacuolar form of neurodegeneration and by a marked increase in the α/β-like shedding of APPL, the fly homolog of human APP (Tschape et al., 2002). Therefore, an increased activity of AMPK extends lifespan in the worm (Apfeld et al., 2004), whereas a functional inactivation induces a dramatic form of neurodegeneration and activates the processing of APPL in the fly (Tschape et al., 2002).

In contrast to worms and flies, mammals have separate receptors for insulin and IGF1. Homozygous disruption of IGF1-R in mice is not viable; however, heterozygous animals lacking only one allele of the receptor have a lifespan that is longer than wild-type (∼30% in females and ∼18% in males; the difference in males did not reach statistical significance) with no apparent manifestation of disease (Holzenberger et al., 2003). Similar results (∼18% increase in lifespan) were obtained with the selective disruption of IR in the adipose tissue (Bluher et al., 2003). This effect is not limited to the receptor itself, but can also be extended to the ligand. In fact, Ames dwarf mice, which have a defect in the pituitary gland and do not secrete IGF-1 in the plasma, live longer than normal animals and exhibit normal cognitive functions (even in old age) (Brown-Borg, 2003; Brown-Borg et al., 1996; Flurkey et al., 2001).

In contrast to the above, hyper-activation of the IGF1-R signaling pathway in $p44^{+/+}$ transgenic mice accelerates the progression of aging and shortens the maximum lifespan (Maier et al., 2004). The $p44^{+/+}$ phenotype is reminiscent of the $p53^{+/m}$ mice, where truncation of the N-terminal domain of p53 generates a hypermorphic p53 and leads to short lifespan and early onset of aging phenotypes (Tyner et al., 2002). The $p44^{+/+}$ mouse system offers a very attractive model by delineating a previously unknown level of regulation of IGF1 signaling (Maier et al., 2004). The involvement of the p53:p44 system in the regulation of lifespan and age-related events has received further demonstration by the observation that the genetic disruption of p53 reverts the accelerated aging phenotype observed in $Zmpste24^{-/-}$ mice (Varela et al., 2005).

It is also important to point out that p53 is found mutated in many forms of cancers, suggesting that the p53:p44 ratio regulates the balance between excessive growth/tumor generation and reduced growth/early senescence (Campisi, 2004). Interestingly enough, $p44^{+/+}$ mice, which have increased p53 activity, show a premature loss of neurogenic capacity in the brain that is linked to accelerated brain aging (Medrano and Scrable, 2005). Notably, the altered p53:p44 activity observed in $p44^{+/+}$ mice affects the balance between cell proliferation and cell death by affecting only the rate of cell proliferation—but not the rate of cell death. Therefore, loss of p53 activity leads to accelerated cell growth (and cancers), whereas increased p53 activity leads to early arrest of cell growth, premature aging, and increased production of Aβ (see later).

Although IGF1 levels in the cerebrospinal fluid (CSF) exhibit no change or a slight increase during aging (Carro and Torres-Aleman, 2004), the expression levels of the receptor (IGF1-R) show a dramatic and progressive increase in both cortex and hippocampus that correlates with learning deficits in aged rats (Chung et al., 2002a,b; Stenvers et al., 1996). A recent study in the brain of normally aged individuals has also found an age-associated increase in the expression of IR (Lu et al., 2004). Finally, correlation studies in normally aged volunteers confirm that disturbances in the IGF1/IGF1-R pathway are associated with poor performance on neuropsychological tests evaluating different cognitive functions normally affected by age (Sytze van Dam and Aleman, 2004). These events are not reverted by administration of GH or normalization of serum IGF1 levels, suggesting – but not proving – a direct involvement of the receptor-mediated signaling pathway in the brain rather than plasmatic IGF1 (Sytze van Dam and Aleman, 2004).

4. IGF1-R, neurotrophin signaling, and AD: a tale of receptors and second messengers

4.1. $p75^{NTR}$ and ceramide: an unexpected link

While trying to analyze the role that the cell-surface sphingolipids GM1 and sphingomyelin (SM) played in the clustering of APP into cholesterol-rich-domains (lipid rafts) and in the metabolism of Aβ, we surprisingly found that ceramide, the product of SM hydrolysis, could regulate the rate of Aβ generation by affecting the molecular stability of BACE1 (Puglielli et al., 2003a). Ceramide is a lipid second messenger that can regulate many of the biochemical and genetic events that occur during senescence/aging, and is, therefore, an attractive candidate as molecular bridge between normal aging and AD.

The existence of a relationship between ceramide and "aging signaling" is mostly indicated by cellular studies showing that both exogenous and endogenous ceramide can reduce the replicative capacities of primary cells and induce a senescent phenotype (Venable et al., 1995; Venable and Obeid, 1999). It is worth remembering that somatic cells have a limited lifespan in culture beyond which they can no longer proliferate (also known as the "Hayflick limit") (Shay and Wright, 2000). The endpoint of this mitotic life in culture has been termed "cellular senescence". Senescence *in vitro* does not imply death in cells any more than *in vivo* senescence; indeed, cells may live for years after they stop dividing. The length of the proliferative lifespan inversely correlates with both the age of the donor organism and the life expectancy of the species from which the cells were obtained (Smith and Pereira-Smith, 1996). Finally, spontaneous senescence is a dominant and irreversible process that can only be overridden by DNA tumor viruses, and delayed (at least in certain instances) by overexpression of telomerases (Cristofalo et al., 2004; Venable and Obeid, 1999). As a result, cellular senescence is acknowledged as a model for aging research. Indeed, many of the factors that influence cellular senescence have also been involved with longevity; conversely, molecular/genetic events that are affected by organismal aging are also affected by cellular senescence (Shay and Wright, 2000; Smith and Pereira-Smith, 1996; Venable and Obeid, 1999).

