Modulation of Amyloid-β peptide aggregation and neurotoxicity in Alzheimer's disease

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Doctoral Thesis UPF 2016

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Biuse Guivernau Almazán: *Modulation of Amyloid-β peptide aggregation and neurotoxicity in Alzheimer's disease,* April 2016

Nothing in this world that's worth having comes easy

— Bob Kelso

'After I got my PhD, my mother
took great relish in introducing me as
"this is my son, he's a doctor
but not the kind that helps people"'

— Randy Pausch

ACKNOWLEDGEMENTS

Moltes gràcies al Paco, per donar-me la oportunitat d'unir-me a aquest gran grup. Per la llibertat de pensament i experimental que m'ha donat. Per aportar sempre un toc d'humor. Per animar-nos a seguir empenyent las *fronteras del conocimiento*, encara que de vegades no es deixin.

Al Miguel, al Chema i al Rubén, per proporcionar-me l'entorn ideal per créixer com a científica i com a persona. Pel *feedback* als seminaris i per fer que li agafés gustillo a l'*obscur món dels ions*. Encara guardo els apunts de fisiologia I i II en un lloc segur per si mai vull refrescar la memòria o he d'escriure 4 pàgines sobre el potencial d'acció[1]. Una menció especial al Rubén per posar sostre a tots els sopars de Nadal i educar-nos el paladar amb la seva selecció de vins i còctels.

A la Fanny, la Selma, la Mercè, l'Alejandro i Pablo. Pels coneixements de biologia molecular, els balls de Bollywood, partits de volei, per suggerir altres punts de vista als seminaris i per totes les kcal en forma de *brownie*. En definitiva, per les hores compartides i les experiències viscudes, tant a dins com a fora del laboratori.

Als Susios molones; us podria dir mil coses i em quedaria curta. A la Cris, per haver estat una pota d'aquesta tesi: Per tota l'ajuda,

1 No és broma.

la paciència, l'estar sempre disposada a escoltar-nos, per no fugir corrent cada cop que he entrat al despatx amb un melós *Criiiiis...* Per ser un engranatge indispensable del laboratori. Per demostrar-me tantes vegades que ets una amiga com poques. Al Gerard, pel seu mentoratge, el seu saber fer, per la introducció de la seva tesi que, usant les seves paraules, ho explica tot sobre el calci. Per una gran amistat, tot i les nostres bronques ocasionals. M'alegro molt d'haver-vos trobat. Per totes les converses fins a altes hores de la matinada, cada birra al marítim, cada nit a cal brut, cada *última i marxem*. Per totes les histories que hem fet viure a tots els bars, platges, terrats i carrers de Barcelona.

A l'Eva, la meva primera mentora. Perquè ha hagut de patir-me com una germana gran. Per tot el que m'ha ensenyat i tot el que hem rigut juntes. Perquè sempre ens quedarà muntar una empresa de fabricació de solucions isotòniques anomenada Webbius. I ho petarem.

A la Marta, perquè he après molt al teu costat. Per introduïr-me al món de la nostra estimada TPI i per tenir tanta paciència. Perquè sempre tens un somriure, una paraula amable i uns minuts per escoltar a tothom, i això s'agraeix molt. A la Mònica, per ser la meva millor *reviewer*. Per totes les discussions conceptuals i sobre protocols durant les hores de berenar. Per deixar el despatx amb olor de taronja i els teus tes de 15 L. Per ser la floreta del laboratori, perquè l'optimisme i el bon rollo que desprens són molt contagiosos. I si alguna cosa hauríem de fer tots és ser una mica floreta. A totes dues, per venir-me a veure a Berlin i pel viatge a Florència. Perquè algun dia hem d'anar a fer una *coppetta di crema di Grom* i sopar a l'*Acqua al 2*, encara que quedi una miqueta lluny.

A la Vicky, altrament anomenada Maria Victòria, per tantes hores compartides de disseccions, cultius i xocolata. Per haver fet més fàcil aquest tram final. Per haver-me ajudat a resoldre cada mini-dubte metodològic, conceptual o de disseny que m'ha sorgit. Perquè ets

una gran inventora d'esports, procediments de laboratori i tècniques de relacions interpersonals. Per haver estat la meva germana petita a fisio.

A l'Ernest. Per rebre'm tan bé a fisio i acollir-me a al seu pis de Madrid. Per les *chancletas* de lacón amb vi blanc. Perquè m'has ensenyat a optimitzar protocols, a ser conscienciosa i exhaustiva amb els experiments i a no rendir-me fàcilment.

A l'Abel, el mindu, per les nostres enriquidores discussions científiques. Pel seu esperit crític i per la seva manera de veure la vida.

A la Carole, pel seu esperit revolucionari. Per les hores que hem compartit al calci, on quan els experiments funcionen t'emociones tant com si fóssin teus. Perquè ens vas obrir literalment la porta del laboratori de Fisiologia l'estiu del 2009, amb el Fèlix a la panxa.

Al Roberto, per ensenyar-nos un idioma nou. Perquè encara que de vegades et queixis *una mica*, sé que és perquè t'importa el que fas i hi poses l'ànima. Perquè aportes un toc distintiu d'humor al laboratori. Acho pijo, perquè treballar al teu costat pot ser de tot excepte avorrit: molts cops m'he preguntat on era la càmera oculta o si m'havia colat accidentalment al plató d'una telenovela mexicana o la casa de Gandía Shore.

Al Carlos, per caminar tants anys al meu costat. Per entendre'm, que no és poc. Per saber riure de tu mateix i riure amb mi quan ets el blanc de la broma fàcil[2]. Per les teves idees i la teva gran habilitat de dissecció de problemes. Perquè admiro la teva capacitat de pensar i de formular hipòtesis. Per seguir aquí després de tot.

To cleisi-Kerstin, my parejita. I still remember the day Rubén asked me if I knew of an available room for his german student, I wouldn't have imagined then everything we would go through. I'm really glad

2 He estat a punt d'imprimir aquest paràgraf de color verd.

we could drive [3] together along this bumpy road. To my favourite roomie and travelmate, for all the tea-times, the meals, the rides, the combinations, the advice, the long hours in the lab, the taxipis, the nitrogen, the language classes, the Scrubs episodes and all the chocolate that we shared. Because we went through everything together, and we made it! For all the laughter tears you made me cry. For all the trucks that ran over us during the nights. For your first, extraofficial night in Badia Paradise. For all the vermouths, to which I'm sorry I got you addicted [4]. For all your unconditional support. *Dios las cría y ellas se juntan*, you will be missed!

A tots els que han compartit el laboratori de Fisio o el despatx amb mi: A la Marisabel, l'Anna, la Gemma, l'Amado, l'Anabel, la Sani i el Gerard Ill. Ha estat un honor treballar amb vosaltres. Per totes les nits, cerveses, riures, consells i històries inversemblants. Per omplir aquests anys d'anècdotes.

A la Lali, la Consuelo i la Melisa, per importar alegria des de l'altra banda de l'Atlàntic. Por los chocolates con churros y el Oktoberfest.

A l'Alberto i el Bertran, per omplir el laboratori de vitalitat. Per fer-nos pixar de riure amb les vostres històries i per la vostra actuació estelar: Bohemian Rhapsody. A tots els estudiants, especialment a la Marta, la Carol, en Xevi, l'Alba, la Cinta, la Judith i la Neus.

A l'Aina i la Marina, que són quasi de fisio. Per tots els riures i totes les beer sessions. Per fer el dia a dia més amè.

Als fàrmacos, per ser el meu segon laboratori d'esperit. A la Cuti, el Thomas, la Carmen. A Sami, por los buenos momentos que hemos vivido, por las clases magistrales de química y neurofarmacología. Por romperte la cabeza conmigo cuando los experimentos no salían.

3 us each other crazy(er)
4 No, I'm not :)

A les meves lavinietes preferides. Per les nostres trobades ocasionals, pels cafès i els sopars, per aportar-me un altre punt de vista i perquè m'alegro molt de conservar-vos després de tants anys. A la Carme, que m'ha ensenyat a lluitar pel que un vol, a deixar-s'hi la pell i a no optar pel camí més fàcil – i ha predicat amb l'exemple. A la Berta, perquè m'encanta presumir de conservar una amistat de 26 anys. Per la seva dolçor, perquè sempre està disposada a escoltar i ajudar a desenterbolir els problemes. Per totes les hores de pati, d'estudi, tots els trajectes d'autobús, els projectes, els aniversaris, els viatges i les excursions. Perquè espero que mai consideris la Biologia com un error; a mi em va permetre retrobar-te.

A la gent de Bio. Al Jordi, la Paula i la Lari, perquè no podeu marxar enlloc on no us vingui a visitar. Perquè encara que ara siguem lluny us porto sempre ben a prop. Per tots els sopars, nits de festa, dies d'estudi, viatges, trobades i tots els riures, que no han estat pocs.

Als fundadors de Badia paradise. Al Víctor, per totes les hores de fotografia, de confidències i de parranda que hem compartit al llarg de tots aquests anys. A la Mimi, perquè t'admiro moltíssim. Pels teus consells, que m'encanten. Perquè m'inspires i sempre em fas sentir millor. Perquè quan tinc dubtes sovint penso en *què faria la Maria* – i sempre funciona!

Al Marc Brut, per patrocinar un equip de volei i posar sostre a tantes nits de discussions filosòfiques i metafísiques. Per la truita collonuda que fa i l'amor que posa a tot el que surt de la barra.

Al Pau, al Pau[5], a l'Alba i al Marc. Perquè m'alegro molt d'haver-vos (re-)trobat. Perquè beneeixo aquell dia de primavera que vaig decidir mudar-me a Aribau. Per alegrar el meu dia a dia mentres vam viure plegats. Pels divendres que el Marc es presentava per sorpresa amb

5 No m'he equivocat; és que hi ha dos Paus

una ampolla de ginebra sota el braç. Per totes les nits d'Apolo[6] i els diumenges de vermut.

Al Miquel, l'agulla, perquè m'ha ajudat molt més del que es pensa. Per culturitzar-me amb les teves històries i per posar-me de bon humor. Perquè saps com arrancar-me un somriure (o un riure histèric). Gràcies per treure'm a passejar, per fer-me somiar i per ajudar a que el castell no faci llenya.

Als castellers de sants, perquè aquests darrers mesos he pogut comprovar que *feina de formiga, força d'elefant!*

A tota la gent que ha compartit pista o sorra amb mi, per les bones estones, crits, suor i nervis que hem passat.

A totes les pedres del camí, per fer-me tornar a aixecar, per forjar la perseverància[7].

A la meva família, especialment els meus pares i l'Ares, per ensenyarme a ser pacient, per ajudar-me a relativitzar les coses i per escoltarme sempre que ho he necessitat. Per la certesa que, passi el que passi, sempre hi haurà algú disposat a donar-me un cop de mà.

I sobretot a tu, lector/a, per tenir aquesta tesi entre les mans. Aquesta tesi que és fruit de, literalment, suor, sang i algunes llàgrimes. Aquesta tesi que ha valgut tant la pena fer i escriure. Espero que, com a mínim, la lectura sigui entretinguda.

6 Sí, inclosa "aquella"

7 Perseverància és una paraula molt bonica, però tots sabem que vull dir tossuderia

ABSTRACT

Aggregation of the amyloid-β (Aβ) peptide to form oligomers and amyloid fibrils is a central event in the pathogenesis of Alzheimer's disease (AD). This thesis aims to provide a better understanding of Aβ toxicity and the impact of changes in Aβ aggregation, both relevant to AD. We have found that Aβ nitrotyrosination inhibits fibril formation, which favours the stabilization of small oligomers. We show that nitro-Aβ oligomers strongly bind to dendrites, altering N-methyl-D-aspartate receptor (NMDAR) physiological function and leading to neuronal dysfunction and cell death. Furthermore, we propose a model for Aβ fibril assembly, according to which fibril elongation is interrupted upon nitrotyrosination, due to the destabilization of interprotofibrillar contacts. Additionally, using a genome-wide screen in *Saccharomyces cerevisiae,* we have identified novel modulators of Aβ toxicity that are potential targets for the development of new AD therapeutic approaches.

RESUM

L'agregació del pèptid β-amiloide (Aβ) en forma d'oligòmers i fibres és un esdeveniment central en la patogènesi de la malaltia d'Alzheimer. Aquesta tesi pretén aprofundir en els coneixements actuals sobre la toxicitat causada per l'Aβ així com en l'impacte que tenen els canvis en l'agregació d'aquest, tots dos rellevants per la malaltia d'Alzheimer. Els nostres resultats indiquen que la nitrotirosinació de l'Aβ inhibeix la formació de fibres, afavorint l'estabilització

d'oligòmers. Demostrem que els oligòmers d'Aβ nitrat s'uneixen a les dendrites, alterant la funció fisiològica dels receptors d'N-metil-D-aspartat (NMDAR) i provocant disfuncions neuronals i la mort cel·lular. A més, proposem un model d'assemblatge per a les fibres d'Aβ, segons el qual la nitrotirosinació interromp l'elongació de la fibra a causa de la desestabilització dels contactes entre protofibres. Addicionalment, utilitzant un cribratge genòmic en *Saccharomyces cerevisiae*, hem identificat nous moduladors de la toxicitat causada per Aβ, que podrien ser clau per al desenvolupament de noves estratègies terapèutiques de la malaltia Alzheimer.

PROLOGUE

Alzheimer's disease (AD) is a devastating disease that affects more than 30 million people worldwide. This number is estimated to double every 20 years, since AD prevalence increases as life expectancy gets higher. AD is a neurodegenerative disorder characterized by a progressive memory loss and cognitive decline. The amyloid-β (Aβ) peptide has been proposed as a causative agent in AD pathology and neurofibrillary tangles, cell loss, vascular damage and dementia are considered a consequence derived from its deposition in the brain.

Currently, there is no effective treatment to stop or slow down the development of the disease. Therefore, a deeper understanding of the mechanisms underlying Aβ aggregation and toxicity is critical to allow the development of new therapeutic strategies.

Nitric oxide (NO) is a gas that acts as a mediator in several biological processes. Nevertheless, it is a free radical and as such it becomes toxic at high concentrations. Under pathological conditions, NO can lead to the production of toxic byproducts like peroxynitrite, which has been reported to nitrotyrosinate proteins, altering their function.

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system. It plays a central role in synaptic plasticity and memory formation. The N-methyl-D-aspartate glutamate receptors (NMDARs) are particularly abundant in the hippocampus and have been extensively linked to AD pathology.

This thesis studies the impact that Aβ nitrotyrosination, a post-translational modification resulting from the oxidative stress characteristic

of AD, has on aggregation and neurotoxicity. Aβ nitrotyrosination critically impairs fibril formation, leading to the stabilization of oligomers, which are more abundant. Nitrotyrosinated Aβ oligomers exert enhanced neuronal toxicity, which appears to be mediated by NMDARs.

In addition, we have identified new molecular players that could be involved in Aβ toxicity, which may help in the search for novel pharmacological targets to treat Aβ-related pathologies. These Aβ toxicity modulators appear to be associated with essential biological processes, such as mitochondrial respiration, gene expression and Ca^{2+} homeostasis.

ACRONYMS

5-HT$_3$R	ionotropic serotonin receptor
3-NT	3-nitro-L-tyrosine
Aβ	amyloid-β
ACh	acetylcholine
AChE	acetylcholinesterase
AD	Alzheimer's disease
ADAM	a disintegrin and metalloprotease
ADDLs	Aβ-derived diffusible ligands
AFM	atomic force microscopy
AMPARs	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors
AP	amyloid plaques
APP	amyloid precursor protein
APOE	apolipoprotein E
BACE1	β-site APP cleaving enzyme 1
BBB	blood-brain barrier
CA	cornus ammonis
CAA	cerebral amyloid angiopathy
CaMKII	calcium/calmodulin-dependent kinase II
cAMP	cyclic adenosine monophosphate

CNS	central nervous system
CREB	cAMP response element-binding protein
CSF	cerebrospinal fluid
EOAD	early-onset Alzheimer's disease
EPSP	excitatory postsynaptic potential
ER	endoplasmic reticulum
FAD	familial Alzheimer's disease
$GABA_AR$	γ-aminobutyric acid receptor A
GlyR	glycine receptor
GSH	glutathione
H_2O_2	hydrogen peroxide
IgG	immunoglobulin G
ISF	intersticial fluid
KARs	kainate receptors
LOAD	late-onset Alzheimer's disease
LDLR	low density lipoprotein receptor
LTD	long term depression
LTP	long term potentiation
MCI	mild cognitive impairment
mGluR5	metabotropic glutamate receptor 5
MRI	magnetic resonance imaging
nAChRs	nicotinic acetylcholine receptors
NFT	neurofibrillary tangles

NMDARs N-methyl-D-aspartate receptors

nNOS neuronal nitric oxide synthase

NO nitric oxide

NOS nitric oxide synthases

NP neuritic plaques

$O_2^{-\bullet}$ superoxide radical anion

$^{\bullet}OH$ hydroxil radical

$ONOO^-$ peroxinitrite

P2X purinergic ATP receptors

PET positron emission tomography

PKA protein kinase A

PrP^c cellular prion protein

PSEN1 presenilin 1

PSEN2 presenilin 2

RAGE receptor for advanced glycation endproducts

RNS reactive nitrogen species

ROS reactive oxigen species

SGA synthetic genetic array

SIN-1 3-morpholinosydnonimine hydrochloride

SOD superoxide dismutase

ThT thioflavin T

TPI triosephosphate isomerase

VDAC1 voltage-dependent anion channel 1

ZAC zinc-activated ion channel

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Part I

INTRODUCTION

1 NEURONAL SIGNALLING

The human brain functioning relies on the organisation of the neuronal circuitry, which interconnects millions of neurons forming a network of bewildering complexity. Each neuron has the ability to receive and process signals coming from other neurons and rely the information to subsequent neurons. Interneuronal communication is mainly accomplished via the release and binding of endogenous molecules called neurotransmitters.

Synapses are the basic structure in the central nervous system (CNS) that permit the communication between neurons. In a typical chemical synapse the axon (presynaptic terminal) comes into close apposition with the membrane (postsynaptic terminal) of the target neuron. The neurotransmitters released into the synaptic cleft by the presynaptic neuron bind to the receptors located at the membrane of the postsynaptic neuron, either triggering an electrical response or initiating a secondary messenger cascade (figure 1).