An interesting connection between replicative capacity of a cell and ceramide comes from the yeast *Saccharomyces cerevisiae*, which shows a marked increase in lifespan when a gene called longevity-assurance gene 1 (*LAG1*) is deleted (D'Mello et al., 1994). Lag1 resides in the endoplasmic reticulum and shows C26-ceramide synthase activity both *in vivo* and *in vitro* (Guillas et al., 2001; Schorling et al., 2001). The extension of lifespan by *LAG1* deletion in the yeast is rescued by the human homolog *LAG1Hs*, which is highly expressed in the brain, along with testis and skeletal muscle (Jiang et al., 1998). Even though it is difficult to draw a direct connection between the yeast phenotype and mammalian aging, the fact that ceramide levels increase during cellular senescence and organismal aging (Cutler et al., 2004; Lightle et al., 2000; Miller and Stein, 2001; Mouton and Venable, 2000) adds support to the concept that this lipid plays an important role in longevity and age-associated events. Of particular interest is also the fact that ceramide levels are higher (approximately three-fold) in the brains of AD patients, when compared to age-matched controls (Cutler et al., 2004; Han et al., 2002).

Following our initial observation, we have recently found that ceramide production in the mouse brain is activated in an age-dependent fashion (Costantini et al., 2005b), and is closely followed by a parallel increase in BACE1 steady-state levels, β cleavage of APP, and Aβ generation. The age-associated activation of ceramide was specifically linked to the expression levels of the tyrosine-kinase A (TrkA) and p75 ($p75^{NTR}$) neurotrophin receptors. Indeed, an expression pattern in which TrkA predominates in younger animals switches to one in which $p75^{NTR}$ predominates in older animals. The TrkA to $p75^{NTR}$ molecular switch is responsible for an age-dependent activation of nSMase, the enzyme that is responsible for the hydrolysis of SM and consequent liberation of the second messenger ceramide (Fig. 3, right panel). The strict requirement for $p75^{NTR}$ was proved by using animals expressing a truncated version of the receptor that lacks the ligand-binding domain ($p75^{NTRExonIII-/-}$), whereas the requirement for ceramide was confirmed by treating wild-type animals with an irreversible and competitive inhibitor of nSMase (Costantini et al., 2005b). In addition, nSMase inhibitors were not effective when used with either primary neurons or animals that express a signaling inactive form of $p75^{NTR}$, therefore positioning ceramide down-stream of $p75^{NTR}$ (Fig. 3, right panel) (Costantini et al., 2005b). Finally, when NGF was administered to neuroblastoma cell lines expressing either TrkA or $p75^{NTR}$, only $p75^{NTR}$ expressing cells responded by increasing ceramide production and the β cleavage of APP (Costantini et al., 2005b). In contrast, TrkA expressing cells showed an apparent decrease in β cleavage of APP, suggesting a dual and differential role in the regulation of the initial enzymatic step for Aβ generation. However, this effect might not be limited to β cleavage since TrkA activation seems also able to reduce the generation of AICD (Tarr et al., 2002).

The use of a cellular system where only one receptor was expressed proved to be very important. In fact, when analyzing TrkA and $p75^{NTR}$ binding to NGF, we must be aware of the fact that TrkA acts as homo-dimer (TrkA–TrkA) or hetero-dimer (TrkA–$p75^{NTR}$) (Fig. 4) forming high-affinity binding complexes with NGF (the normal stoichiometry of the NGF:TrkA binding is 2:2), whereas $p75^{NTR}$ can bind to NGF as a monomer, with a stoichiometric ratio of 2:1 (NGF:$p75^{NTR}$) (He and Garcia, 2004; Zampieri and Chao, 2004). One "bizarre" consequence of this is that the TrkA–$p75^{NTR}$ complex forms a high-affinity binding site ($K_d \sim 10^{-10}$ to 10^{-11} M instead of 10^{-8} to 10^{-9}) for the mature NGF that transduces TrkA – but not $p75^{NTR}$ – signals (Kalb, 2005). This issue, which was initially interpreted as a simple controversy caused by the different experimental approaches used, is now being reinterpreted as a fundamen-