1.1 SYNAPTIC TRANSMISSION

Dendritic spines are membranous protrusions arising from the dendritic shaft of postsynaptic neurons. They receive most of the excitatory synapses in the CNS. Thus, they are key elements in neuronal transmission and they have been widely and tightly related to neuronal plasticity and remodelling. Dendritic spines were first described in the 19th century by Santiago Ramón y Cajal (figure 18) [1], who speculated that they could function as synaptic contacts between

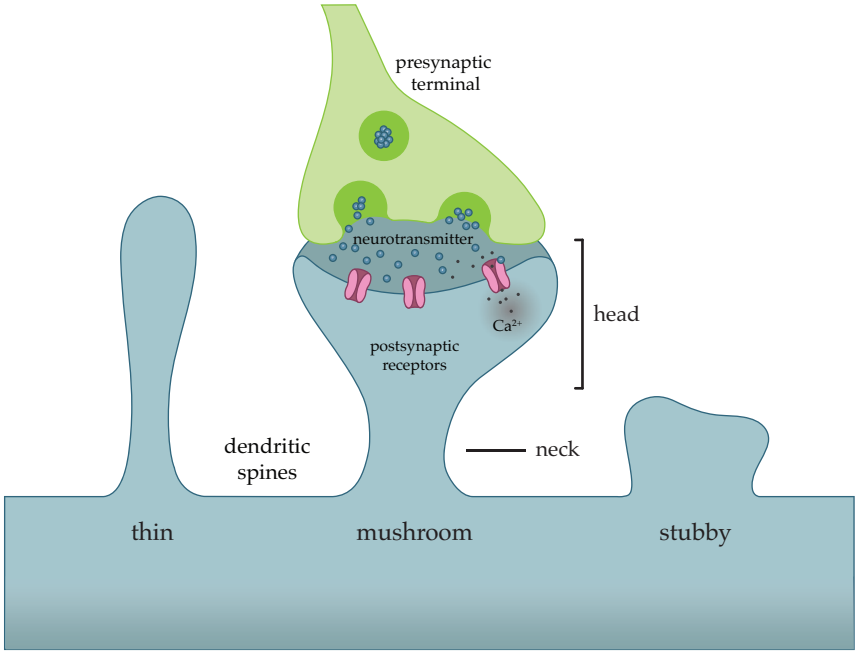

Figure 1: Chemical synapse

The excitatory presynaptic terminal contacts the dendritic spine. Upon the release of a neurotransmitter, the postsynaptic neuron triggers a signalling cascade initiated by a secondary messenger, in this case calcium (Ca^{2+}). Spines are composed of a bulky head connected to the dendritic shaft by a narrowing of the membrane; the neck. Dendritic spines can be classified morphologically in three major types: thin, mushroom and stubby.

neurons. This hypothesis was confirmed more than 50 years later with the emergence of electron microscopy [2].

Ever since their discovery, the role of these femtoliter-sized structures has been the focus of attention, speculations and debate. The essential conundrum regarding dendritic spines is that, even though some synapses, mostly inhibitory, involve an axon directly contacting the dendritic shaft, all excitatory inputs occur between an excitatory axon and a dendritic spine. The importance of dendritic spines cannot be underestimated; given the prevalence of dendritic spines in the CNS, one might think that they cannot be the primary site of excitatory

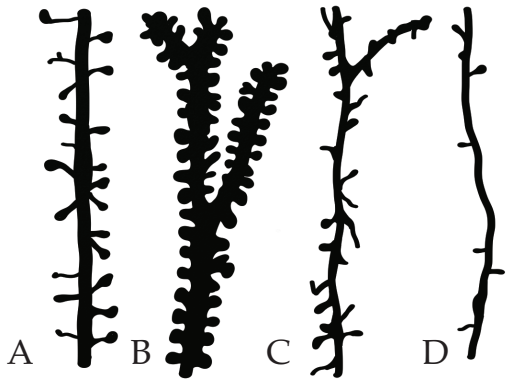

Figure 2: Dendritic spines
Adaptation of a drawing by Ramón y Cajal [3] showing dendritic spines of pyramidal (A), Purkinje (B), basket (C) and Golgi cells (D)

synapses on cortical pyramidal neurons, cerebellar Purkinje cells and a variety of other neuron types just by chance. This is the so-called spine problem, and there are many proposals aiming to explain the rationale of dendritic spines [4]. These hypotheses can be grouped into three categories:

1. Spines enhance synaptic connectivity. Axons have vertical, straight trajectories and basal dendrites are well positioned to intercept them [5–8]. Spines could be arranged in helixes along dendrites to minimize the number of spines used while maximizing their chances to contact passing axons. These structural features, straight axons and helicoidal spines, reveal a consistent logic of the connectivity of spiny circuits: Excitatory axons distribute information to as many neurons as possible while spiny neurons make contacts to as many different axons as possible [9].

2. Spines are electrical compartments that serve to enhance synaptic connectivity, enabling linear integration of inputs. Postsynaptic neurons receive many inputs that need to be integrated, not only in intensity but also in time, avoiding interference

between them and summing them up [10, 11]. Spine neck morphology can impact the kinetics and propagation of excitatory synaptic potentials, avoiding shunts and allowing input-specific plasticity [12–14].

3. Spines are biochemical compartments that implement input-specific plasticity. These distributed connections need to be plastic for the circuit to learn how to adapt to novel situations [15]. A circuit could change its function by either altering its connections or their strength. Spines are ideally suited to enhance circuit plasticity and regulate synaptic strength in an input-specific fashion because of their biochemical compartmentalisation (local calcium signals), electrical filtering by the spine neck and their extensive contacts with a variety of axons [9].

More recently, an integrated view of all three has been proposed, evidencing that these three functions of dendritic spines go hand in hand pursuing a same goal: To achieve an integrating and distributed circuit in the brain [9].

Dendritic spines are typically categorized in three different groups (figure 16). Thin spines are the most abundant, and have long neck and small bulbous heads (< 0.6 µm in diameter). Mushroom spines have constricted neck and large head (> 0.6 µm) and are considered to be mature and fully functional. At last, stubby spines have similar head and neck widths [16]. These categories have been further revised since live imaging of dendritic spines has revealed that they are dynamic structures, changing their shape and size within minutes [17, 18].

Table 1: Ionotropic receptors

Cys-loop receptors	nAChR
	$GABA_AR$
	$5\text{-}HT_3R$
	GlyR
	ZAC
Ionotropic glutamate receptors	NMDARs
	AMPARs
	KARs
ATP-gated receptors	P2X

nicotinic acetylcholine receptors (nAChRs); γ-aminobutyric acid receptor A ($GABA_AR$); ionotropic serotonin receptor ($5\text{-}HT_3R$); glycine receptor (GlyR); zinc-activated ion channel (ZAC); NMDARs; AMPARs; kainate receptors (KARs); purinergic ATP receptors (P2X).

1.2 NEUROTRANSMITTER RECEPTORS

The presynaptic terminal releases different neurotransmitters, which act on their correspondent receptors. The receptors are predominantly located in the postsynaptic membrane and can be classified in two main types: ionotropic receptors (ligand-gated ion channels) and metabotropic receptors, which transduce signals typically through G protein coupled receptors [19].

Ionotropic receptors can be classified in three superfamilies depending on the ligand and the structure of the channel (table 1). Glutamatergic ionotropic receptors, specifically N-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) have a major role in learning and memory.

NMDARs are heterotetrameric, cationic channels, typically composed of two copies of the glutamate-binding GluN1 subunit and two of the glycine-binding GluN2 (A-D) and/or GluN3 subunits [20]. The activation of NMDARs requires the binding of the two agonists, glycine and glutamate, together with the release of Mg^{2+} block by membrane depolarization [21]. The opening of NMDARs leads to an influx of cations, predominantly Ca^{2+}but also Na^{+}, which initiates signalling cascades that in turn modulate synaptic strength. The kinetics of activation and deactivation of NMDARs are markedly slower than those of non-NMDA receptors. Its slow deactivation rates are the ones that define the duration of the excitatory postsynaptic potential (EPSP), a measure of the strength of synaptic signalling [22, 23].

AMPARs are also heterotetrameric, cationic channels. Most AMPARs are composed of a *dimer of dimers* of GluA2 subunit and GluA1, GluA3 or GluA4 subunits [24]. The presence of a GluA2 subunit confers impermeability to Ca^{2+}, thus being Na^{+} and K^{+} the principal ions gated by AMPARs [25].

1.3 SYNAPTIC PLASTICITY: LEARNING AND MEMORY

In the human brain, the hippocampal formation is a cerebral structure located in the medial temporal lobe. It is formed by several substructures that differ greatly anatomically and functionally from each other: The cornus ammonis (CA) (subfields 1-4), the dentate gyrus, the subiculum and the surrounding perirhinal, parahippocampal and entorhinal cortex (figure 3) [26].

Most of the knowledge about the structure-function relationship of the hippocampus is derived from the patient Henry G. Molaison, widely known as H.M. in the literature, who was subjected to a bilateral medial temporal lobectomy to treat his epilepsy and suffered

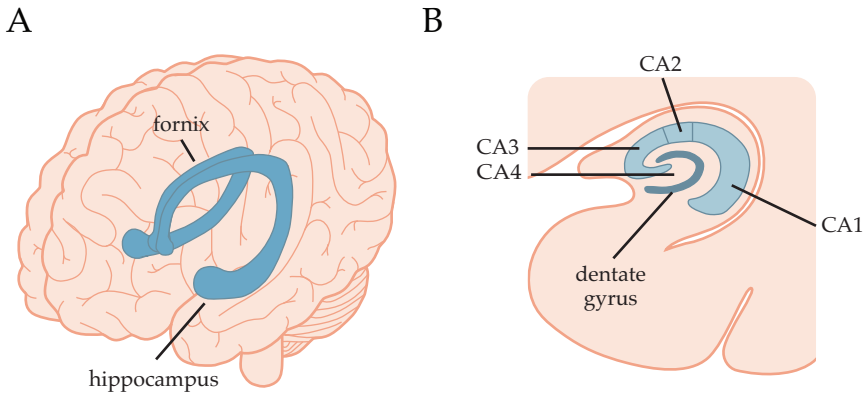

Figure 3: The Hippocampus
(A) Localization of the hippocampal formation within the human brain. (B) Representation of a coronal section of the human brain

severe anterograde amnesia as a consequence of the resection [27]. It became then clear that the hippocampus has a fundamental role in the consolidation of information from short-term to long-term memories, in the maintenance of long-term memory as well as in declarative memory [28].

Synapses are not static units that simply transmit bits of information from one neuron to the next. Instead, they are highly plastic structures, whose strength can be adjusted either up or down. The plasticity of this network in response to environmental stimuli allows the brain to adapt to new demands and allows learning and the formation of memories. Furthermore, functional changes of the synapses are correlated with morphological modifications of dendritic spines [16, 29, 30], including changes in spine number, size and shape [31].

Synaptic plasticity is exemplified by two well characterized paradigms: long term potentiation (LTP) and long term depression (LTD), a gain and loss of synaptic strength, respectively. In the hippocampus, synapses between Schaffer collateral and CA1 neurons can undergo either LTP or LTD depending on the frequency and duration of stimulation. A 100 Hz train of action potentials for just 1 s triggers LTP,

whereas 1-3 Hz stimulation for much longer periods (10 min) can induce LTD [32, 33].

The property of associativity of both processes relies on a mechanism that detects coincident pre- and post-synaptic activity [34]. At most glutamatergic synapses in the CNS the NMDAR performs the function of biochemical coincident detector, and initiates the synaptic plasticity processes by increasing intracellular Ca^{2+} in the dendritic spine [35].

The presynaptic neuron releases glutamate into the synaptic cleft as a result of an action potential (figure 4). Glutamate binds to ionotropic glutamate receptors at the dendritic spine of the postsynaptic neuron. This is not sufficient to activate NMDARs, since at near-resting action potentials the channel is blocked by Mg^{2+} ions [36]. Only when the postsynaptic neuron is sufficiently depolarized the Mg^{2+} block is released from the channel, allowing an influx of Ca^{2+} and Na^{+}. This Ca^{2+} influx is thought to initiate LTP induction [37, 38]. Then, calcium-sensitive signalling mechanisms, such as the calcium/calmodulin-dependent kinase II (CaMKII) or the cyclic adenosine monophosphate (cAMP)-depending pathways, are activated. The early phase of LTP is not dependent on protein synthesis and involves the Ca^{2+} influx in the dendritic spine (EPSP) as well as an increase of AMPARs in the postsynaptic membrane by exocytosis of endosomes and lateral diffusion [39–42]. The late phase of LTP is tightly related to protein synthesis, which can rely on pre-existing mRNA or *de novo* synthesis mediated by transcription factors. During LTP, the cAMP-dependent protein kinase A (PKA) activates cAMP response element-binding protein (CREB). CREB is a transcription factor necessary for the late stage of LTP and the formation of memories [43, 44]. It also has an important role in the development of drug addiction and in psychological dependence [45].

On the other hand, some forms of LTD are also dependent on NMDARs and are triggered by low concentrations of postsynaptic Ca^{2+}[46]. The phosphatase PP2B, also known as calcineurin, is activated at low concentrations of Ca^{2+} / calmodulin, dephosphorylating kinase targets such as glutamate receptors and the kinases themselves [47, 48]. The activity of PP2B and other phosphatases leads to clathrin- and dynamin-mediated endocytosis of AMPARs [49]. LTD is proposed as a homeostatic mechanism to ensure that CNS synapses are not saturated by learning. It also plays an important role in behavioural extinction and in cerebellar motor learning [50].

1.4 SPINE DEGENERATION AND NEURONAL LOSS

Many psychiatric and neurological disorders are characterized by structural and functional alterations of dendritic spines (table 2) [51]. Whether these abnormalities are a direct cause of cognitive deficits or are secondary to another event is still unclear. Nevertheless, synaptic pathology precedes neuronal death in many neurodegenerative diseases and, in particular, it has been reported to correlate well with cognitive decline in AD [52, 53].

Table 2: Spine abnormalities in neurological disorders

Schizophrenia	Decreased spine density in prefrontal cortical pyramidal neurons [54, 55]
Epilepsy	Loss of spine density in the hippocampus [56–58]
Fragile X syndrome	Reduced number of spines, filopodia-like spines [59, 60]
Autism	Increased number of spines, synaptic dysfunction [55, 61]
Drug addiction	Increased spine density in nucleus accumbens [62, 63]
Down's syndrome	Fewer mushroom spines, more stubby spines [64]
Alzheimer's disease	Reduced spine density in hippocampus and cortex, LTP impairment [65, 66]

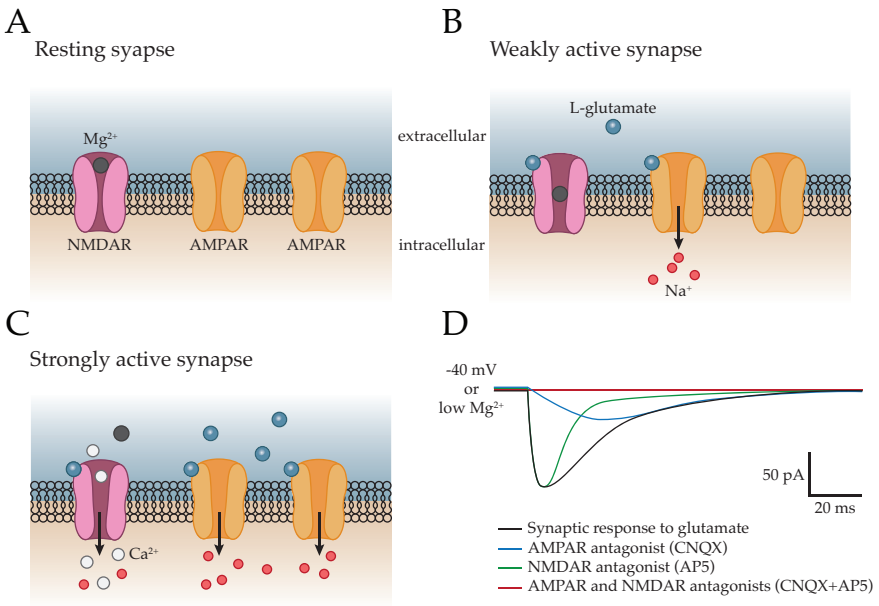

Figure 4: LTP

The NMDAR as a coincident detector. (A) Under resting conditions NMDAR is blocked by Mg^{2+} ions. (B) Low concentrations of released glutamate in the synaptic cleft do not depolarize the postsynaptic membrane sufficiently to release the Mg^{2+}block of NMDAR . (C) High concentrations of glutamate produce strong depolarization of the post-synaptic membrane, resulting in NMDAR opening and Ca^{2+} entry. (D) Representation of intracellular recordings of excitatory synaptic currents under conditions of low magnesium or membrane depolarization (either of which result in unblocked NMDAR and ion permeability when glutamate is bound to the receptor; black trace). Application of antagonists of AMPAR (CNQX; blue trace), NMDAR (AP5; green trace) or both (red trace) results in selective abolition of fast or slow components of EPSP, respectively. Adaptation from [34].

2 ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a neurodegenerative disorder that was first described by Alois Alzheimer in 1907 [67] and is characterized by a progressive memory loss. Dementia is a medical condition characterized by a decline in memory, language, problem solving and other intellectual abilities that hinders daily life. AD is the most common form of dementia, as it accounts for 60-80% of the estimated 46.8 million cases worldwide. Other causes of dementia are Lewy body dementia, Creutzfeldt Jakob's disease, frontotemporal dementia and vascular dementia [68, 69].

2.1 CAUSES AND RISK FACTORS

Based on the age of onset AD can be classified as early-onset Alzheimer's disease (EOAD) and late-onset Alzheimer's disease (LOAD). In EOAD the age of onset ranges roughly from 30 to 65 years old, and it accounts for approximately 1-5% of AD cases. LOAD, which is the most common form of AD (more than 95%), is defined by an onset at an age later than 65 years old [70]. AD can be classified by heritability as well; familial Alzheimer's disease (FAD) *vs.* sporadic. Both EOAD and LOAD may occur in people with a positive family history of AD . Approximately 60% of EOAD patients have multiple AD cases in their families and among these cases, 13% are inherited in an autosomal dominant manner with at least 3 generations affected [71, 72]. Actually, AD exists as a continuum across these four categories, with most of the cases involving multiple susceptibility genes and environmental factors. For instance, EOAD can occur in

Table 3: AD-causing mutations

Gene	Protein	Chromosome	Mutations	Phenotype
APP	amyloid precursor protein	21q21	24	increased $A\beta_{42}/A\beta_{40}$ ratio increased Aβ production increased Aβ aggregation
PSEN1	presenilin 1	14q24.2	185	increased $A\beta_{42}/A\beta_{40}$ ratio
PSEN2	presenilin 2	1q42.13	14	increased $A\beta_{42}/A\beta_{40}$ ratio

families with a LOAD history [73]. AD appears to be sporadic and to have a later age of onset (LOAD) in more than 90% of the patients [74]. Nevertheless, reviews of twin and family studies suggest the existence of an heritable component in LOAD [75, 76].

A small percentage of AD patients (less than 1%) develop the disease as a consequence of mutations to any of these three specific genes: presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) and *APP* (table 3) [77]. Those inheriting a mutation in the *APP* or *PSEN1* genes are guaranteed to develop AD. Those inheriting a mutation in the *PSEN2* gene have a 95% chance of developing the disease [78]. Patients with mutations in any of these three genes tend to develop EOAD, whereas the vast majority of AD patients have LOAD.

The cause for most AD cases is still unknown, except for those cases where it is caused by a genetic mutation. AD is likely to be developed as a result of a combination of environmental risk factors and a genetic background. Age is the major risk factor for AD: The risk of developing AD doubles every five years after the age of 65 [79].

The only gene that has been consistently linked with LOAD in multiple genetic studies is the *apolipoprotein E (APOE)* gene. *APOE* ε4 allele increases the risk of developing AD by 3-fold in heterozygous carriers and by 8 to 10-fold in homozygous carriers [80, 81]. On the

Table 4: Genetic variants associated to AD

Gene	Protein	p value *
APOE	apolipoprotein E	<1E-50
BIN1	bridging integrator 1	1.59E-26
CLU	clusterin	3.37E-23
ABCA7	ATP binding cassette subfamily A member 7	8.17E-22
CR1	complement component 3b/4b receptor 1	4.72E-21
PICALM	phosphatidylinositol binding clathrin assembly protein	2.85E-20
MS4A6A	membrane spanning 4-domains A6A	1.81E-11
CD33	CD33 molecule	2.04E-10
MS4A4E	membrane spanning 4-domains A4E	9.51E-10
CD2AP	CD2-associated protein	2.75E-09

* *p values* refer to systematic meta-analyses of the publicly available AlzGene database [83]

other hand, *APOE* ε2 is a protective allele [82]. Unlike inheriting a genetic mutation that causes AD, inheriting the ε4 form of *APOE* gene does not guarantee that an individual will develop the disease. In addition, genome-wide association studies (GWAS) have allowed the identification of several genetic variants with AD (table 4).

Many factors that increase the risk of cardiovascular disease are also associated with a higher risk of AD. Some of these factors are obesity [84–86], diabetes [87, 88], hypertension [89–91] and hyperlipidemia [92, 93].

2.2 MOLECULAR PATHOPHYSIOLOGY: PROTEIN AGGREGATION

Brain atrophy caused by neuronal loss leads to a progressive shrinkage of the areas where protein deposition is more abundant, *i.e.* the hippocampus and the cerebral and entorhinal cortices, as well as the enlargement of the ventricles.

AD is characterized by the pathological aggregation of proteins in the brain. The two major neuropathological hallmarks of AD are the extracellular accumulation of Aβ in amyloid plaques (AP) and the intracellular neurofibrillary tangles (NFT) formed of hyperphosphorilated tau protein [67, 94](figure 5). NFT become extracellular when tangle-bearing neurons die. AP are commonly classified in diffuse and dense-core, based on their morphology and staining. Diffuse plaques are amorphous amyloid deposits that are negative for thioflavin-S and congo red staining. On the other hand, dense-core plaques have a compact core of amyloid fibrils that stains with thioflavin-S and congo red. Dense-core plaques are typically neuritic plaques (NP), *i.e.* they are surrounded by dystrophic neurites, therefore containing both Aβ and tau. This classification is relevant to the disease because thioflavin-S-positive dense-core plaques are associated with deleterious effects in the surrounding neuropil, including dystrophic neurites, synaptic loss, neuronal loss, and recruitment and activation of astrocytes and microglia [95–100](figure 6).

Aβ not only deposits in the brain parenchyma in the form of AP, but also in the vessel walls. In cerebral amyloid angiopathy (CAA), Aβ deposits in the tunica media of leptomeningeal arteries and cortical capillaries, small arterioles and medium-size arteries. Some degree of CAA, usually mild, is present in approximately 80% of AD patients [103].

2.3 THERAPEUTICS

Six pharmacological treatments have been approved by the U.S. food and drug administration (FDA) to temporarily ameliorate the symptoms of AD by modulating certain neurotransmitters in the

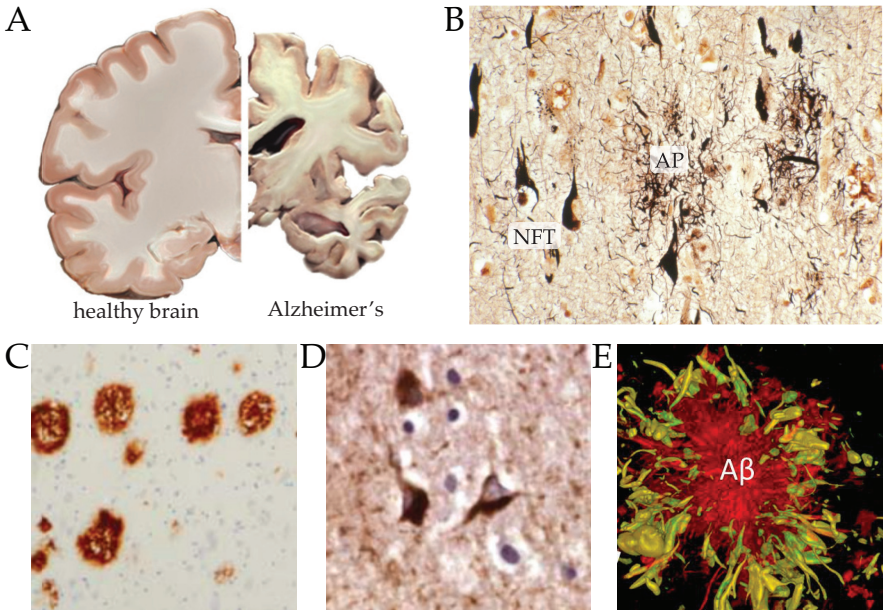

Figure 5: Histopathology of AD

(A) Cross sections of a healthy brain and a brain with severe AD. The hemibrain from the right (AD) shows atrophy or shrinkage of the cerebral and entorhinal cortex and the hippocampus and enlargement of the ventricles [101]. (B) Neurofibrillary tangles (NFT) and amyloid plaques (AP) in the hippocampus. Modified Bielschowsky silver impregnation (magnification 251x) [102]. (C) Immunohistochemistry against Aβ highlights AP and (D) immunohistochemistry against tau highlights NFT [103]. (E) Double labelling with a tau antibody (green) and an Aβ antibody (red) shows a classic neuritic plaque (NP) in which an amyloid fibrillar plaque is associated with dystrophic neurites [104].

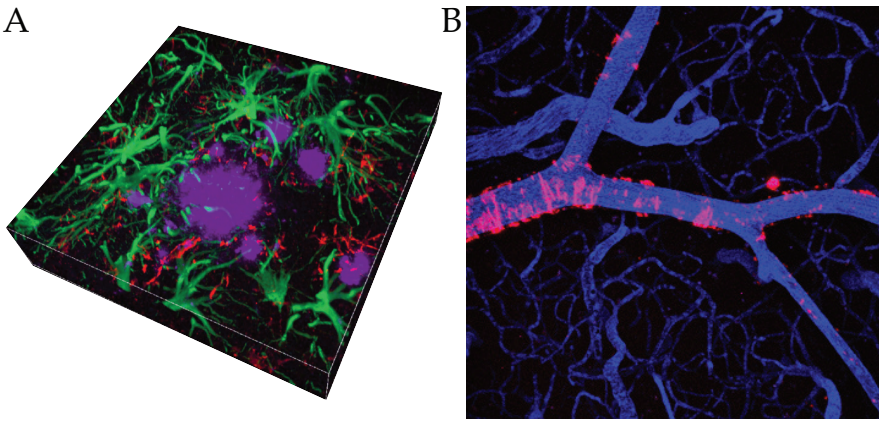

Figure 6: Astrogliosis, microgliosis and CAA

(A) 3D reconstruction of amyloid plaque deposition and glial accumulation in APP mouse brain. Cortical section of Tg2576 was stained with thioflavin S (compact amyloid plaque, purple pseudocolor), anti-IBA1 (microglia, red pseudocolor) and anti-GFAP (astrocytes, green pseudocolor) [105]. (B) Cerebral amyloid angiopathy (CAA)-affected vessel of a living Tg2576 mouse (model of amyloid deposition) imaged with multiphoton microscopy. Vascular and parenchymal Aβ deposits are identified by fluorescence from systemically administered methoxy-XO4 (red pseudocolor). Fluorescent angiograms with Texas Red dextran were performed to identify fiduciary markers (blue pseudocolor) [106].

Table 5: FDA-approved drugs to treat AD

Name	Type	Approved for	Year
tacrine *	AChE inhibitor	mild to moderate	1993
donezepil	AChE inhibitor	all stages	1996
rivastigmine	AChE inhibitor	all stages	2000
galantamine	AChE inhibitor	mild to moderate	2001
memantine	NMDAR inhibitor	moderate to severe	2003
memantine + donezepil	mixed	moderate to severe	2014

* the use of tacrine has been discontinued in the U.S. since 2013 due to concerns over safety

CNS (table 5). However, there exists no cure to AD and current treatments do not modify the course of the disease.

Current pharmacological treatment of AD focuses in two main strategies:

1. Increase the levels of acetylcholine (ACh), which are reduced in the brain of AD patients, by inhibiting the activity of the enzyme acetylcholinesterase (AChE).
2. Prevent dysfunctional glutamatergic transmission and excitotoxicity by inhibiting NMDARs.

Most of the investigation targeting AD therapeutics focus on Aβ and the reduction of its accumulation, either by limiting its production or by increasing its clearance.

Currently, there are 58 ongoing clinical trials in phase II, III and IV (table 6). Among them, there are 7 studies based on immunotherapy: via administering Aβ antibodies (passive immunotherapy) or inducing a humoral immune response (active immunotherapy). This has been the most extensively studied approach in Aβ-targeted therapy. Both passive and active immunotherapies have been shown to reduce Aβ accumulation in preclinical trials [107–109] but have failed

Table 6: Ongoing clinical trials to treat AD

Target type	Phase II	Phase III	Phase IV
Aβ-related	12	10	-
Cholesterol	-	-	1
Cholinergic system	-	1	-
Other neurotransmitters	6	2	2
Inflammation	4	2	-
Tau	-	1	-
Other	13	2	2

Information extracted from https://clinicaltrials.gov

spectacularly in clinical trials: Phase III studies on Gammagard™ (Baxter), a preparation of pooled human plasma antibodies, and Bapineuzumab™ (Johnson & Johnson, Pfizer), a humanized monoclonal anti-Aβ antibody, were discontinued in 2013. Phase III studies on the humanized monoclonal anti-Aβ antibody Solanezumab™ (Eli Lilly & Co.), which targets the soluble pool of Aβ instead of amyloid plaques, showed a slight improvement of cognition only in patients with mild AD. Solanezumab™ is currently being tested in asymptomatic or very mildly symptomatic patients either carrying the autosomal-dominant mutations of AD (APP, *PSEN1* and *PSEN2*) or testing positive for biomarker evidence of brain amyloid deposition (preclinical AD).

A recent study analyzed the outcome of 244 compounds tested in 413 AD clinical trials between 2002 and 2012 [110]. Of those 244 compounds only one was approved for marketing by the FDA. The authors report that the overall success rate of approval is 0.4% (99.6% of failure), which is among the lowest for any other therapeutic area [111, 112].

There are mainly two obstacles for therapeutic candidates to success: First, the delivery of peripherally-administered drugs to the brain is

very limited due to the blood-brain barrier (BBB) [113]. Second, most of clinical trials are performed in patients with clinical AD. It is estimated that AD symptoms show up about 10 years after the disease starts at a molecular level. Then, it seems feasible that when attempting to treat the disease the damage in the brain is too extensive and practically irreversible. This is why an increasing number of studies are being performed in people carrying genetic-causing mutations of AD who do not have clinical symptoms [114, 115]. Researchers are also pursuing the development of new biomarkers in order to diagnose AD at earlier clinical stages [116–119].

3 THE AMYLOID-β PEPTIDE

Amyloid-β (Aβ) is a peptide of 36-43 amino acids that is the main component of AP in AD. It is the product of the sequential cleavage of the amyloid precursor protein (APP), a ubiquitously expressed transmembrane protein. Its physiological role is not well understood, but there are some studies that point to Aβ as a synaptic plasticity regulator and a neurotrophic factor [120–123].

3.1 THE AMYLOID CASCADE HYPOTHESIS

In the early 1990s, many scientists postulated that Aβ is the causative agent in AD pathology and that neurofibrillary tangles, cell loss, vascular damage and dementia follow as a direct result of its deposition [124–128]. This constitutes the basis of the amyloid cascade hypothesis. There are many events in AD that support this hypothesis:

- FAD is caused by mutations in *PSEN1*, *PSEN2* and *APP*. The products of these genes are directly involved in Aβ production [129].
- Additionally, mutations in the Aβ-coding region in the carboxy-terminal part of *APP* cause FAD and CAA by increasing the aggregation propensity or inhibiting the degradation of Aβ, without affecting its production [130–133].
- Duplication of the *APP* locus on chromosome 21 increases Aβ production in people with Down syndrome, who have trisomy

21. This causes a greatly increased risk of developing AD or AD-like dementia in Down syndrome patients [134].

- Recently, a rare genetic variant of *APP* was found to be protective for AD, by diminishing amyloidogenic Aβ production [135].
- Aβ is toxic to cultured neurons [136]
- The AD-susceptibility *APOE* ε4 genotype is strongly associated with increased vascular and plaque Aβ deposits in patients with LOAD [137] and in knock-in mice expressing human *APOE* ε4 along with FAD-linked APP transgenes [138, 139]. Conversely, Aβ cannot form amyloid deposits in mice in which this gene has been knockout [140].
- AD mouse models, typically relying on strong promoters to drive expression of human APP containing single or multiple FAD mutations, show an AD-like phenotype. Furthermore, clearance of AP by active and passive immunization improves cognitive performance in transgenic mice, being their performance in memory tests similar to wild-type animals [141].

Given all that, Aβ seems the most likely candidate for initiation of the cascade, ultimately leading to full-blown AD pathology decades after.

Nevertheless, there is controversy on that matter and some authors argue that there are inconsistencies surrounding the amyloid hypothesis of AD [142–145].

There is evidence, from autopsy studies and from live imaging using Aβ-binding PET ligands, reporting individuals with few or nonexistent clinical symptoms of dementia who yet carry substantial amyloid burden in their brains [146, 147]. This is the Aβ deposition paradox: It is possible to have AP without dementia, therefore Aβ deposition

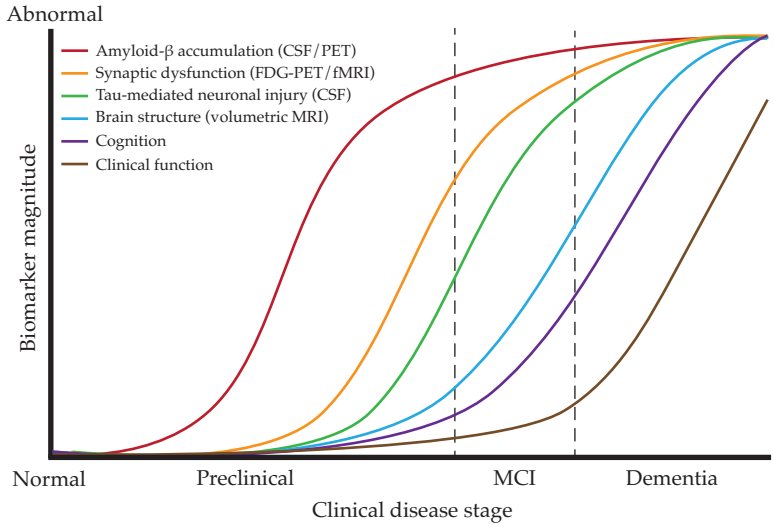

Figure 7: Biomarkers of AD pathological cascade
Chronological sequence of molecular and anatomical events during the development of AD. cerebrospinal fluid (CSF); positron emission tomography (PET); functional magnetic resonance imaging (MRI).

is not sufficient to cause the disease. In addition, Aβ deposition does not correlate with the degree of dementia as well as tangle density or synapse loss (figure 7).

However, amyloid deposits of asymptomatic patients are almost exclusively diffuse forms of AP, which are not associated with surrounding neuritic and glial pathology [148]. Moreover, the degree of cognitive decline in AD associates much better with oligomeric Aβ species than with histologically determined plaque counts, which is consistent with the hypothesis that soluble Aβ oligomers, rather than plaques, are responsible for neurodegeneration in AD [149–152].

It is generally accepted that memory impairment in AD starts with subtle alteration of hippocampal synaptic efficacy and that this synaptic dysfunction is caused by soluble oligomeric assemblies of Aβ [65]. Accordingly, synapse loss in AD brains is the major correlate with cognitive decline and severity of the disease [52, 153, 154].

Aβ oligomers bind to postsynaptic terminals *in vitro* [155], decreasing presynaptic vesicle markers and reducing synaptic spine density in glutamatergic, but not GABAergic, cultured neurons [156]. Furthermore, the addition of Aβ oligomers to primary hippocampal or cortical neurons has deleterious effects on synapse formation, neurite outgrowth and arborisation in a concentration-dependent manner [157].

In organotypic slices, Aβ dimers and trimers, but not monomers, induce a progressive loss of hippocampal synapses that requires the activation of NMDARs through a pathway involving cofilin and calcineurin [158]. In addition, subtoxic concentrations of Aβ oligomers reversibly decrease spine density, increase spine length and subdue spine motility *ex vivo* [159].