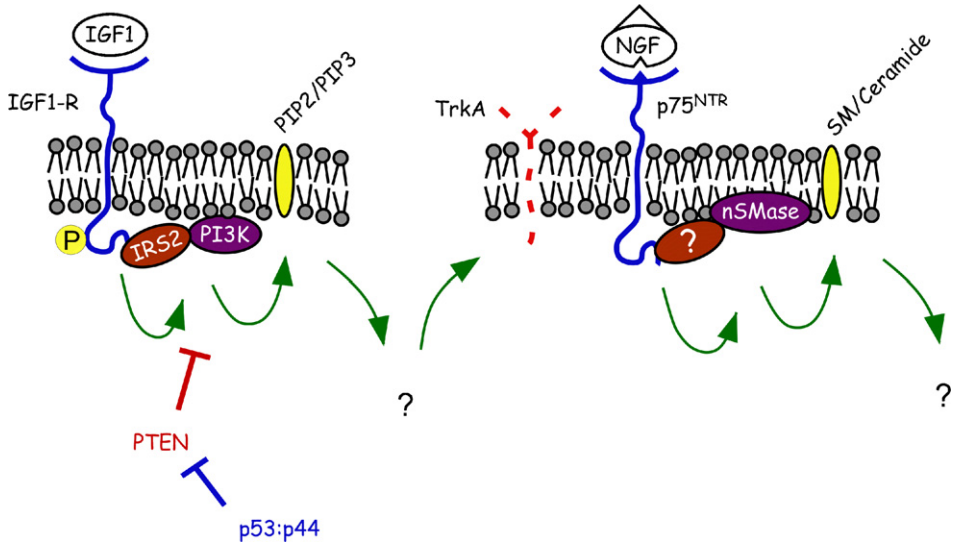

Fig. 3. IGF1-R acts upstream of $p75^{NTR}$ in regulating Aβ generation during aging. The progressive activation of IGF1-R expression levels and signaling activity in the brain that characterizes aging leads to a switch in the expression levels of neurotrophin receptors TrkA and $p75^{NTR}$ (Costantini et al., 2005b, 2006). This event requires the adaptor protein IRS2 and class I PI3Ks, which phosphorylate PIP2 at position 5 of the inositol head group activating the second messenger PIP3. The activation of $p75^{NTR}$ leads to a progressive and age-associated activation of nSMase, which results in hydrolysis of SM and liberation of the active second messenger ceramide (Costantini et al., 2005b). Ceramide, in turn, is responsible for the molecular stabilization of BACE1, the rate-limiting enzyme for the generation of Aβ (Puglielli et al., 2003a). The question marks indicate the signaling cascades that act downstream of PIP3 and ceramide, which are still unknown. Similarly, the adaptor protein that allows $p75^{NTR}$ to activate nSMase has not been identified yet.

tal and important property of neurotrophin signaling (Chao, 2003; Kalb, 2005; Nykjaer et al., 2005). Therefore, overexpression of $p75^{NTR}$ on a TrkA background may very well potentiate TrkA-, rather than $p75^{NTR}$-mediated events (Fig. 4). Indeed, TrkA can "silence" $p75^{NTR}$ signaling in cellular systems (Kalb, 2005; Plo et al., 2004), in addition to promoting $p75^{NTR}$ proteolytic clearance (Domeniconi et al., 2005; Jung et al., 2003; Kanning et al., 2003; Weskamp et al., 2004) through a mechanism that seems to involve both trans-activation (Kanning et al., 2003) and ligand-binding

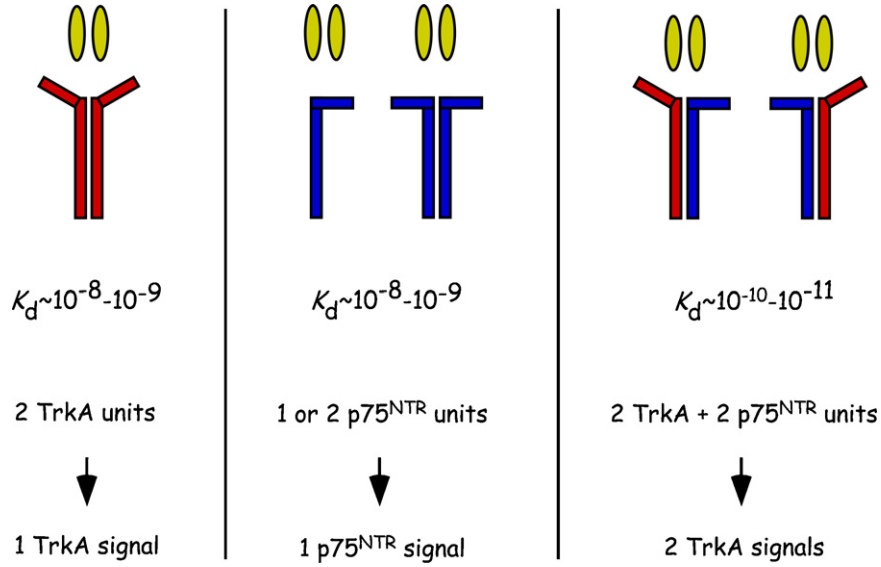

Fig. 4. The stoichiometry of binding with NGF influences the signals transduced by TrkA and $p75^{NTR}$. TrkA can bind NGF both as homo-dimer (TrkA + TrkA, left panel) or hetero-dimer (TrkA + $p75^{NTR}$, right panel) (He and Garcia, 2004; Kalb, 2005; Zampieri and Chao, 2004). In contrast, the crystal structure of the $p75^{NTR}$/NGF complex indicates that the functional NGF dimer binds a single $p75^{NTR}$ monomer (middle panel)—even though it does not rule out the possibility for NGF to engage $p75^{NTR}$ as a homodimer (He and Garcia, 2004). The affinity binding of the NGF dimer:TrkA homodimer does not differ from the NGF dimer:$p75^{NTR}$ monomer (or homodimer). However, the TrkA + $p75^{NTR}$ complex has a higher affinity ($K_d \sim 10^{-10}$ to 10^{-11} *vs.* $K_d \sim 10^{-8}$ to 10^{-9}) for NGF (discussed in the text). Therefore, expression of $p75^{NTR}$ on a TrkA background may facilitate the generation of bimolecular (1 TrkA + 1 $p75^{NTR}$) signaling complexes transducing TrkA – and not $p75^{NTR}$ – signals.

(Domeniconi et al., 2005). These events may all be part of a rather complex form of regulation of neurotrophin signaling, potentially influenced by aging itself (Bergman et al., 1999; Costantini et al., 2005b, 2006; Johnson et al., 1999).