When directly injected into mice hippocampi, oligomers extracted from human AD brain inhibit LTP, enhance LTD, reduce synaptic density and disrupt memory and learning *in vivo* [160]. Moreover, there is also evidence of AD-like pathology development secondary to Aβ seeding in humans: The presence of amyloid deposits and severe CAA in people who contracted Creutzfeldt-Jacob disease from human postmortem pituitary-derived growth hormone injections has been recently reported [161]. These are the most direct evidence supporting the central role of Aβ oligomers in synapse impairment and as a causative agent in the pathogenesis of AD.

3.2 PRODUCTION

Aβ is the product resulting from the amyloidogenic processing of APP. APP is a ubiquitously expressed integral membrane protein encoded by a gene in chromosome 21, which contains 18 exons and spans 290 kilobases [162]. Several alternative splicing forms of APP have been observed in humans, ranging from 695 to 770 amino

acids in length, including the membrane-spanning domain from which Aβ derives. In APP695, the most abundant isoform in the brain, this domain ranges from amino acids 597 to 638. APP exact physiological function is still not known, but it has been implicated in cell proliferation [163], differentiation [164], cell adhesion [165], synapse formation [166], neuronal plasticity [167] and iron export [168].

There are three main enzymatic complexes involved in APP processing: α-secretase, β-secretase and γ-secretase. The α-secretase activity is performed by members of the a disintegrin and metalloprotease (ADAM) family, including ADAM9, ADAM10 and ADAM17. ADAM10 is the most expressed in the brain [169]. The β-secretase cleavage is performed by β-site APP cleaving enzyme 1 (BACE1), an integral membrane aspartyl protease that is localized in the intracellular compartments of the secretory pathway, particularly in the Golgi apparatus and endosomes . BACE1 active site is localized in the lumen and has an acidic optimal pH [170]. The γ-secretase complex consists of four independent integral membrane proteins. Presenilins (PSEN1 and PSEN2) are aspartyl proteases that constitute the active site of the complex. The other proteins in the complex are nicastrin, anterior pharynx-defective 1 and presenilin enhancer 2 [171]. The β- and α-secretase activities are mutually exclusive and can render or not Aβ production, leading to the amyloidogenic or non-amyloidogenic pathways of APP processing, respectively.

In the non-amyloidogenic pathway, which is predominant in non-neuronal cells, APP is cleaved by α-secretase within the fragment that gives rise to Aβ, precluding the formation of Aβ and releasing the soluble fragment sAPPα into the medium (figure 8). APP is cleaved by α-secretase both at the cell surface and along the secretory pathway, where ADAM10 is abundantly expressed [172]. The membrane-anchored fragment C83 can be further processed by γ-

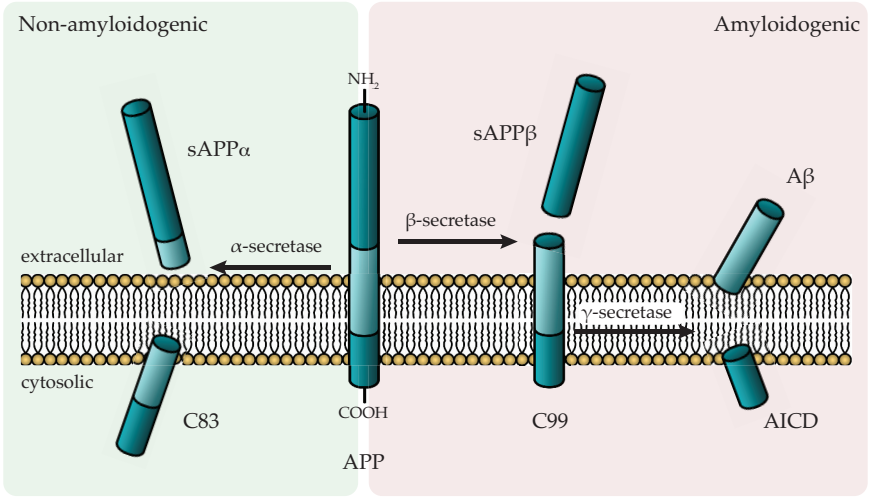

Figure 8: APP processing

Proteolytic processing of APP via non-amyloidogenic (left) and amyloidogenic (right) cleavage. Non-amyloidogenic cleavage occurs when α-secretase cleaves APP to produce sAPPα and C83. Amyloidogenic cleavage by β-secretase liberates C99 and sAPPβ, which in turn is cleaved by γ-secretase to produce Aβ and AICD.

secretase, generating the P3 peptide and AICD [173]. The generally accepted model proposes that α- and γ-secretase shedding is spatially and temporally separated. However, the existence of a multi-protease complex containing both α- and γ-secretase has been recently proposed [174].

In the amyloidogenic pathway, BACE1 cleaves APP in the N-terminal region of Aβ, releasing sAPPβ to the lumen and C99, which remains bound to the plasmatic membrane. BACE1 cleavage of APP is sequence specific [175] and takes place mostly in early endosomes [176]. The γ-secretase complex at the plasma membrane then cleaves C99, producing Aβ and AICD [171]. Depending on the γ-secretase cleaving site, different Aβ species are produced, being $A\beta_{40}$ and $A\beta_{42}$ the most abundant. Processing by BACE1 occurs mainly in the late compartments of the secretory pathway [177], but the γ-secretase cleavage is performed at the plasma membrane, releasing Aβ at the cell surface [178].

3.3 CLEARANCE

The pathological accumulation of Aβ in AD is thought to be the result of an imbalance between Aβ production and clearance. Presenilin mutation carriers that have EOAD present an increased Aβ production paired with a decreased Aβ clearance [179], whereas only a decreased Aβ clearance has been reported in patients with LOAD [180]. Apart from intrinsic defects in the clearance systems, Aβ aggregation in the brain parenchyma, which is associated with decreased $A\beta_{42}$ concentrations in the CSF, has been proposed as an important factor contributing to impaired clearance in AD [179, 181]. There exist different overlapping systems involved in Aβ removal, but their relative contribution to the overall clearance is currently unknown (figure 9):

- Degradation clearance. Neuronal intracellular Aβ is degraded by proteases, such as the insulin-degrading enzyme [182], by the proteasome in the ubiquitin-proteasome pathway [183] and by lysosomal enzymes through the endosome-lysosomal [184] or the autophagy-lysosomal [185] pathways. Extracellular Aβ can be degraded by extracellular enzymes such as neprilysin [186], insulin-degrading enzyme [187], matrix metalloproteases [188], plasmin and tissue plasminogen activator factor [189], among others. It can also be phagocyted by astrocytes and microglia in the intersticium and by vascular smooth muscle cells, macrophages and astrocytes in the perivascular space [190].

- BBB clearance. Aβ transport from the brain to the blood through the BBB is assisted directly or indirectly by several families of receptors. First, by low density lipoprotein receptor (LDLR) family members, such as LRP1 and LRP2 [191], the latter only when forming complexes with clusterin [192], which is also known as apolipoprotein J. LRP1 is considered to be the pre-

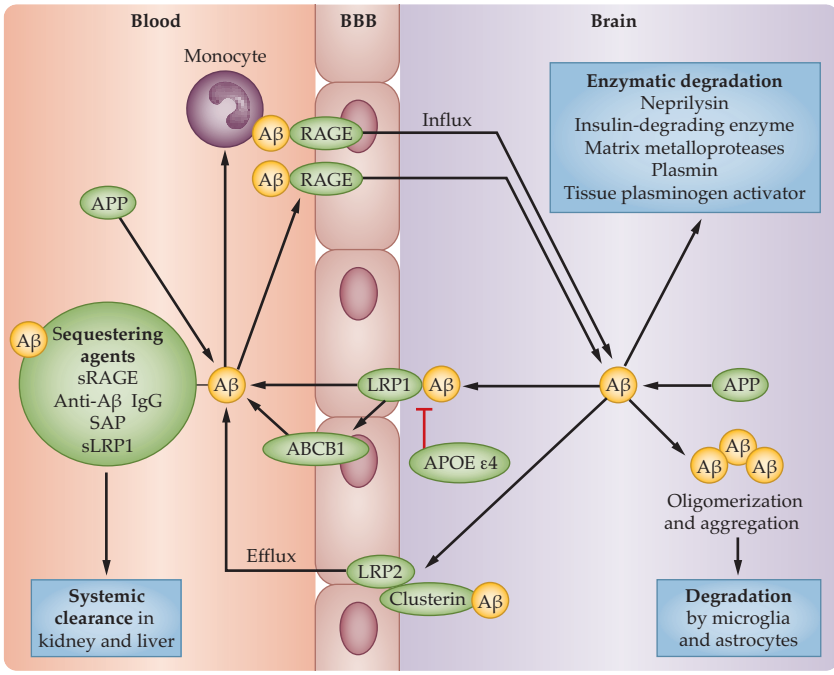

Figure 9: Aβ clearance

Aβ is produced from APP both in the brain and in peripheral tissues. Aβ clearance from the brain normally maintains its low levels in the interstitium. Sequestering agents chaperone Aβ for systemic degradation and prevent its influx to the brain. Aβ is eliminated from the brain enzymatically or by efflux through the BBB. LRP1 mediates efflux of Aβ with the help of ABCB1 and APOE ε4 inhibits such transport. LRP2 eliminates Aβ that is bound to clusterin through transportation across the BBB. RAGE is responsible for the influx of peripheral Aβ into the brain. Impairment of Aβ vascular clearance from the brain or increased re-entry across the blood vessels can elevate Aβ levels in the brain parenchyma. Modified from [181].

dominant form of Aβ efflux from the intersticium through the BBB [193]. Second, ABC-binding cassette transporters, such as ABCB1, also known as MDR1, and ABCA1, via an uncharacterized APOE-dependent mechanism [194]. Third, by other proteins such as macroglobulin or insulin, which is likely to be involved because BBB clearance is sensitive to insulin levels [195]. Transportation of Aβ to the bloodstream facilitates systemic degradation by the kidneys and liver [196].

- Glymphatic clearance. Because the brain lacks a lymphatic circulation, the CSF acts as a sink for the brain extracellular residues. A portion of subarachnoid CSF circulates into the brain intersticium and is cleared along large-caliber draining veins. This paravascular intersticial fluid (ISF) bulk-flow is dependent on astroglial aquaporin-4 and has been proposed as a potential Aβ clearing mechanism [197].

There are other mechanisms indirectly involved Aβ clearance: Aβ influx to the brain, which is performed predominantly by receptor for advanced glycation endproducts (RAGE) [198], and soluble Aβ transporters or sequestering agents, such as soluble RAGE and LRP (sRAGE and sLRP, respectively), serum-amyloid component (SAP) and anti-Aβ immunoglobulin G (IgG), which bind Aβ in the blood thereby preventing its entrance to the intersticium [199].

3.4 AGGREGATION

Aβ is secreted by the cells as a soluble protein with predominantly α-helical and random-coiled structure. Some conditions such as peptide concentration, salt content, temperature, agitation and pH favour the switch of Aβ secondary structure to β-sheet folding *in vitro* [200, 201]. The β-sheet conformation is particularly promoted at pH 4 - 7, resulting in the maximum β-sheet formation at pH 5.4, close to the

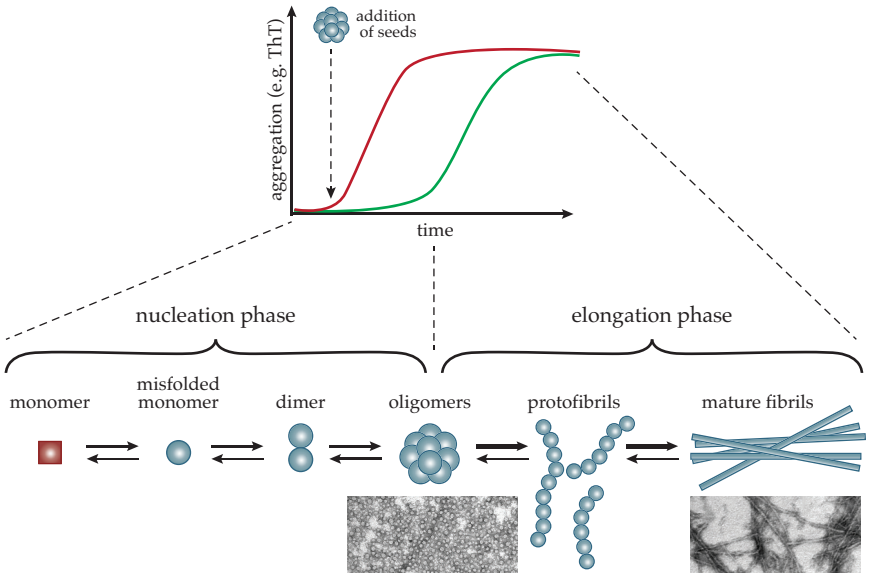

Figure 10: Aβ aggregation

The kinetics of amyloid formation consist in two phases: a slow nucleation / lag phase and a fast elongation / growth phase (green sigmoidal curve). The rate-limiting step in aggregation is the formation of misfolded seeds that promote aggregation, thus amyloid formation can be substantially accelerated by adding preformed oligomeric seeds or truncated fibrils (red sigmoidal curve). The assembly of misfolded Aβ monomers leads to the formation of oligomers, protofibrils and fibrils.

isoelectric point of the peptide (pI $\approx$ 5.5), at which Aβ precipitation and aggregation propensity are maximal [202]. Abnormal β-sheet folding is associated with Aβ assembly and the formation of pathological aggregates, *i.e.* oligomers, protofibrils and fibrils (figure 10).

Some AD-causing mutations in the Aβ fragment of APP have been proposed to differentially alter Aβ oligomerization [203]: English (H6R) [204], Tottori (D7N) [205], Italian (E22K) [206], Arctic (E22G) [207], Osaka (E22Δ) [208], Dutch (E22Q) [209] and Iowa (D23N) [210] Aβ variants all increase its aggregative propensity. On the other hand, V18A is a synthetic mutation, *i.e.* it has not been reported in humans, that impairs Aβ aggregation [211] (figure 11).

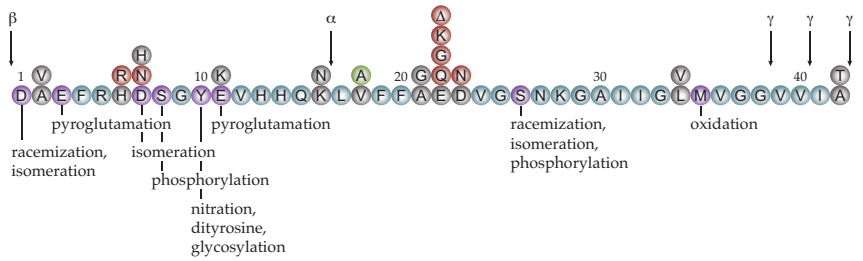

Figure 11: Aβ described mutations and post-translational modifications
Described Aβ mutations appear in grey, green and red: the green amino acid depicts a mutation that inhibits aggregation propensity, whereas a red residues refer to mutations that increase aggregation propensity. Purple amino acids are subject to post-translational modifications. Sites of α, β and γ cleavage are marked with arrows.

Apart from FAD mutations, Aβ aggregation also relies on some post-translational modifications and the peptide length. Different Aβ species can be generated depending on the cleavage site by γ-secretase. The most abundant is $A\beta_{40}$, followed by $A\beta_{42}$, which is detected about 10-fold lower levels [212]. The two extra residues confer more hydrophobic properties to $A\beta_{42}$, thus making it more aggregative than $A\beta_{40}$. But $A\beta_{40}$ and $A\beta_{42}$ not only have markedly different aggregation propensities; their kinetics of aggregation differ on the relative importance of primary nucleation versus fibril-catalyzed secondary nucleation processes [213]. $A\beta_{42}$ is the major component of AP in AD, whereas $A\beta_{40}$ is only present in a subset of plaques, suggesting that $A\beta_{42}$ precedes $A\beta_{40}$ aggregation [214]. Remarkably, in presenilin-mutation carriers there is an increase of $A\beta_{42}$ levels relative to $A\beta_{40}$, *i.e.* a shift in the $A\beta_{42}/A\beta_{40}$ ratio [215]. Even a slight increase in this ratio has dramatic effects in neurotoxicity [216] and its reduction inhibits amyloid deposition in transgenic mice [217].

In addition, several post-translational modifications of Aβ, including oxidation, phosphorylation, nitration, racemization, isomerization, pyroglutamylation and glycosylation, have been proposed to alter the

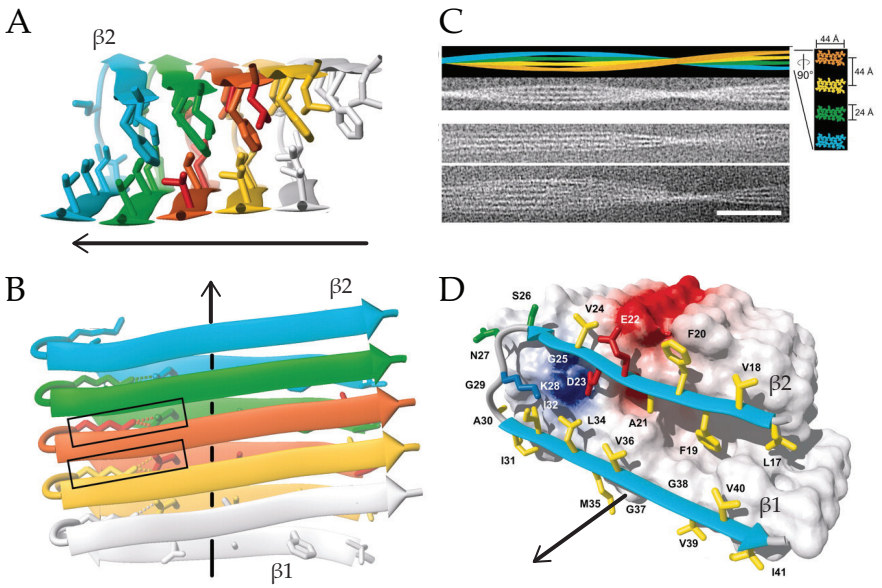

Figure 12: $A\beta_{42}$ fibril structure

Ribbon diagrams of the core structure of residues 17-42, with the two antiparallel β-sheets; β1 and β2. (A) and (B) illustrate the molecular nature of intrafibrillar interactions. (C) and (D) represent fibril cross-sections. (C) depicts interprotofibrillar interactions and the supramolecular structure of the fibril. Black arrows represent the fibril axis. Modified from [219].

aggregative and pathogenic properties of the peptide [218] (figure 11).

The three-dimensional structure of $A\beta_{42}$ fibrils was experimentally determined in 2005 (PDB ID: 2BEG). Each protofibril consists of two antiparallel β-sheets comprising the residues L17-S26 (β1) and I31-I41 (β2) (figure 12). Hydrophobic interactions, backbone hydrogen bonds and intermolecular salt bridges, between D23 and K28, likely contribute to the stabilization of this structure [219]. However, interprotofibrillar interactions are also necessary to achieve the supramolecular architecture of the fibril. It has been hypothesized that the residue S26 and the fragment D1-H16 are involved in these interactions [220–222].

3.5 TOXICITY

Aβ oligomers, rather than Aβ fibrils, are likely the main toxic species accountable for the synaptic impairment and neurotoxicity present in AD [223, 224]. However, the exact mechanism of oligomer-induced neurotoxicity remains elusive: There are many molecular pathways by which Aβ oligomers could be inducing cytotoxicity, but the relevance of each is still unknown.

Extracellular oligomers have been reported to bind the cell surface. In the membrane, Aβ interacts with nAChRs, AMPARs and NMDARs, impairing cholinergic and glutamatergic transmission, respectively [225–227]. Notably, Aβ oligomers modulate NMDA activity [158, 228, 229] and render neurons more vulnerable to excitotoxic insults [230, 231], in a process dependent on NMDAR activation and Ca^{2+} entry. By altering the functionality of AMPARs and NMDARs, Aβ oligomers disrupt Ca^{2+} homeostasis. Ca^{2+} dyshomeostasis can promote several neurodegenerative processes, including free radical formation [232] and the phosphorylation of tau [233]. Aβ has also been reported to bind the nerve growth factor receptor [234], which can induce cell death through the p75 neurotrophin receptor [235], insulin receptor [236], frizzled receptor [237] and the cellular prion protein [238]. Furthermore, oligomers can directly disrupt the plasmatic membrane, causing an increase in its conductance [239].

Additionally, Aβ oligomers can accumulate in pyramidal neurons [240], possibly through a mechanism of endocytosis [241]. The internalization of nAChRs secondary to Aβ-binding can also be a mechanism for oligomer internalization [242]. Aβ binds to RAGE in neurons, initiating a cascade that leads to oxidative stress and NF-κB production [243] and the internalization of the Aβ-RAGE complex [244]. APOE receptors and LDL receptor-related protein are also likely to contribute to Aβ uptake [245]. The intracellular mechanisms

of Aβ toxicity include the dysfunction of the ubiquitin-proteasome system, which leads to impairment of receptor trafficking [246], build-up of tau protein and further accumulation of Aβ [247]. Additionally, Aβ can increase the sensitivity of neuronal inositol triphosphate and ryanodin receptors, which are endoplasmic reticulum (ER) channels that release Ca^{2+} from the internal stores [248].

In mitochondria, the progressive accumulation of Aβ can lead to the inhibition of the respiratory chain complexes III and IV [243, 249]. This consideration correlates with the abnormal mitochondrial distribution and morphology, as well as mitochondrial fission observed in AD [250, 251]. Together with Aβ-dependent Ca^{2+} hypersensitivity, these can lead to the initiation of the intrinsic pathway of Ca^{2+}-induced apoptosis by mitochondria, via the mitochondrial permeability transition pore.

Lastly, a substantial proportion of the toxicity triggered by Aβ is mediated by oxidative stress (reviewed in [252]), which plays a central role in the pathogenesis of the disease. Aβ induces oxidative stress and, simultaneously, oxidative stress promotes Aβ production and enhances its toxicity. This relationship is presented in more detail in the following chapter.

4 NITRO-OXIDATIVE STRESS

On a molecular basis, modifications resulting from increases in reactive oxygen species (ROS) and reactive nitrogen species (RNS) have also been shown to play a detrimental role in AD [253, 254]. ROS and RNS are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. They include the superoxide radical anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxil radical ($^{\bullet}OH$), nitric oxide (NO) and peroxinitrite ($ONOO^-$), all of which have been shown to play an important role in AD pathogenesis [255].

Oxidative stress is a condition where there is an imbalance between the production of these reactive species and antioxidant defences [256–258].

The brain is specially vulnerable to nitro-oxidative stress because its biochemical composition involves a pool of unsaturated lipids, which are susceptible to peroxidation and oxidative modifications [259, 260]. In addition, the brain accounts for an elevated fraction (20%) of total O_2 consumption, compared to its relatively small weight (2%) [261], but is not particularly enriched in antioxidant defences, unlike other organs such as the liver.

4.1 ROS AND RNS PRODUCTION

Mitochondria are an important source of ROS in most mammalian cells: within mitochondria, $O_2^{-\bullet}$ is produced by the one-electron reduction of O_2 . The efficiency of electron transport chain reactions

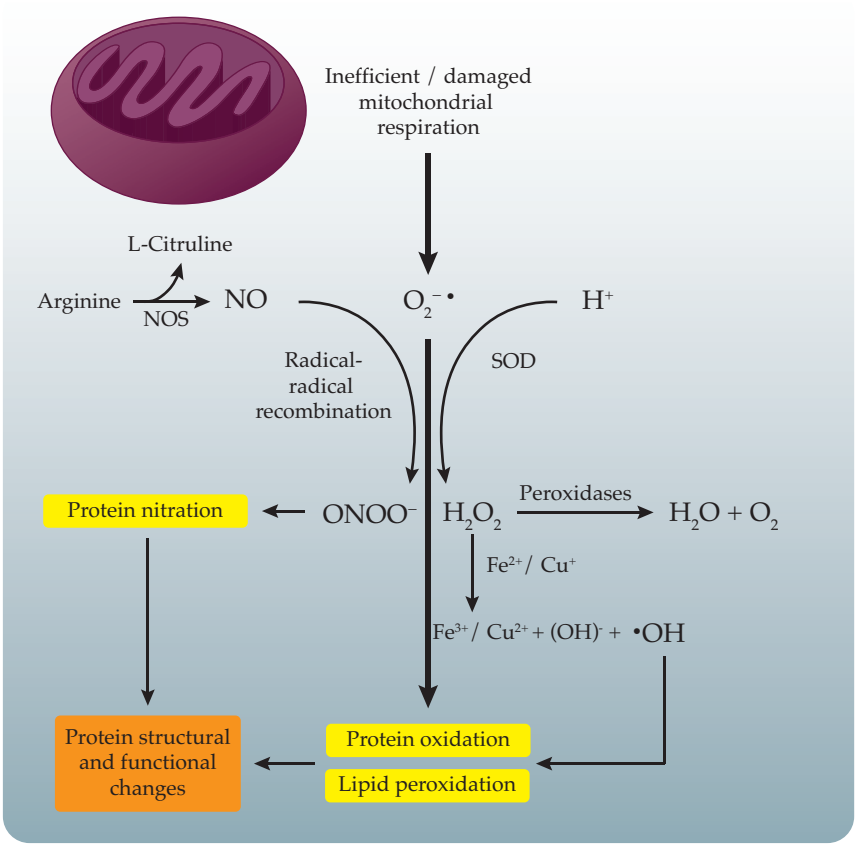

Figure 13: ROS and RNS production

ROS formation involving mitochondria-derived superoxide free radicals, subsequent processing by SOD, Fenton chemistry and RNS formation following NO production. Modified from [269].

decreases with age, increasing the amount of $O_2^{-\bullet}$ leaked from mitochondria (figure 13) [262–265]. Reactive $O_2^{-\bullet}$ oxidizes biomolecules directly through protein oxidation and lipid peroxidation and indirectly inducing oxidative / nitrative stress in the brain through the production of other reactive species [266–268].

$O_2^{-\bullet}$ is converted into H_2O_2 by the action of the mitochondrial scavenger superoxide dismutase (SOD). $O_2^{-\bullet}$ and H_2O_2 can interact with transition metals, such as Fe^{2+} and Cu^+, via the metal-catalyzed Fenton reaction to produce the extremely reactive $^{\bullet}OH$, which is

responsible for most of the downstream indirect damage of H_2O_2 [268, 270].

$$Fe^{2+}/Cu^{+} + H_2O_2 \longrightarrow Fe^{3+}/Cu^{2+} + OH^{-} + {}^{\bullet}OH$$

NO is a gas that acts as a mediator in several biological functions, such as neurotransmission and vascular smooth muscle relaxation. Nevertheless, it is a free radical and as such it becomes toxic at high concentrations. NO is synthesized from L-arginine by endothelial, inducible and neuronal nitric oxide synthases (NOS) [271].

NO reacts with $O_2^{-\bullet}$ quickly enough to avoid antioxidant defences, forming the $ONOO^{-}$ anion [272]. Given the short half-life of NO (3-5 s) [273] and the fact that $O_2^{-\bullet}$ has higher affinity for NO than for SOD [274, 275], the amount of NO and its diffusion coefficient are the limiting factors in this equation [276].

4.2 PROTEIN NITRO-TYROSINATION

$ONOO^{-}$ has a short half-life (1-2 s) and is highly reactive. It is one of the main molecules responsible for protein nitration, which consists in the addition of a nitro group (NO_2) to proteins. This modification occurs mainly, but not exclusively, to tyrosine residues, resulting in 3-nitro-L-tyrosine (3-NT). The susceptibility to nitration increases for tyrosines that are surrounded by negatively charged residues [277]. Tyrosine residues play an important role in regulating the function of a protein. Tyr is a site of phosphorylation, a prominent regulatory function. Thus, protein nitrotyrosination has a relevant biological role because it alters the normal activity of proteins by preventing the phosphorylation at Tyr residues or by changing its structure [278](table 7).

Proteins are significantly more nitrated in MCI and AD [295–299] as well as in other neurodegenerative diseases such as Parkinson's

Table 7: Effects of protein nitrotyrosination

Protein	Effect
MnSOD	Inactivation [279, 280]
Actin	Morphologically distinct disorganization of filamentous actin [281]
GS	Loss of catalytic activity [282]
HO	Reversible, concentration-dependent loss of activity [283]
IRP-1	Inhibition of aconitase activity and IRE-binding activity [284]
HDAC2	Reduction of activity [285]
ALDA	Impaired glycolytic activity [286]
p53	Inhibition of DNA-binding ability [287]
PGI(2)-S	Inhibition of activity [288]
PKC	Inhibition of enzymatic activity [289]
Cyt c	Increased peroxidatic activity [290]
Fibrinogen	Acceleration of clot formation [291]
GST	Increased enzymatic activity [292]
JNK	Activation [293]
PARS	Stimulation [294]

MnSOD (manganese superoxide dismutase); GS (glutamine synthetase); HO (heme oxygenase); IRP-1 (iron regulatory protein-1); HDAC2 (histone deacetylase 2); ALDA (aldolase A); PGI(2)-S (prostacyclin synthase); PKC (protein kinase C); Cyt c (cytochrome c); GST (glutathione S-transferase); JNK (c-Jun NH(2)-terminal kinase); PARS (poly(ADP-ribose) synthetase).

disease [300] and amyotrophic lateral sclerosis [301]. Several proteins have been reported to be affected by nitrotyrosination in AD brain: presenilins [302], albumin [303] the glycolytic enzymes triosephosphate isomerase (TPI) [304–307], α-enolase [295] and glutamate dehydrogenase [308], voltage-dependent anion channel 1 (VDAC1) [296], tau [309] and Aβ [310], among others.

4.3 Aβ-INDUCED NITRO-OXIDATIVE STRESS

The AD brain is characterized by increased oxidative stress, manifested as increased lipid peroxidation [311–313], protein oxidation [314–316], oxidized nucleotides in nuclear and mitochondrial DNA [317–319] and altered antioxidant enzyme levels [320, 321] in brain regions affected by AD pathology. The link between amyloid deposits and oxidative stress in AD is difficult to establish because postmortem tissue studies are unable to reveal whether the increase of nitro-oxidative stress is a byproduct of neurodegeneration and protein aggregation or precedes the degenerative process. However, there is increasing evidence that Aβ itself may be associated with nitro-oxidative stress.

Aβ aggregation to β-sheet induces the production of ROS through the reduction of transition metals: Misfolded Aβ directly produces H_2O_2 by a mechanism involving the reduction of Fe^{3+} and Cu^{2+} to Fe^{2+} and Cu^{+}, respectively, setting up the conditions for Fenton-type chemistry [322, 323]. Aβ-mediated ROS production has been reported in an aqueous solution [324] and in cultured hippocampal neurons, assessed by the increased fluorescence of dichlorofluorescein [325]. In addition, Aβ causes lipid peroxidation, a hallmark of oxidative damage, in neuronal cultures and synaptosomal membranes [326–328].

Aβ can promote the indirect production of ROS and RNS as well by activating glial cells: It activates microglia to produce RNS and interferon-γ [329] and it can activate astrocytes in the presence of cytokines, playing a role in AD neuronal damage via the production of NO [330].

4.4 ANTI-OXIDANT DEFENCE

Oxidative damage in the CNS of AD patients may result from an increased production of free radicals or from the failure of antioxidant defences.

Antioxidants serve to counterbalance the effect of ROS and RNS. There are many endogenous free radical scavenger systems, which involve enzymatic and non-enzymatic reactions. One of the oxidative stress handling enzymes is the Cu/Zn- and Mn-SOD, which converts $O_2^{-\bullet}$ to H_2O_2. Glutathione peroxidase and catalase will then convert H_2O_2 to H_2O. These three enzymes together with glutathione (GSH) provide the primary antioxidant defence mechanism [331–333].

Additionally, some exogenous antioxidants that can be obtained from the diet and supplements include ascorbic acid (vitamin C), α-tocopherol (vitamin E), β-carotene and vitamin A.

The aggregation of Aβ can be promoted by the addition of metal-catalyzed systems. Accordingly, the addition of radical scavengers such as ascorbic acid, vitamin E and amino acids prevents the aggregation process induced by the radical initiators [334].

Besides preventing Aβ aggregation, antioxidants also revert Aβ-mediated ROS production and Aβ toxicity. Continuous intracerebroventricular infusion of Aβ stimulated the H_2O_2 generation in isolated neocortex mitochondria, caused by an increase in SOD activity and a decrease in the activities of catalase and glutathione peroxidase [335].

Catalase protects from Aβ toxicity and cell lines that are selected for resistance to Aβ toxicity are also highly resistant to exogenously applied H_2O_2 [336]. In addition, mouse neuroblastoma cells (Neuro 2a), which are resistant to cytotoxicity driven by Aβ, H_2O_2 and glutamate, contain high levels of the antioxidant GSH [337].

The increased protein oxidation and ROS formation induced by Aβ in primary rat embryonic hippocampal neuronal culture are prevented by the free radical scavenger and antioxidant vitamin E [338]. Vitamin E also inhibits Aβ-induced lipid peroxidation [339] and protects neurons [336, 340] and endothelial cells [341] against Aβ toxicity *in vitro* . In addition, vitamin E is being tested as a putative treatment for AD in phase III clinical trials.

Resveratrol, a polyphenol with antioxidant properties, reduces plaque pathology in a mouse model of AD [342] and is currently being evaluated as a dietary supplement for the treatment of AD (phase III) and mild cognitive impairment (MCI) (phase IV).

Curcumin is another polyphenol with many potential neuroprotective properties: It acts as an antioxidant and anti-inflammatory compound [343], it inhibits tau [344] and Aβ [345, 346] aggregation and it promotes metal chelation [347] and neurogenesis [348]. Curcumin is in clinical trials for various cognitive conditions, also including AD (phase II) and MCI.

5 *S. CEREVISIAE* AS A MODEL ORGANISM

Saccharomyces cerevisiae is a species of yeast. The genome of these unicellular eukaryotic microorganisms was the first to be entirely sequenced in 1996; its 12,068 kilobases organized in 16 chromosomes constitute 5,885 potential protein-encoding genes [349]. The availability of single-gene deletion or overexpression libraries has allowed its use as a model organism in many research areas, including neurological disorders [350, 351]. Some of the few advantages of working with yeast as a model include its convenient growth in culture, with doubling times around 1.5 h at 30 °C, the possibility to easily express human genes, by transformation or homologous recombination, and the ability to undergo meiosis, allowing sexual genetics research.

S. cerevisiae can live as a diploid or an haploid organism (figure 14). Both can divide asexually by mitosis, through the budding mechanism. In contrast, the mating of yeast, or sexual reproduction, can only occur between haploid cells of opposite mating. Haploid yeast cells contain either the *a* or α mating-type allele determined by the single locus MAT. When diploid cells undergo meiosis, in response to nitrogen starvation in the presence of a poor carbon source, they produce 2 MATa and 2 MATα haploid spores [353].

Large-scale genetic analysis have proven the robustness and redundancy of biological pathways in *S. cerevisiae*: Only around 20% of the yeast genes are essential for viability when deleted individually in haploids growing under standard conditions [354, 355]. Synthetic lethality refers to the observation that often a pairwise combination of mutant alleles appear inviable, whereas the same alleles are viable when deleted individually. High-throughput synthetic lethal analysis

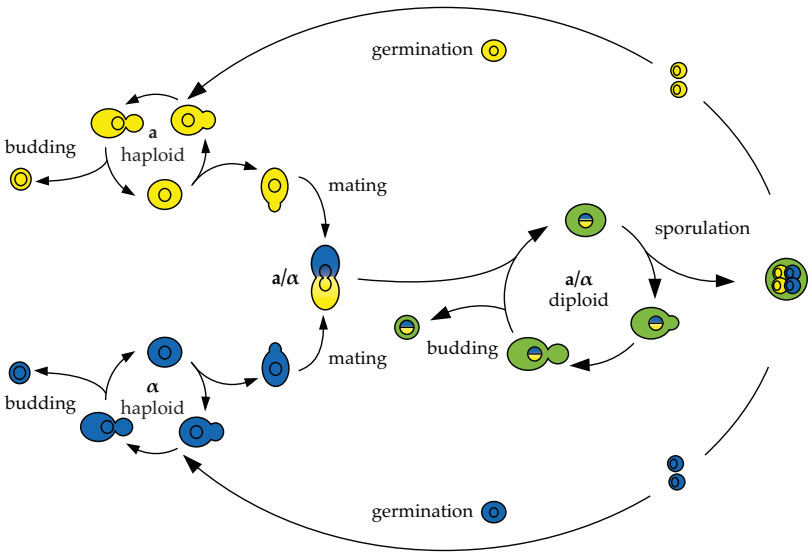

Figure 14: *S. cerevisiae* life cycle

Yeast cells can exist in haploid and diploid state and both can proliferate asexually by budding. Haploid cells have either mating type a or α, being able to mate with a cell of the opposite mating type. When exposed to nutrient-poor conditions, diploids can undergo meiosis, resulting in four haploid spores that can germinate to haploid cells under better nutrient conditions. Modified from [352].

has allowed the identification of proteins that are functionally related by disturbing the same essential cellular processes [356]. Synthetic genetic array (SGA) analyses have been used to map large-scale genetic-interaction networks using a yeast deletion-mutant collection of non-essential genes in *S. cerevisiae*. In these screenings, a strain with a query mutation is mated with an array of single-mutants, resulting in an output array of double mutants (figure 15). After the selection of the meiotic progeny, each double-mutant viability is assessed by pinning them onto solid medium and evaluating their growth [357].