$p75^{NTR}$ is a member of the tumor necrosis factor receptor superfamily (TNFRSF) and interacts with all the different members of the neurotrophic factor family: the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4 (reviewed in Ibanez, 1998). In addition to $p75^{NTR}$, the above neurotrophins also bind to tyrosine kinase receptors (TrkA, TrkB, and TrkC). Signaling specificity is determined by ligand-binding affinities to the different receptors. Several studies have shown the existence of cross-talk between the different neurotrophins and the Trk receptors: more than one neurotrophin may bind to the same Trk receptor, and more than one Trk receptor may bind to the same neurotrophin (reviewed in Ibanez, 1998). Even in the case of $p75^{NTR}$, there seem to be subtle differences in the ligand-binding affinities, which allow $p75^{NTR}$ to discriminate between the different neurotrophins.

Like all other TNFRSF members, $p75^{NTR}$ can transduce both death and survival signals (Dechant and Barde, 2002). This may involve either different affinities to the ligand(s) (Ibanez, 1998), in concert with co-receptors that would determine the exact message to be translated (Mi et al., 2004; Nykjaer et al., 2004), or cytoplasmic interactors, which modulate the interaction of the receptor with the "active" signaling molecule (Dechant and Barde, 2002; Zampieri and Chao, 2004). On this front, it is important to stress the fact that $p75^{NTR}$ is devoid of intrinsic catalytic activity and, therefore, its signaling abilities rely on intracellular interactors. In the case of the second messenger ceramide, it is not known how $p75^{NTR}$ activates nSMase. Whether such activation requires adaptor proteins, physical "connection", or molecular interaction with nSMase remains to be determined.

Mature NGF normally interacts with both TrkA and $p75^{NTR}$ with similar affinities (dissociation constant K_d in the 10^{-8} to 10^{-9} M range) (Kalb, 2005). However, pro-NGF, the uncleaved form of NGF, binds to $p75^{NTR}$ with five times greater affinity than mature NGF, and shows very low affinity binding to TrkA (Lee et al., 2001a). This might be particularly relevant, since the levels of pro-NGF found in the brain of AD patients are higher than in age matched controls (Fahnestock et al., 2001; Peng et al., 2004), therefore providing a further level of risk over that already provided by normal aging. This line of thought seems to find its own validation from the AD11 mouse model expressing transgenic antibodies that neutralize brain NGF (reviewed in Ref. (Capsoni and Cattaneo, 2006)). In fact, these mice develop several features of AD neuropathology, which include high Aβ levels, amyloid plaques, and neurofibrillary tangles. This phenotype was initially interpreted as solely caused by the lack of NGF signaling in the areas of the brain that are typically affected by AD pathology. However, it was soon realized that the transgenic NGF-neutralizing antibody produced by AD11 mice had very low or no affinity to pro-NGF, suggesting an alternative model in which the lack of NGF signaling (most likely through TrkA) would facilitate pro-NGF signaling through $p75^{NTR}$. This model found its own validation when it was observed that genetic disruption of $p75^{NTR}$ completely abolished the high Aβ levels, the intracellular deposits of Aβ, and the plaque pathology observed in these mice. In conclusion, the different experimental approaches employed with the AD11 mice (reviewed in Capsoni and Cattaneo, 2006) overall suggest that when NGF is neutralized by the recombinant anti-NGF antibodies, pro-NGF signals through $p75^{NTR}$, and activates the production of Aβ and its consequent aggregation/accumulation into intracellular and extracellular (amyloid plaques) deposits. Conversely, when $p75^{NTR}$ is removed or NGF signal (through TrkA) is restored, the amyloid pathology is also abolished. Therefore, a complex set of interactions between ligands (NGF and pro-NGF) and receptors (TrkA and $p75^{NTR}$) might be acting upstream of the cascade of events that leads to AD (also discussed below).

The connection between the TrkA/$p75^{NTR}$ receptor system and AD neuropathology seems to find additional validation from the association between gender and AD. In fact, it is well known that women experience a sharp increase in the risk of developing AD after menopause (Henderson, 1997; Manly et al., 2000). This is further strengthened by the fact that ovariectomy promotes the amyloidogenic processing of APP (Jaffe et al., 1994; Petanceska et al., 2000; Xu et al., 1998; Zheng et al., 2002), and that these effects are partially or totally reverted by estrogen treatment (Petanceska et al., 2000; Zheng et al., 2002). Animal studies have also shown that surgical ovariectomy increases the expression of $p75^{NTR}$, while decreasing TrkA, at both mRNA and protein levels. These effects are completely abrogated by exogenous estrogens (Gibbs, 1998; Jover et al., 2002; Lanlua et al., 2001; Ping et al., 2002). Therefore, the loss of ovarian function after menopause would only aggravate the age-associated changes in neurotrophin receptors, further increasing the risk for AD in normally aging women.

It is also worth noting that different studies have found a selective and marked reduction in the expression of cortical TrkA in early and late stages of AD, when compared to age-matched controls (Counts et al., 2004; Hock et al., 1998; Mufson et al., 2002, 2003). This seems to occur in the face of stable (Counts et al., 2004; Hock et al., 1998; Mufson et al., 2002) or increased (Hu et al., 2002; Mufson and Kordower, 1992) $p75^{NTR}$, and increased pro-NGF (Counts and Mufson, 2005; Fahnestock et al., 2001; Peng et al., 2004). Because pro-NGF preferentially binds to $p75^{NTR}$ (reviewed in Lee et al., 2001a), the above changes ultimately lead to increased activation of $p75^{NTR}$ signaling. Interestingly, $p75^{NTR}$ expressing cholinergic neurons seem to be more susceptible to cell death (reviewed in Coulson, 2006) and are also characterized by increased accumulation of hyperphosphorylated tau (Hu et al., 2002). Therefore, the above studies seem to support the notion that the balance between TrkA and $p75^{NTR}$ signaling in the hippocampus and cortex might be directly linked to the three classical features of AD: Aβ, tau, and neuronal death.

4.2. IGF1-R and PIP3: the common culprit

The fact that caloric restriction was able to block/revert the TrkA to $p75^{NTR}$ switch in mice (Costantini et al., 2005b) led us to explore the potential role of IGF1-R, the common regulator of lifespan. Studies *in vitro* with both human neuroblastoma cell lines and primary neurons showed that indeed IGF1-R acts upstream of both receptors (Costantini et al., 2006) (Fig. 3). Both genetic silencing of IGF1-R or biochemical inhibition of PI3K, downstream of IGF1-R, were able to block the TrkA to $p75^{NTR}$ switch (Costantini et al., 2006). Interestingly enough, primary neurons in culture were found to go through a neurotrophin receptor switch reminiscent of that previously observed in the brain of normally aging animals: an expression pattern in which TrkA predominates in days 0–3 (in culture) neurons switches to one in which $p75^{NTR}$ predominates in days 18–24 neurons. This was closely followed by activation of the second messenger ceramide, stabilization of BACE1, and increased production of Aβ (Costantini et al., 2006). Gene-silencing approaches directed toward *Igf1-r* were able to block the entire cascade of events, therefore proving that IGF1-R acts upstream. Finally, analysis of both primary neurons and brain tissue (cortex and hippocampus) from $p44^{+/+}$ mice, which have a constitutive hyper-activation of IGF1-R signaling, showed an early and accentuated TrkA to $p75^{NTR}$ switch, together with a parallel activation of ceramide and Aβ generation. The use of both genetics and biochemistry indicated that these events require the adaptor protein IRS2, PI3K, and conversion of PIP2 into the active second messenger PIP3 (Fig. 3).

The above events were found to be under the regulatory control of PTEN and p44: PTEN can block the TrkA to $p75^{NTR}$ switch downstream of IGF1-R by converting PIP3 into its inactive precursor PIP2, whereas p44 can block PTEN, therefore releasing the inhibitory function of PTEN (Costantini et al., 2006). It is worth noting that AD brains are characterized by decreased levels of PTEN (similarly to $p44^{+/+}$ mice) and hyper-activation of Akt downstream of IGF1-R (An et al., 2003; Griffin et al., 2005; Li et al., 2005). It is also worth mentioning that *ex vivo* studies revealed that the IRS "branch" of IR/IGF1-R signaling can promote tau phosphorylation (Lesort and Johnson, 2000; Schubert et al., 2003), potentially linking the two major hallmarks of AD (Aβ and tau) to the same upstream molecular pathway. Therefore, the studies implicating IGF1-R signaling to lifespan and aging have now been connected to those that implicate neurotrophin signaling to AD neurobiology, providing a molecular bridge between aging itself and one of the most common forms of age-related diseases.

Even though the main topic of this review is the signaling pathway acting downstream of IGF1-R, and its role in aging and AD neuropathology, we must be aware that IGF1, the preferential ligand for IGF1-R, is also a growth factor with trophic activities that not always require IGF1-R. In fact, IGF1 can protect from cell death *in vitro* (even in cells that do not express IGF1-R) and can exert neuroprotective effects *in vivo* (reviewed in Carro et al., 2003). Some of the neuroprotective effects documented *in vivo* seem to involve the cell-surface receptor megalin/LRP2 (Carro et al., 2005, 2006a,b) and/or transport/clearance across the BBB (Carro et al., 2005, 2006b). Additionally, plasmatic IGF1 can be naturally cleaved into des-N (1–3)-IGF-1 and the N-terminal tripeptide, glycine-proline-glutamate (GPE-IGF1) (Sara et al., 1989; Yamamoto and Murphy, 1994, 1995a,b). Without interacting with IGF1-R, GPE-IGF1 can cross the BBB, can stimulate dopamine and acetylcholine release *in vitro*, and can protect neurons from hypoxic–ischemic injury (Guan et al., 2000a,b; Nilsson-Hakansson et al., 1993). Finally, some of the anti-apoptotic/pro-survival effects induced by IGF1 *in vitro* do seem to involve signaling molecules that traditionally act downstream of IGF1-R. However, these actions also occur in cells that do not express IGF1-R and must not be confused with IGF1-R signaling. Therefore, we must always be aware of the fact that IGF1 means more than just IGF1-R.

5. PIP3 and ceramide: two different lipids with the ability to act as second messengers

The sequence of events that leads from IGF1-R to Aβ requires two lipids, PIP3 and ceramide, that share a few similarities, even though they are very different. They both are found – mostly, but not only – in the plasma membrane; they both have a fatty acid moiety that anchors them to the lipid bilayer (Fig. 5A and C); they both exist as inactive precursors (PIP2 and SM) under static conditions and are activated only following extracellular signals (Fig. 5B and D); and finally, they both can act as second messengers and transduce messages from the extracellular *milieu* to the inside of the cell.