Yeast are less complex and yet share many characteristics with mammalian cells: Approximately 30% of human genes have yeast orthologs and many signalling pathways involved in neurodegenerative diseases are homologous and functionally conserved [359, 360]. *S. cerevisiae* has been used to study the role of proteins that are in the pathogenesis of AD, including APP, BACE1 and tau [361–363]. APP does not have an ortholog in yeast, but Aβ can be easily expressed to study the relationship between its aggregation propensity and toxicity [364–367]. Aggregation propensity can be evaluated by quenching of fluorescently-tagged Aβ, a phenomenon that is prevented when a linker is added between Aβ and the fluorophore [368]. Thus, the addition of a short sequence between Aβ and a fluorescent marker allows the study of oligomerized Aβ toxicity without losing its fluorescence [369]. In *S. cerevisiae*, oligomeric Aβ has been proven to be toxic when expressed extracellularly or in the secretory pathway, in agreement with its predominant localization in neurons, but not when expressed in the cytosol [369, 370]. This findings stress out the importance of intracellular traffic pathways regarding Aβ toxicity and confirm the involvement of some endocytic factors already related to AD.

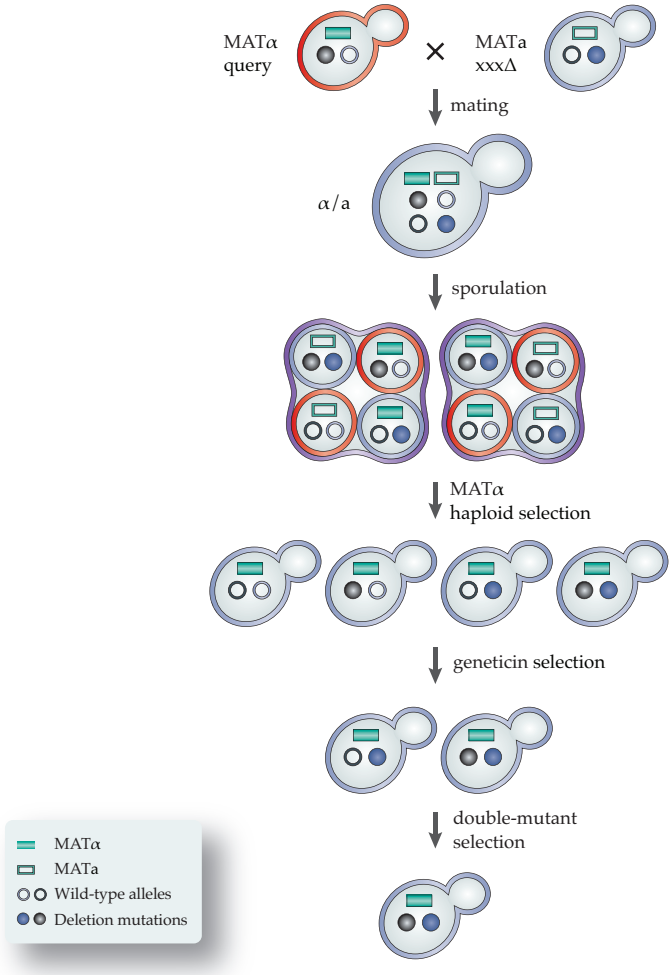

Figure 15: The SGA methodology

A MATα strain carries a query mutation linked to a selectable marker, such as URA3, and the reporter MFA1pr-HIS3 . This MATα query strain is crossed to an ordered array of the MATa deletion mutants (xxxΔ), which contain a geneticin-resistance marker (KanMK). The resultant heterozygous diploids are transferred to sporulation medium. Spores are transferred to a synthetic medium that lacks His, which allows the selective germination of MATα meiotic progeny because cells express MFA1pr-HIS3. Finally, the double-mutant meiotic progeny is selected in a medium with geneticin lacking Ura and His. Modified from [358]

Part II

OBJECTIVES

The main goal of this thesis was to study the molecular behaviour of Aβ peptide in terms of aggregation propensity and toxicity upon oligomerization and nitrotyrosination.

It is widely accepted that Aβ is a causative agent in AD pathology and that nitro-oxidative stress plays a central role in the pathophysiology of the disease. Peroxynitrite, a byproduct of nitric oxide and superoxide, has been reported to alter the structure and function of several proteins by irreversibly nitrating specific tyrosine residues. Remarkably, Aβ is among the proteins that have been found to be nitrated in AD brains. We hypothesized that the pathological role associated to nitro-Aβ would be due to the alteration of its aggregation propensity, thus modifying the toxicity it exerts.

In addition, we sought to explore the toxic effect that oligomeric Aβ has in yeast cells and to find novel molecular mediators of Aβ toxicity using a genome-wide screening in *S. cerevisiae*.

The specific objectives of this thesis are as follow:

- Study of the effect that Aβ nitrotyrosination has in aggregation propensity and oligomer stability.
- Evaluation of the impact that Aβ and nitro-Aβ oligomers have on NMDAR function and neuronal survival.
- Definition of the molecular mechanism underlying nitro-Aβ behaviour using an *in silico* model of aggregation.
- Determination of new molecular candidates involved in Aβ toxicity.

Part III

RESULTS

CHAPTER I

Guivernau B, Bonet J, Valls-Comamala V, Bosch-Morató M, Godoy JA, Inestrosa NC, et al. Amyloid-β Peptide Nitrotyrosination Stabilizes Oligomers and Enhances NMDAR-Mediated Toxicity. J Neurosci. 2016 Nov 16;36(46):11693–703. DOI: 10.1523/JNEUROSCI.1081-16.2016

Keywords: Aβ, alzheimer, oligomers, peroxynitrite, nitrotyrosination

Running title: Aβ nitrotyrosination impairs fibril formation

CHAPTER II

Identification of novel molecular players in Aβ toxicity using gene deletion mutants of *Saccharomyces cerevisiae*

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Keywords: Aβ, toxicity, alzheimer, *S. cerevisiae*

Running title: Novel molecular players of Aβ toxicity in *S. cerevisiae*

1 These authors contributed equally to this work. M.B.M. conducted the genetic screening in *S. cerevisiae*. B.G.A. performed the revalidation in yeast and the experiments with the ortholog in *H. sapiens*.

ABSTRACT

Aggregated forms of amyloid-β peptide (Aβ) trigger neurotoxicity in Alzheimer's disease (AD). We have carried out a genome-wide screen in *Saccharomyces cerevisiae* mating a library of ~5154 gene knock-out strains with an Aβ-expressing strain to identify novel players in Aβ-mediated toxicity . Our data suggest that several genetic risk factors in AD may modify Aβ toxicity; especially *EPHA1*, *RABGEF1* and *ADSSL1*. Several Aβ toxicity modulators are involved in the mitochondrial respiratory chain, including many cytochrome b and cytochrome c oxidase subunits, and in the Ca^{2+} homeostasis signalling pathway, including Calcineurin A, the transcription factor Crz1 and Erv29. Finally, a revalidation study confirmed some of the results from the screen and identified new modulators such as the yeast ortholog of *DYRK1A* and *VDAC*. Our findings suggest that genome wide analysis in yeast is a high-throughput model for screening molecular mechanisms underlying Aβ toxicity with potential therapeutic applicability.

INTRODUCTION

The Amyloid-β peptide (Aβ) is a hallmark of Alzheimer's disease (AD) [148, 397], Down's syndrome [373], cerebral amyloid angiopathy (CAA) [398], sporadic inclusion body myositis (sIBM) [399] and UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) myopathy [400]. All these diseases are characterized by the accumulation of Aβ either intracellularly, in the myopathies, or both intracellularly and extracellularly, in the other diseases. Aβ is generated by the amyloidogenic processing of the amyloid precursor protein

(APP) [170, 401], which takes place in the secretory pathway, releasing Aβ into endosomal compartments and mainly to the extracellular space [402, 403], from where it can be internalized [404, 405]. It is widely accepted that Aβ monomers can aggregate into oligomers, which are the most toxic species of the peptide [223, 224]. Although much research in the past years has focused on revealing the cytotoxic effects of Aβ, the molecular mechanisms responsible for Aβ toxicity remain elusive.

Accumulating evidence associates Aβ toxicity to several crucial cellular functions. Aβ has been proposed to accumulate in mitochondria inducing a decrease in mitochondrial respiration and triggering the production of free radical oxidative stress that could lead to apoptosis through caspase-3 activation [243, 251, 252]. Besides this, Aβ clearly affects cellular calcium homeostasis, including impaired mitochondrial Ca^{2+} regulation [406–408]. Additional pathological effects of Aβ accumulation are the perturbation of bilayer membranes [239], disturbance of protein folding system through the inhibition of the chaperones or the proteasome [409, 410] and dysregulation of autophagic processes [411, 412]. Despite the association of many cellular functions to Aβ toxicity, there is no agreement about which one is the main responsible for Aβ harmful effects. In addition, few specific molecules have been described as mediators of Aβ toxicity.

A cellular model used in Aβ aggregation studies is the *Saccharomyces cerevisae* yeast [364, 365], due to its reduced complexity and the similarity of most of its molecular signalling pathways compared to mammals, including autophagy, apop-

tosis, mitochondrial processes, cellular trafficking and protein homeostasis [413].

Aβ toxicity has also been evaluated in yeast, with most of the studies focused on extracellular Aβ effects showing a dose-dependent cytotoxicity for the oligomeric species [414], in agreement with mammalian cell studies. On the other hand, intracellular Aβ has been reported to be slightly toxic or innocuous to yeast when expressed in the cytosol whereas it is highly cytotoxic when directed to the secretory pathway, in an attempt to reproduce Aβ localization in human cells [364, 369]. Therefore, yeast has been recently proposed in two different reports as a proper and advantageous model to evaluate intracellular Aβ toxicity. In the first report, they transformed an overexpression library of 5,532 proteins with Aβ and they look for modulators of Aβ toxicity [370]. They demonstrate a link between endocytic factors, already associated to AD, and Aβ toxicity. In the second study, they evaluate Aβ toxicity in few yeast strains knocked out for genes with a key role in intracellular traffic routes [369]. Both studies pointed to the importance of endocytic processes to modulate Aβ toxicity.

RESULTS AND DISCUSSION

Aβ is toxic to yeast cells

In order to evaluate the toxic effects of Aβ in *S. cerevisiae*, we overexpressed the most aggregative and pathogenic isoform of the peptide, $A\beta_{42}$ (referred to as Aβ). Cytosolic Aβ expressed in frame with GFP (Aβ-GFP) does not induce toxicity [365], but when Aβ-GFP expression is directed to the secretory pathway

it is remarkably toxic to yeast [369, 370]. This is why in the present study Aβ is fused to the mating factor α (MFα) prepro-leader sequence secretion signal derived from the precursor of the *S. cerevisiae* MFα [415]. To enable the detection of a fluorescent signal, Aβ was fused to a GFP tag by a linker nucleotide sequence, as previously described [369] (Fig. 1A). Aβ expression was under the control of the GAL1 promoter, hence Aβ production was induced by growing yeast strains in galactose medium (Fig. S1). Apart from the Wild-type Aβ (WT), we evaluated the Dutch (E22Q) and Arctic (E22G) mutant forms of Aβ. Dutch and Arctic mutations are reported in AD patients and increase the aggregation propensity of the peptide [209, 211, 416, 417].

We transformed yeast cells with WT, Dutch and Arctic Aβ-GFP constructs. Cells were cultured in inducing medium (galactose) and Aβ production and processing was assessed with GFP (Fig. 1B) and Aβ (6E10, fig. 4B) immunodetection by Western blot. Aβ fused to GFP resulted in a band of ~41 kDa when the MFα is still not processed (MFα-Aβ-GFP) and a band of ~31 kDa when the protein is mature and MFα has been processed (Aβ-GFP). In addition, the band observed at ~45-50 kDa could represent a glycosylation modification of MFα, according to a previous report [369]. As a positive control, expression of GFP alone resulted in a band of 26 kDa (Fig. 1B).

In addition, we assessed Aβ cellular localization by confocal microscopy imaging of cells grown in inducing medium (Fig. 1C). The Aβ-expressing yeast showed a punctate pattern with a few filamentous inclusions, probably evidencing an endomembranous location, whereas a cytosolic diffuse pattern was observed in the GFP control.

The main objective of this study was to investigate Aβ toxicity, thus we analyzed the growth rate of Aβ-expressing yeast (Fig. 2A). When WT Aβ expression was induced, yeast growth was strongly impaired, evidencing Aβ toxic effects. In contrast, this effect was not observed in yeast transformed with an empty vector (control). The growth rate was quantified as the ratio between growth in inducing and non-inducing media (gal/glu), after 3 days onto solid plates (Fig 2B). WT Aβ significantly reduced growth rate in yeast cells ($p<0.001$). In addition, both Arctic Aβ and Dutch Aβ showed enhanced cytotoxicity compared to WT Aβ ($p<0.05$), suggesting that Aβ toxicity is dependent, at least partially, on the aggregative propensity of the peptide, in agreement with previous findings [369].

Taken together, these data confirm that Aβ overexpression in *S. cerevisiae* is an appropriate model to evaluate intracellular Aβ cytotoxicity.

Screening for modulators of Aβ toxicity

In other amyloid-related pathologies, such as sIBM and GNE myopathy, no Aβ mutations have been described. That is why, in order to have the maximum applicability of results, WT Aβ (referred to as Aβ from this point onwards) is used to determine modulators of Aβ toxicity in most of the studies.

We identified modulators of Aβ toxicity by screening ~5154 mutants from a *S. cerevisiae* genome-wide deletion library that were mated with an Aβ-expressing strain using an automated system [418]. Sporulation was induced after mating and selection of diploid cells and the resultant haploid cells, which contained the Aβ plasmid and were knock-out for a specific gene,

were sequentially selected. Then, the selected cells were pinned onto synthetic minimal medium lacking Uracil (SD–URA), given that Aβ plasmid contains the URA3 selectable marker. Notably, the SD–URA medium contained either glucose, to avoid Aβ expression, or galactose, to induce it. Plates were incubated for 3, 4 and 5 days and the growth rate of the colonies was assessed by the ratio between growth in inducing medium at each time point and uninducing medium at day 3 (gal/glu, Fig. S2A). The WT strain with an empty plasmid, consequently not expressing Aβ, showed a growth rate of 1.04 after 4 days and 1.83 after 5 days of induction whereas the Aβ-expressing WT strain presented a growth rate of only 0.09 after 4 days and 0.39 after 5 days of induction. These results confirm the toxic effects of Aβ previously shown in this report (Fig. 2).

When Aβ expression was induced in the ~5154 knock-out strains, Aβ had substantially different toxic effects in several strains, suggesting that these knock-out genes have a potential implication Aβ toxicity. Knock-out strains with a minimum growth rate of 0.7 after 4 days of induction were considered revertants or inhibitors of Aβ toxicity and knock-out strains with a growth rate of 0 after 5 days of induction were considered enhancers of Aβ toxicity (Fig. S2B). Knock-out strains with reported deficient growth in galactose medium and strains with observed deficient growth in glucose medium were discarded. Taking into consideration these premises, we identified a total of 141 strains as inhibitors of Aβ toxicity (revertants; Table S1), and a total of 312 strains as enhancers of Aβ toxicity (enhancers; Table S2).

From these results, it can be hypothesized that the genes the deletion of which reverts Aβ toxicity probably mediate this

toxicity, hence when they are knocked out Aβ does not exert its cytotoxic effects. On the other hand, the genes the deletion of which enhances Aβ toxicity can be identified as protectors from Aβ toxicity, given that when they are knocked out Aβ toxicity is increased. Alternatively, they can be interpreted to belong to a critical molecular pathway responsible for Aβ toxicity, whose absence would cause an increased vulnerability to some other proteins from the same cellular pathway.

Functional annotations of Aβ toxicity modulators

The sets of knocked out genes that resulted in an enhancement or revertance of Aβ toxicity were analyzed for overrepresentation of Gene Ontology (GO) categories. This was accomplished by an analysis with BiNGO and cytoscape software taking the list of knock-outs used in the present screen (~5154 genes) as the background reference. The top 10 significant hits with the lowest p values are displayed in figure 3.

Regarding the cellular localization, the set of genes the deletion of which increases Aβ toxicity are significantly enriched in mitochondria related compartments including membranes, lumen, matrix and mitochondrial ribosomes (Fig. 3A, S3). Accordingly, enhancer genes are enriched in mitochondrial-associated processes such as mitochondria organization, translation, respiration, energy derivation by oxidation of organic compounds and mitochondrial respiratory chain complex assembly (Fig. 3B, S4). Two different conclusions can be inferred from the analysis of enhancer genes: The first one is that mitochondrial-related processes may be protecting cells from Aβ toxicity, thus when mitochondrial-related genes are knocked out, the protective ef-

fect is not observed. The second hypothesis is that the deletion of some mitochondrial-related genes causes a slight damage in the mitochondria that makes the cell more susceptible to Aβ toxicity. Interestingly, Aβ toxicity has been previously associated to mitochondrial dysfunction and fragmentation in neurons and skeletal muscle cells [251, 419, 420]. The present findings provide evidence of some specific mitochondrial-related genes that may be Aβ targets or at least be involved in the main responsible mechanisms of Aβ toxicity.

Regarding the genes the deletion of which reverts Aβ toxicity, their location is enriched in the nuclear and nucleolar compartments, among others (Fig. 3C, S5). They seem to be involved in biosynthetic and metabolic processes, specifically in nucleobase-containing metabolic processes and gene expression (Fig. 3D, S6). According to this analysis, it seems plausible that Aβ requires from *de novo* gene expression to exert its toxicity, since most of the functional annotations for these genes are related to gene transcription and translation.

Some of the Aβ toxicity modulators identified in the present study appear as modulators of Aβ aggregation in a genome-wide analysis recently published [421]. The cited study uses an alternative Aβ construct that does not show fluorescence when is aggregated, which allows them to classify modulators of Aβ aggregation between those that show intense fluorescence and those that show weak or nonexistent fluorescence. Several revertants from our screen are reported to strongly modify Aβ aggregation in the cited publication, including *INO2*, *CTK1*, *MOT2*, *ADE12*, *BIM1* and some a with weak effect on Aβ aggregation, such as *DUN1*, *YGR015C*, *YCR061W* and *YDR537C*. In addition, the enhancers from our screen that show

a strong effect on Aβ aggregation in their study are *YDR230W* and *YOR364W* whereas the enhancers with a weak effect on Aβ aggregation are *MRP1*, *CCM1*, *COX6*, *MRPL32*, *COQ9*, *MRPL16*, *ATP5*, *TED1*, *MRPL17*, *COX11*, *MRPL51*, *QRI7*, *COQ2*, *ASI1*, *SAF1*, *APM3*, *IMG1*, *MRPL4*, *EAF3*, *IML3*, *QCR7*, *RMD9*, *SCS3*, *OXA1*, *AIM10*, *MRPL20*, *COX19*, *YDR442W*, *YHR050W-A*, *YJL007C* and *YKL202W*. This parallelism between both studies suggests that the association of these specific genes with Aβ toxicity is probably due to its influence on Aβ aggregation.

Aβ modulators include several orthologs of human AD risk factors

Prior to the detailed analysis of the revertants and the enhancers identified in the present study, orthologs of several human genes linked to AD by genome-wide association studies were recognized as modulators of Aβ toxicity in this screen (Table 1).

The strain knock-out for *NPR1*, ortholog of the human *EPHA1*, showed increased Aβ toxicity. However it is not included in the enhancers list (Table S2) because its basal growth in glucose medium does not reach the threshold that we set as normal growth. *EPHA1* is a member of the ephrin receptor subfamily involved in synapse formation and development [422] with some SNPs described as protective for late-onset AD [423] and linked to decreased risk of Aβ deposition in the brain [424]. In addition, the strain knock-out for *VPS9*, ortholog of the human *RABGEF1*, is also an enhancer of Aβ toxicity. *RABGEF1* is a potential risk factor for AD, which has been associated to Aβ toxicity in a recently published yeast screen [370].

On the other hand, strains knocked out for *YOL075C*, *VTH1*, *GBP2* and *ADE12* partially prevented Aβ toxicity. It should be noted that although these strains resulted in a higher growth than the WT strain, they are not considered completely revertants since its growth rate is lower than the threshold that we established at 0.7.

The human ortholog of *YOL075C*, *ABCA7*, is a member of the ATP-binding cassette genes which are responsible for lipid transport [425] and several variants of this gene have been associated with late-onset AD [426] and a higher risk of Aβ deposition in the brain [424]. The human ortholog of *VTH1*, *SORL1*, belongs to the vacuolar protein sorting 10 (*VPS10*) domain-containing receptor family and to the low density lipoprotein receptor family. It has been associated with an increased risk for AD [427] and it has been proposed to regulate APP trafficking. The human ortholog of *GBP2*, *CELF1*, encodes a member of the protein family that regulates pre-mRNA alternative splicing [428], which has been also associated to AD [427, 429]. At last, the human ortholog of *ADE12*, *ADSSL1*, codifies for the muscle isozyme of adenylosuccinate synthase recently reported as a potential risk factor for AD [370].

Notably, *ADE12* has been also reported to modify Aβ toxicity in a recently published yeast screen but in our hands it shows a protective role instead of the deleterious role previously observed [370]. This discrepancy could be due to the differences on the used Aβ construct that may lead to a substantially different amount of Aβ in each model, which could cause diverse effects in the absence of this gene.

To sum up, the presence of AD risk factors orthologs in our screen confirms the adequacy of this model to seek novel

Aβ toxicity modulators. In addition, these findings suggest a connection between Aβ toxicity and some AD risk factors whose role in AD pathology is completely unknown.

Aβ toxicity modulators are involved in crucial biological processes

Apart from the orthologs of several genetic AD risk factors, many other genes were found to modify Aβ toxicity (Table 2), as previously classified as revertants (Table S1) and enhancers (Table S2). When we analyzed these modulators in detail, we inferred that critical cellular and biochemical pathways are affected by Aβ toxicity, as previously pointed out by the functional annotation analysis.

The knock-out strains of the main components of the superoxide radical removal pathway behaved as enhancers of Aβ toxicity: superoxide dismutase (*SOD2*), catalase A (*CTA1*) and thiol peroxidase (*HYR1*), in accordance with the widely accepted notion that Aβ induces an increase of reactive oxygen species (ROS) [252, 307]. To cope with a ROS increment, cells can either convert ROS to less reactive products or reverse the oxidation. Interestingly, *SOD2*, *CTA1* and *HYR1* and their human orthologs *SOD2*, *CAT* and *GPX* have a common function of buffering oxidative stress converting ROS to less harmful molecules. Therefore, a knockout of any of these genes would be expected to increase Aβ toxicity, which is precisely what we observed in the yeast knock-out for these three genes.

As previously described, a high percentage of Aβ toxicity modulators are components of the mitochondrial respiratory chain, according to the GO enrichment analysis performed in the enhancer strains (Fig. 3A,B). The knock-outs for the cytochrome b

additional subunits *COR1* and *QCR7* as well as the knock-outs for proteins responsible for the mRNA synthesis of the catalytic cytochrome b subunit *COB* (*CCM1*, *CBP2*, *CBP1*, *CBP6*, *CBS1* and *CBS2*) showed an increment in Aβ toxicity. Moreover, the knock-outs for *BCS1*, the product of which is a translocase and chaperone required for cytochrome b-c1 complex assembly, increased Aβ toxicity. Furthermore, the knock-outs for cytochrome c heme lyase (*CYC3*), for several subunits of cytochrome c oxidase (COX) (*COX6,9,12*) and for proteins involved in COX assembly (*COX11,18,19*, *COA2,3*, *PET100,117*) were also enhancers of Aβ toxicity. On the other hand, the knock-out for *COA1*, which is also involved in COX assembly, behaved as a revertant of Aβ toxicity. This results are in good agreement with the literature, considering that there is a well-documented association between COX and Aβ toxicity [430, 431]. On the contrary, regarding cytochrome b, there is only one report showing a reduction in the mitochondrial complex I+III oxidoreductase activity using the TgCRND8 mouse model of AD [432]. Thus, cytochrome b has not been directly associated to Aβ harmful effects until now.

The clathrin-mediated endocytosis pathway is also enriched in several Aβ toxicity modulators. The knock-out for *SLA1*, which encodes a cytoskeletal protein binding protein, increased Aβ toxicity. This is consistent with a report that demonstrated a decrease of Aβ toxicity in a strain overexpressing *SLA1* [370]. The knock-out for *BZZ1*, involved in clathrin-dependent endocytosis but without a clear human ortholog, is a revertant of Aβ toxicity. Furthermore, *ENT5* knock-out is another revertant of Aβ toxicity, in agreement with the findings from a similar study [369]. The *ENT5* human ortholog *EPSIN1* encodes a clathrin accessory protein that has been shown not to participate in APP

endocytosis [433]. *APM2*, homologous to the medium chain of mammalian clathrin-associated protein complex (AP-1) and *APM3*, which codifies for a subunit of the clathrin-associated protein complex (AP-3) are enhancers of Aβ toxicity. Remarkably, Aβ toxicity has been reported by others to be affected by clathrin related genes although there is controversy over the directionality of Aβ toxicity modification (revertant *versus* enhancer) [369, 370]. Furthermore, clathrin-mediated endocytosis is considered one of the main mechanisms of Aβ internalization in neurons [434].

Therefore, these data reinforce the importance of pathways already associated to AD and to Aβ toxicity and it proposes novel concrete proteins from these pathways that could act as targets or mediators of Aβ toxicity.

Modulators of Aβ toxicity associated with Ca^{2+} homeostasis

It is widely accepted that Ca^{2+} homeostasis is impaired in AD and, specifically, Aβ has been proved to affect Ca^{2+} signalling [406]. A recently described Ca^{2+} channel known as calcium homeostasis modulator 1 (CALHM1) present polymorphisms associated with AD that affect its Ca^{2+} permeability [407, 435]. Despite the evident associations between Aβ toxicity and Ca^{2+} homeostasis impairment, the exact molecules that constitute this relationship remain unclear. Interestingly, most of the genes involved in yeast Ca^{2+} homeostasis behaved as modulators of Aβ toxicity in the present screen (Table 2). These genes are key members of the main pathways in Ca^{2+} homeostasis: Ca^{2+}/ calcineurin and Ca^{2+}/ calmodulin kinases signalling pathway and the secretory pathway.

CNA1 and *CRZ1* knock-out strains were found to inhibit Aβ toxicity, suggesting that the proteins codified by these genes may mediate Aβ toxicity, at least partially. *CNA1* encodes calcineurin A, the catalytic subunit of calcineurin, which is a serine/threonine protein phosphatase. Calcineurin dephosphorylates several proteins, including Crz1, a zinc-finger transcription factor [436]. Dephosphorylation of Crz1 induces a conformational change that exposes a nuclear localization signal triggering its transport into the nucleus [437], where it activates transcription of specific genes [438, 439]. Notably, we found that several of the Crz1 inducible genes are also revertants of Aβ toxicity modulators including *RCN1* and *PMR1* knock-outs. Rcn1 is a protein involved in calcineurin regulation [439] and Pmr1 is a high affinity Ca^{2+}/ Mn^{2+} P-type ATPase involved in Ca^{2+} dependent protein sorting and processing [440, 441].

Apart from Crz1, calcineurin dephosphorylates Mms22 and Frt2, their knock-outs being enhancers of Aβ toxicity. Both proteins seem to be involved in trafficking processes [442, 443]. Additional enhancers of Aβ toxicity are the knock-out for *CMK1*, a stress response gene, and *ECM7*, which encodes a putative membrane protein with a role in Ca^{2+} uptake.

Furthermore, an extremely strong revertant of Aβ toxicity was identified: *ERV29* knock-out. Erv29 protein, localized to COPII-coated vesicles, is involved in vesicle formation and incorporation of specific secretory cargo. Its human ortholog, Surf4, has been recently proposed to modulate STIM1-mediated store-operated Ca^{2+} entry (SOCE) [444].

Therefore, the pathways regulating Ca^{2+} homeostasis seem to be highly involved in Aβ toxicity, specially the calcineurin-Crz1 signalling. These findings are consistent with previous

research showing that *PPP3CA*, the human ortholog of *CNA1*, mediates Aβ-induced synaptic failure [445] and that *RCAN*, human ortholog of *RCN1*, is associated to AD [446]. In addition, we propose novel Aβ toxicity modulators, among which Surf4 is the most promising candidate because of its extremely strong revertant phenotype.

Revalidation of hits of interest

In an attempt to further confirm the results obtained in the screen, we performed a revalidation study of several knock-out strains, previously identified as Aβ toxicity modulators in the screen. First, the yeast orthologs of two genes already known to be associated with AD, *SOD2* [447] and *MTHFR* [448], were included in the revalidation study. In addition, some modulators associated with Ca^{2+} homeostasis were also assessed (Fig. 4A), since Ca^{2+} signalling pathway was one of the most affected by Aβ toxicity. Ent5Δ was included as a representative member of the also widely affected clathrin-dependent endocytosis pathway and mot2Δ and rrt2Δ were reanalyzed in this assay because they have never been linked to Aβ or AD until now.

For the revalidation assay, each knock-out strain was individually transformed with the Aβ construct or an empty plasmid, instead of the mating procedure used in the screen. After selection, strains were pinned into non-inducing or inducing medium for 2 to 4 days and growth rate was calculated as previously described. It is worth to mention that Aβ expression analyzed by Western blot seems to be unaltered in the knock-out strains cultured in inducing media (Fig. 4B). Hence, the

inhibitory or enhancer behaviour of these strains should not be attributed to an effect on Aβ differential expression.

Regarding the genes with a key role in the Ca^{2+} signalling pathway, the knock-outs erv29Δ, cna1Δ, crz1Δ and rcn1Δ behave as revertants of Aβ toxicity whereas ecm7Δ and mms22Δ behave as enhancers, confirming the effect observed in the screen. Regarding the enhancers identified in the screen, sod2Δ increases Aβ toxicity whereas the revertants, rrt2Δ, ent5Δ and mot2Δ are shown to inhibit Aβ toxicity (Fig. 4A). On the contrary, met13Δ was identified as an enhancer in the screen and shows a slight inhibition of Aβ toxicity in the revalidation assay.

In addition to these knock-out strains, we reevaluated with the method described above some genes of interest which showed a controversial phenotype in the screen. We identified yak1Δ and dnf2Δ as revertants of Aβ toxicity and por1Δ as an enhancer (Table 3). *YAK1* is the ortholog of the human *DYRK1A/B*, which has been already associated to AD [449]. *DNF2* is the ortholog of the human *ATP8B1*, which encodes an aminophospholipid translocase with a role in endocytosis and cell polarity, and has not been related to AD until now; from these results we postulate it could play a role in Aβ endocytosis. Finally, *VDAC*, the human ortholog of *POR1*, is the most abundant protein of the outer mitochondrial membrane and mediates the exchange of ions and metabolites between the cytoplasm and mitochondria. Taken together, these findings reinforce the role of the cellular processes previously identified in the screen.

Among the 14 knock-out strains revalidated, which show a minimal growth in glucose and galactose, 13 were confirmed to have the same enhancer or revertant phenotype in this altern-

ative approach as in the screen, demonstrating the feasibility of our genetic screen to identify novel Aβ toxicity modulators.

Evaluation of the revertant erv29 and its human ortholog Surf4

In addition, the knock-out strain showing the highest effect on Aβ toxicity, erv29Δ, was further evaluated. First, as we had observed in the revalidation study, the growth of erv29Δ pinned to inducing solid media (erv29Δ Aβ) was completely restored compared to the WT (WT Aβ; Fig. 5A), which was consistent with the results of the screen (Table S1). Then, we assessed the growth rate in liquid media for the WT and erv29Δ containing either Aβ or an empty vector: The Aβ-expressing WT strain (WT Aβ) also showed a slower growth compared to the WT strain containing an empty vector (WT) (Fig. 5B). Notably, when Aβ is expressed in the knock-out strain for *ERV29*, the growing curve shifts to the left indicating an evident improvement in cell growth. Therefore, these data reinforce the strong revertant behaviour of erv29Δ.

Erv29 has been reported to participate in collecting soluble cargo proteins into coat protein complex II (COPII)-coated vesicles in the ER. Given that the MFα prepro-leader sequence secretion signal targets Aβ to the secretory pathway, we wanted to rule out that the strong revertant effect observed in erv29Δ was artifactual. To discard that the different behaviour was due to a differential intracellular location of the peptide, we visualized Aβ-GFP using super-resolution microscopy stacks, where the same endomembranous pattern was observed (Fig. 5C). In addition, Aβ-GFP expression in this mutant was similar to the WT, as it had been already proven by Western blot

(Fig. 4B). In these experiments, long times of incubation in inducing media (15 h) were used to guarantee the formation of Aβ aggregates [365, 369].

In order to assess whether the absence of erv29 reverted Aβ toxicity in mammals, we knocked down its human ortholog, Surf4, in human neuroblastoma cells (SH-SY5Y). The *SURF4* gene belongs to the human surfeit locus, which contains 6 sequence-unrelated housekeeping genes (*SURF1-6*) [450]. Not much is known about the function of Surf4, but it has been associated to STIM1-dependent calcium entry and the maintenance of the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) architecture [444, 451]. Transiently transfecting the cells with the Surf4 siRNA significantly reduced mRNA levels compared to the control siRNA (Fig. 5D). However, when we tested the cell viability incubating the cells with two different concentrations of Aβ, no significant differences were observed (Fig. 5E). This observation could be due to the great variability of Aβ toxicity between batches or to the fact that transfecting the cells with Surf4 siRNA seemed slightly toxic *per se*.

CONCLUSIONS

The present report confirms that Aβ overexpression in the yeast secretory pathway is an appropriate model for identifying modulators of intracellular Aβ toxicity among the entire yeast genome. Additionally, we suggest previously unknown association between several genetic AD risk factors and Aβ toxicity, including the EPHA1 and ADSSL1 genes. Furthermore, we provide compelling evidences that Aβ toxicity involves several cellular pathways that have been already linked to AD

such as the removal of oxidative stress pathway, the mitochondrial respiratory chain and the clathrin-dependent endocytosis and we suggest the involvement of specific novel molecules such as the cytochrome b. Interestingly, this study reveals the importance of Ca^{2+} homeostasis in Aβ toxicity pointing to the calcineurin/Crz1 signalling pathway as a mediator of Aβ harmful effects and *SURF4* ortholog knock-out strain as the strongest inhibitor of Aβ toxicity.

A revalidation study confirmed some of the results from the screen and identified new Aβ modulators such as the yeast ortholog of *DYRK1A* and *VDAC*. Future work should include validation of the genes identified here as Aβ toxicity modulators in a more physiological relevant model, either neurons or skeletal muscle cells. Assuming positive validation in mammalian cells, we could consider this study a high-throughput model for screening molecular mechanisms responsible for Aβ toxicity with potential therapeutic applicability.

MATERIALS AND METHODS

Media and growth conditions.

Yeast strains were grown in YPD medium (2% w/v dextrose, 2% w/v peptone, 1% yeast extract), or synthetic minimal medium (SD; 2% w/v glucose, 0.17% yeast nitrogen base without amino acids, 5% w/v ammonium sulfate, 0,003% adenine). Solid medium contained also 20 mg/L agar. When indicated, SD was complemented with 0.7 g dropout medium lacking Uracil (SD -URA). The inducing medium was SD -URA that contained 2% w/v of galactose instead of glucose as carbon

source. Since ammonium sulfate inhibits the function of the antibiotic geneticin (G418), synthetic medium G418 was made with monosodium glutamate as a nitrogen source (MSG; 2% w/v glucose, 1.7 g/L yeast nitrogen base without ammonium sulphate and amino acids, 1 g/L monosodium glutamic acid, 2 g/L amino acid dropout). Medium preparation and yeast culturing was carried out according to standard techniques.