5.1. Ceramide

The initial step in ceramide biosynthesis occurs in the endoplasmic reticulum with the condensation of serine and palmitoyl-CoA, followed by reduction of 3-ketosphinganine, acylation of the amide group of sphinganine, and final dehydrogenation of dihydroceramide. The predominant long-chain base of ceramide is sphingosine (shown in red in Fig. 5A), although sphinganine is also found in very low amounts. Once these initial steps are completed, ceramide is transported to the Golgi apparatus by two different mechanisms: one protein-mediated, which requires a ceramide-specific transfer protein (CERT), and another vesicular-mediated (Hanada et al., 2003; Kumagai et al., 2005). The ceramide that will serve as substrate for the generation of sphingomyelin is transported by CERT, whereas the one that will serve as substrate for glycosylation is translocated by the vesicular-mediated mechanism, indicating that the biosynthesis of glysphingolipids is segregated and compartmentalized very early in the pathway (for review, see also

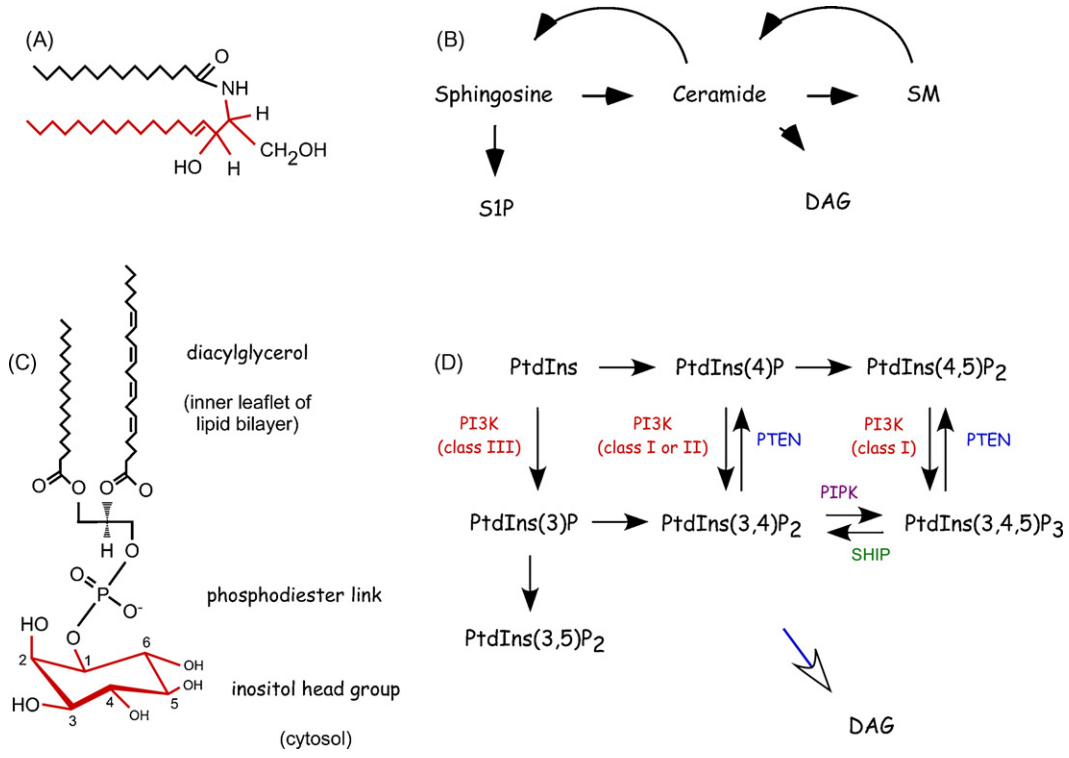

Fig. 5. Schematic view of the ceramide and PIP3 "cycles". The full description of the structures and pathways is in the text.

Futerman and Riezman, 2005; Hannun and Luberto, 2004; Munro, 2003).

The initial step in the biosynthesis of major glycosphingolipids requires the generation of glucosylceramide by a ceramide-specific glucosyltransferase, which then will serve as backbone for further glycosylation. All these glycosylation steps occur in the Golgi apparatus. In contrast, the biosynthesis of sphingomyelin (SM), which also occurs in the Golgi apparatus, requires only the addition of choline. The donor of the choline moiety is phosphatydylcholine (PC) and the enzyme that carries out the reaction is the sphingomyelin synthase (Futerman, 1994). Even though most of the SM is generated very early in the Golgi apparatus (*cis*- and *medial*-Golgi), a SM synthase activity has also been described at the plasma membrane (PM), which could account for as much as 10–15% of total cell SM synthase (Futerman, 1994; Futerman and Riezman, 2005).

Following its biosynthesis in the Golgi apparatus, SM is translocated to the PM, where it is mostly clustered into lipid rafts in tight association with cholesterol. At the PM, SM can undergo hydrolysis and liberate ceramide plus choline. The enzyme that carries out this reaction is called neutral sphingomyelinase 2 (nSMase2) (Hofmann et al., 2000; Marchesini et al., 2003; Zumbansen and Stoffel, 2002) and is different from the lysosomal-based SMase that is active at low pH (hence the name acid SMase) and involved in the catabolism of ceramide-containing sphingolipids in the lysosomal compartment (Sandhoff and van Echten, 1994; Venkataraman and Futerman, 2000).

The ceramide generated at the cell surface following hydrolysis of SM has signaling abilities and acts as a lipid second messenger. It is not clear whether SM hydrolysis occurs on the outer or inner leaflet of the PM bilayer. However, ceramide can rapidly equilibrate ("flip-flop") across the membrane bilayer; therefore this issue does not pose any topographical obstacle to the activity of the second messenger (Venkataraman and Futerman, 2000). Cell surface ceramide can then recycle back to SM. Whether this step requires retrotranslocation to the Golgi apparatus is not clear yet. However, the fact that both the donor of choline, PC, and the enzyme, SM-synthase, are found in the PM may suggest a local form of regulation of the "ceramide cycle" (Futerman and Riezman, 2005). Even though the above cycle is regarded as the main pathway for the metabolism and regulation of the signaling activities of ceramide, it is important to stress that local pools of SM/ceramides are also found in other cellular membranes. Whether they can elicit signaling events limited to a specific organelle is still under debate.

When dealing with the signaling activities of ceramide, it is always important to consider the fact that the transfer of the choline phosphate head group from PC to ceramide by SM synthase results in the formation of SM and diacylglycerol (DAG). In addition, following SM hydrolysis, ceramide can be converted to sphingosine, which can be phosphorylated to sphingosine-1-phosphate (S1P). Since DAG, sphingosine, and S1P can also act as signal transducers, it is not difficult to conceive how one or more of the enzymatic steps delineated above (and schematically shown in Fig. 5B) can result in

very complex (and sometimes opposite) biological responses (reviewed in Yang et al., 2004). Indeed, it is not unusual to consider ceramide, DAG, sphingosine, and S1P as a "biostat sensor" system that allows the cell to adapt and respond to very diverse situations.

5.2. PIP3

Phosphoinositides (PtdIns) are low abundant lipids (less than 5% of total membrane phospholipids) that are mainly found in the cytoplasmic leaflet of cellular membranes. They can be phosphorylated in three different positions – 3, 4, and 5 – of their inositol head group (Fig. 5C), generating different species (Fig. 5D) that have different biological functions. PtdIns(3)P and PtdIns(4,5)P_2 (also known as PIP2) are the most abundant and seem quite static. They are mostly found in the PM, endosomes, and, although in smaller amounts, in the Golgi apparatus, where they serve as an anchor for the sorting/targeting of proteins containing the pleckstrin homology (PH) domain and as regulators of endocytosis (Vanhaesebroeck et al., 2001). The bis-phosphoinositide, PtdIns(3,4)P_2, and the tris-phosphoinositide, PtdIns(3,4,5)P_3 (also known as PIP3) are not abundant and seem to be very dynamic, showing rapid changes in response to extracellular stimuli.

The critical phosphorylation of the inositol head group at position 3 requires a specific set of enzymes collectively called phosphatidylinositol-3-kinases (PI3Ks) or phosphoinositide 3-kinases (reviewed in Deane and Fruman, 2004). Depending on their subunit structure and substrate selectivity, PI3Ks are normally categorized as classes I–III. The conversion of PtdIns into PtdIns(3)P is mainly performed by class III PI3Ks, whereas the conversion of PtdIns(4)P into PtdIns(3,4)P_2 can be performed by both classes I and II PI3Ks. Finally, class I are the only capable of converting PIP2 into the critical and better characterized second messenger PIP3 (Fig. 5D). PI3K is a hetero-dimer composed of a catalytic and regulatory subunit. The catalytic subunit exists in three isoforms (p100α, p110β, and p110δ), whereas the regulatory subunit exists in five different isoforms (p85α, p85β, p55α, p55γ, and p50α) produced by differential splicing of three genes (reviewed in Deane and Fruman, 2004). When inactive, PI3K is found in the cytoplasm while its substrate, PI(4,5)P_2, resides on the cytoplasmic leaflet of the PM. Upon binding to its extracellular ligand, IGF1-R associates and phosphorylates IRS proteins. The phosphorylated-Tyr-X-X-Met sequence on IRS proteins then serves to recruit and activate the regulatory subunits of PI3K. This event is followed by the assembly of the active PI3K heterodymer that phosphorylates PI(4,5)P_2 to produce the second messenger PI(3,4,5)P_3.

In addition to the above and more studied PI3K-mediated pathway, PIP3 can also originate following phosphorylation at position 5 of the inositol group on PtdIns(3,4)P_2 by type I phosphatidylinositol phosphate kinase (PIPK) (reviewed in Giudici et al., 2004). This previously unknown biochemical reaction can occur both *in vitro* (Zhang et al., 1997) and *in vivo* (Halstead et al., 2001), and seems to act downstream of oxidative stress rather than classical growth factors, potentially providing signal specificity to the signal transduced by PIP3. PIPK seems to be more promiscuous than PI3K and could be involved in the phosphorylation of additional positions of the inositol head group, playing an even more complex regulatory role (Hinchliffe, 2001).

The levels of PIP2 and PIP3 are tightly controlled by a dynamic equilibrium between the rate of phosphorylation (by PI3Ks) and dephosphorylation (by PTEN) at position 3 (Fig. 5D). PTEN has both protein (Li et al., 1997; Tamura et al., 1998) and lipid (Maehama and Dixon, 1999; Myers et al., 1998; Stambolic et al., 1998) phosphatase activities; however, most of the functions in signal transduction ascribed to PTEN seem to be mediated by its lipid phosphatase activity, which dephosphorylates position 3 of the inositol head group of PIP3 (Maehama and Dixon, 1999; Myers et al., 1998; Stambolic et al., 1998). In addition, PIP3 can also be dephosphorylated at position 5 by SH2-containing inositol 5′-phosphatase proteins (SHIP), which counteract PIPK (Fig. 5D). The recognition/binding capacity of SHIP seems to require the Src homology 2 (SH2) domain, which mediates binding of several additional proteins to PIP3 (Blazer-Yost and Nofziger, 2005). Therefore, generation of PIP3 not only recruits proteins that will transduce the signal, but also proteins that will limit the signal by deactivating PIP3 itself.

Mutations in PTEN and SHIP generate non-overlapping phenotypes, therefore underscoring the importance and differential roles played by the above lipids and enzymes (Blazer-Yost and Nofziger, 2005; Maehama and Dixon, 1999; Rohrschneider et al., 2000). The importance of the dynamic equilibrium between the different PtdIns species is also stressed by the fact that PIP2 is slowly emerging as a previously unrecognized signal transducer, rather that a simple inert molecule (Czech, 2000; Hinchliffe, 2000; Insall and Weiner, 2001). This is further complicated by the fact that phosphatidylinositols can be cleaved at the *myo*-inositol group, producing the soluble inositol phosphate and the lipid anchored DAG (Fig. 5D), which can also act as signal transducer (Berridge, 1987).

6. Conclusion

The last decade has surprisingly shown that the lifespan of an organism can be modulated by the signaling pathway that acts downstream of the IGF1-R, indicating that there is a "program" that drives the process of aging. New studies have now shown that the same pathway can regulate neurotrophin signaling, the neurogenic capacity of the brain, and the rate of Aβ generation. Therefore, a complex molecular cascade seems to link the normal process of aging to one (or more) of the initial events involved with the pathogenesis of AD. Obviously, we still need to identify all the signaling molecules that act downstream of PIP3 and ceramide (Fig. 3). This might

be easier for PIP3, since many target molecules that are activated/recruited by PIP3 are already known. In addition, the presence of the PH domain can serve to screen for new potential targets that have not yet been linked to PIP3 signaling. In contrast, ceramide signaling is still mostly uncertain; specifically, it is not know how the signal is transferred and whether ceramide requires a specific targeting sequence/domain(s) to recruit downstream molecules. In addition, the mechanism(s) that allow $p75^{NTR}$ itself, which is devoid of any catalytic activity, to activate nSMase are still completely unknown. This might require initial dissection of the function(s) of the cytosolic tail of the receptor. For example, the identification of the specific sequence that is responsible for the $p75^{NTR}$-mediated activation of ceramide might lead to the identification of the adaptor protein(s) responsible for the recruitment/activation of nSMase.

Finally, the specific mechanism that leads to the TrkA-to-$p75^{NTR}$ switch downstream of IGF1-R remains to be determined. In theory, the expression levels of $p75^{NTR}$ could be regulated at three different levels: increased transcription/translation, reduced clearance, and increased molecular stability. The last two events are intimately related because a reduction in clearance would also lead to increased half-life of $p75^{NTR}$. On this regard, the fact that TrkA can stimulate the proteolysis of $p75^{NTR}$ (Kanning et al., 2003) seems to support a possible post-translational event. Indeed, the down-regulation of TrkA would reduce the proteolytic clearance of $p75^{NTR}$. However, transcriptional regulation is normally regarded as the primary mechanism used by aging to control the expression levels of a certain protein. Therefore, the primary mechanism could still involve an independent transcriptional regulation of TrkA and $p75^{NTR}$; the downregulation of TrkA, in turn, would further potentiate the effects on $p75^{NTR}$ by reducing the proteolysis of the receptor.

One additional argument that needs to be addressed – the role of IGF1-R in the progression of AD pathology – will require the generation of appropriate mouse models. In fact, even though the connection between IGF1-R and Aβ has been identified and characterized at the molecular level, we still need to analyze whether hyper-activation of IGF1-R signaling can accelerate the progression of AD-like pathology in a mouse model. This approach, if successful, would also allow testing possible biochemical strategies aimed at the modulation of IGF1-R signaling for the prevention of AD. Such a strategy cannot be performed with the current mouse models of AD, since they harbor human genes (APP and/or presenilins) carrying mutations associated with familial-forms of AD, and, therefore, not under the control of the aging program.

The main focus of this review was to bring together the findings that implicate IGF1-R in the regulation of lifespan to those that connect IGF1-R to neurotrophin signaling, the progressive loss of neurogenic capacity of the aging brain, and to the pathogenesis of AD. This connection is particularly relevant because aging itself is acknowledged as the single most important risk factor for AD. In fact, the prevalence of AD increases sharply after the age of 60 and doubles with every decade of life reaching ∼50% in individuals that are 85 years of age or older (Hebert et al., 2003; Thal et al., 2004). In addition, with increasing age, the frequency of higher neurofibrillary stages and higher Aβ stages in non-demented individuals increases as well, underscoring a strong relationship between aging and AD pathology (Thal et al., 2004). However, we still must remember that, by definition, risk factors (in this case, age) are neither necessary nor sufficient for the development of a disease. Indeed, approximately 50% of the individuals that are 85 years of age or older are not affected by any sign of dementia, proving that a successful aging is possible. Additional risk factors (either environmental or genetic) might be required to develop the disorder. Obviously, protective factors might also be involved in the protection of certain individuals. Therefore, a broad combination of different approaches (both age- and disease-oriented), combining different areas of biomedical research, including biochemistry, molecular biology, and genetics will be required to completely resolve the rise of the tragic and devastating disease described by Alois Alzheimer a century ago.

Conflict of interest

The author has no conflict of interest to disclose.

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While preparing the reference section, I tried to maintain a balance between original papers and reviews on selected topics. The reviews provide additional breadth for those who wish to gain more insights, whereas the original papers acknowledge and describe the original findings. However, due to the large number of references, I apologize to those whose work could not be included in the final version of this manuscript.

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