Yeast strains and plasmids

S. cerevisiae strain BY5563α was transformed with a multicopy yeast-expression plasmid (pRS426) with the URA3 selectable marker and CYC1-GAL1 promoter controlling the expression of MFα-Aβ1-42-GFP (kindly gifted by C. Marchal, Université Bordeaux 2, France), which was completely sequenced (Table S1). The construct contains a BamHI restriction site followed by the α-factor prepro sequence, the Aβ1-42 coding sequence, the linker GGTGCTGGCGCCGGTGCT and the GFP sequence followed by a Bsu36I restriction site. Plasmid transformations were performed using the lithium acetate method [452]. Transformants were selected in solid synthetic minimal medium lacking uracil (SD –URA). Colonies were grown for 4 days at 30 ºC. Expression of Aβ was induced by growth in inducing medium (2% w/v galactose) instead of non-inducing medium (2 % w/v glucose) for at least 5 h at 30 ºC.

Systematic genetic screen

The library used for the screen is constituted by ~5154 homozygous knock-outs (Saccharomyces Gene Deletion Project, EUROSCARF, Open Biosystems). These knocked out cells were

produced in the BY4741 strain background (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) and present G418 resistance. Solutions of Canavanine (L-canavanine sulfate salt, Can), and G418 were previously dissolved in water at 100 mg/L, filtered sterilized and stored in aliquots at 4ºC. The screen was performed with an automated system using the ROTOR HDA Singer Instruments following the Synthetic Genetic Array (SGA) protocol with some modifications [418, 453]. Briefly, MATα Aβ-expressing strain (BY5563) was grown overnight in rich medium and transferred to a 96-well plate. Then the cells were pinned onto YPD plates and grown for one day. After pinning the query strain onto fresh YPD plates, the ~5154 MATa library strains (BY4741) were pinned on top of the query strain and plates were incubated for one day at 30 ºC to allow the mating. Heterozygous MATa/α diploid cells were pinned onto SD with 200mg/L G418 lacking uracil (SD –URA G418). After a recovery in YPD medium, sporulation was induced for 7 days at 22ºC in medium with low amount of nutrients (2% agar, 1% potassium acetate, 25% Drop out –URA supplemented with Uracil). The MATa spore progeny was selected by pinning the spores onto specific selectable medium and grown for 2 days. Specifically, three selection rounds were performed with MSG and the specific markers. First selection medium was MSG –His –Arg Can (50mg/L) G418 (200mg/L). Second selection medium was MSG –His –Arg –URA Can (50mg/L) and third selection medium was MSG –His –Arg –URA Can (50mg/L) G418 (200mg/L). The resulted haploid cells containing both the deleted gene and the Aβ plasmid were pinned onto SD -URA plates. Cells were placed in either glucose (uninduced) or galactose (induced) medium and incubated at 30°C for 3, 4 and 5 days before scoring. The screen was performed in

duplicate. Analysis of the dots was accomplished with Cell Profiler software and the growth rate was quantified through the ratio of the growth observed in the induced plate after 3, 4 and 5 days over the growth observed in the uninduced plate after 3 days (Gal/Glu). Yeast strains considered enhancers and revertants of Aβ toxicity were analyzed with BiNGO plugin using Cytoscape software. Human orthologues were obtained from Ensembl (http://www.ensembl.org) and from Drosophila RNAi Screening DRSC Integrative Ortholog Prediction Tool (http://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl). Further analyses were performed in the Saccharomyces Genome Database (http://www.yeastgenome.org/).

Western blot

Yeast cells were harvested and lysed with TCA 85% for 10 min at RT. Supernatants were resolved in SDS-PAGE. Gels were transferred in polyvinylidene fluoride membranes (ImmobilonP, Millipore, USA), which were blocked for 1 h in tween-tris buffer saline (TTBS) 5% milk or 3% Albumin from Bovine Serum (BSA). Membranes were incubated o.n. at 4 ºC with the following primary antibodies: anti-GFP 1:500 (Sigma) and anti-Aβ 6E10 1:500 (Covance). Membranes were washed thrice with TTBS and incubated for 1 h with anti-mouse secondary antibodies (GE-Healthcare) at 1:2000 dilutions. Three washes with TTBS were performed and membranes were developed with Super signal West Pico and Femto Chemiluminiscent substrate (Thermo Scientific Pierce). Blotting quantification was done with Quantity One software.

Confocal microscopy

For microscopy analysis yeast strains were cultured in inducing medium 6 or 15 h at 30 ºC. Then, they were incubated 30 min in poly-lysinated chambered cover glass and digital images were taken with a Leica TCS SP5 II CW-STED confocal microscope, deconvolved with Huygens (SVI) and analyzed with Image J software.

Spotting assays

Strains were grown overnight at 30 °C in SD –URA containing glucose. Cell concentrations (OD660) were adjusted at OD660 0.3 and after 5 h growing in SD –URA containing raffinose, three dilutions 1:10 were spotted in plates with SD –URA containing glucose (uninduced) or galactose (induced). Plates were incubated at 30°C for 3 days before analysis.

Revalidation assays

The selected knock-out strains were grown in YPD G418 solid medium at 30 ºC for 24-48 h. One colony of each strain was grown in YPD liquid medium at 30 ºC o.n. Cell concentrations were adjusted at OD660 0.2 and after 4 h of growing in YPD liquid medium at 30 ºC, cells were transformed with the Aβ construct or an empty vector pRS426 as previously described. Transformants were selected in SD –URA solid medium for 4 days at 30 ºC. Then, two colonies of each knock-out strain were selected and used for spotting assay using the ROTOR HDA Singer Instruments as previously described. Briefly, knock-

out strains transformed with Aβ construct or empty vector were grown in SD –URA liquid medium o.n. at 30 ºC. Cell concentrations were adjusted at OD660 0.2 and after 4 h of growing in SD –URA, cells were pinned into either glucose (uninduced) or galactose (induced) medium and incubated at 30°C for 2 to 4 days before scoring.

Growth curve

Knock-out strains transformed with Aβ construct or empty vector were grown in SD –URA solid medium o.n. at 30 ºC. Cells were pinned into SD –URA liquid medium containing either glucose or galactose and incubated at 30 ºC for 48 h in a fluorimeter while OD660 values were being registered. OD660 results were used to obtain the growth curves of each mutant.

Cell culture

Human neuroblastoma cells (SH-SY5Y) were grown in DMEM /F-12 media (Invitrogen) supplemented with 15% FBS (Invitrogen) and 1% penicillin / streptomycin (Invitrogen). Cells were transfected with a Surf4 siRNA (Invitrogen) using Lipofectamine RNAiMAX (Invitrogen) according to manufacturer instructions.

MTT

Neuroblastoma cells were seeded in 96-well plates at a density of $7.5 \cdot 10^3$ cells / well. The corresponding treatments (100 μL / well) were added to the equivalent culture media without

phenol red and cells were treated for 24 h at 37ºC. Cell viability was assessed by methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction

Briefly, 10 μL of MTT (Sigma-Aldrich) stock solution (5 mg/mL) were added to the treatments and after 2h the media were replaced with 100 μL of dimethylsulfoxide (DMSO) to solubilize tetrazolium salts. MTT absorbance was determined in an Infinite 200 multiplate reader (Tecan) at A540 nm and corrected by A650 nm.

ACKNOWLEDGEMENTS

This work was supported by Fundació la Marató de TV3 (100310); the Spanish Ministry of Economy and Competitiveness (Fondo de Investigación Sanitaria PI13/00408 and Red HERACLES RD12/0042/0014); FEDER Funds; Generalitat de Catalunya (SGR09-1369).

FIGURES

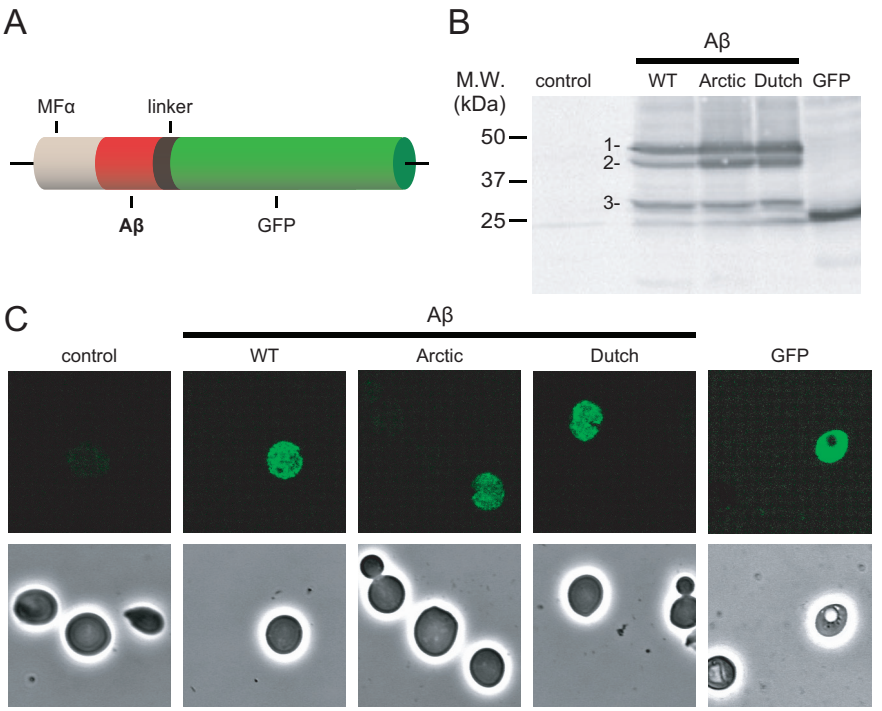

Figure 1. Expression of Aβ in yeast. (A) $A\beta_{42}$ construct contains the mating factor α (MFα) prepro-leader sequence secretion signal at the N-terminal and GFP tag at the C-terminal fused with a gly-ala linker (in dark grey). (B) Western blot analysis of Aβ-GFP expression using an anti-GFP antibody in yeast transformed with WT, Dutch or Arctic Aβ or with an empty vector (control) and cultured for 6 h at 30 ºC in inducing (galactose) medium. A strain constitutively expressing GFP was used as a positive control. 1 represents glycosylated MFα-Aβ-GFP, 2 indicates non-glycosylated MFα-Aβ-GFP and 3 represents Aβ-GFP. (C) Representative confocal images of Aβ-GFP in yeast transformed with WT, Dutch or Arctic Aβ or with an empty vector (control) and cultured for 6 h at 30 ºC in inducing (galactose) medium. A strain constitutively expressing GFP was used as a positive control.

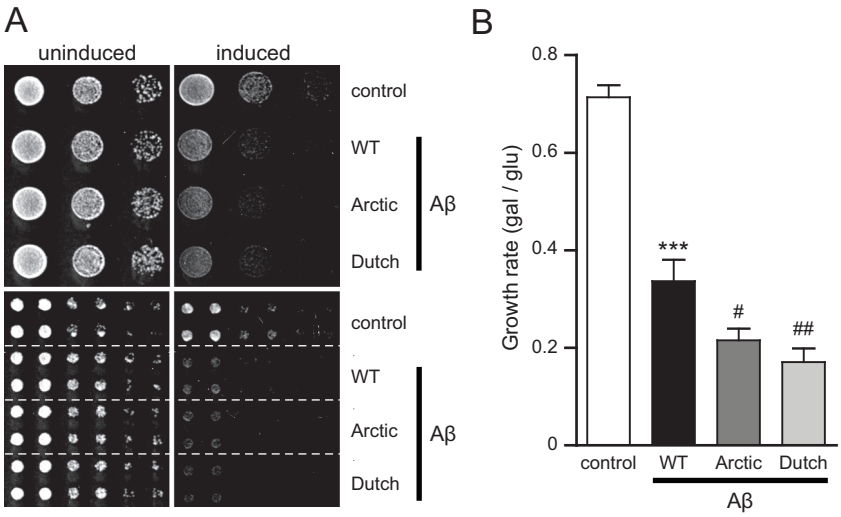

Figure 2. Intracellular Aβ impairs yeast growth. (A) Serial dilutions of yeast transformed with WT Aβ, Dutch Aβ, Arctic Aβ or with an empty vector (control) and spotted on inducing (galactose) and non-inducing (glucose) medium for 3 days at 30 ºC. (B) Quantification of mean growth observed in (A) calculated as the growth detected after 3 days in inducing medium (A, right panel) divided by the growth in non-inducing medium (A, left panel): (Gal / Glu). Data are the mean ± SEM of n = 11-16 experiments ***p<0.001 vs control, ##p<0.01, #p<0.05 vs. WT by One-Way Anova and Tukey's post-test.

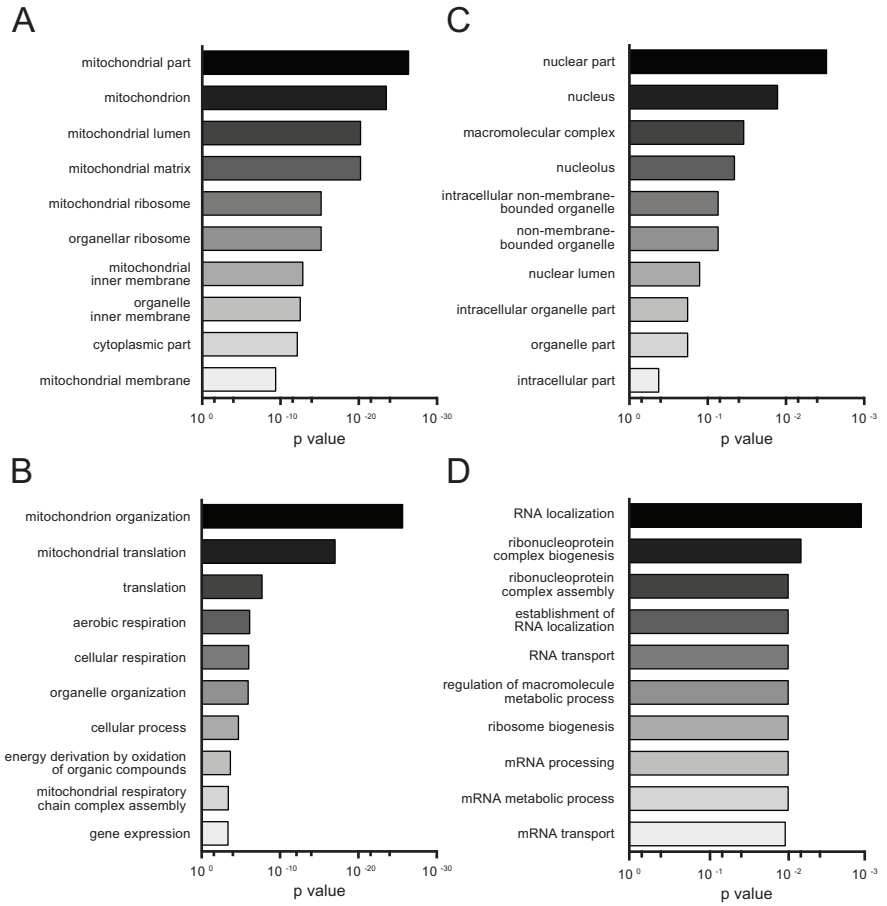

Figure 3. Gene ontology enrichment of Aβ toxicity modulators. (A) Cellular compartment enrichment of genes the knock-out of which enhances Aβ toxicity. (B) Biological process enrichment of genes the knock-out of which enhances Aβ toxicity. (C) Cellular compartment enrichment of genes the knock-out of which reverts Aβ toxicity. (D) Biological processes enrichment of genes the knock-out of which reverts Aβ toxicity.

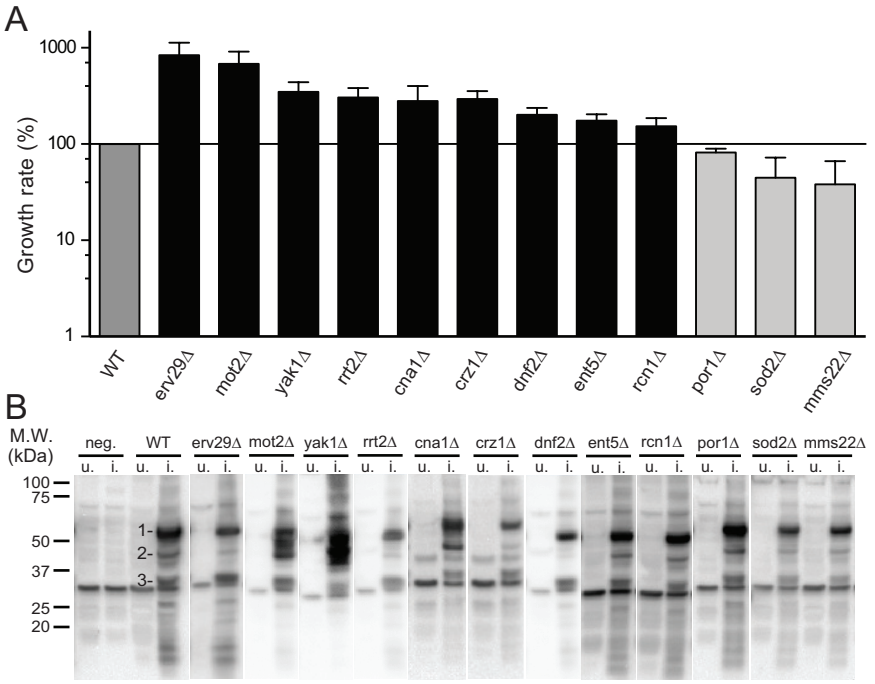

Figure 4. Revalidation of Aβ toxicity modulators. (A) Growth rate was assessed in knock-out strains transformed with the Aβ construct. First, growth observed in the Aβ containing strain in inducing medium was divided by growth in non-inducing medium and then this value was corrected by the same value calculated in the strain transformed with an empty plasmid: (Aβgal/Aβglu) / (Øgal/Øglu). Results shown are the percentage of the growth rate of each knock out compared to the WT strain. (B) Western blot showing Aβ expression in the WT and knock-out strains grown in uninducing (u.) or inducing (i.) medium for 15 h at 30 ºC; 1 represents glycosylated MFα-Aβ-GFP, 2 indicates non-glycosylated MFα-Aβ-GFP and 3 represents Aβ-GFP.

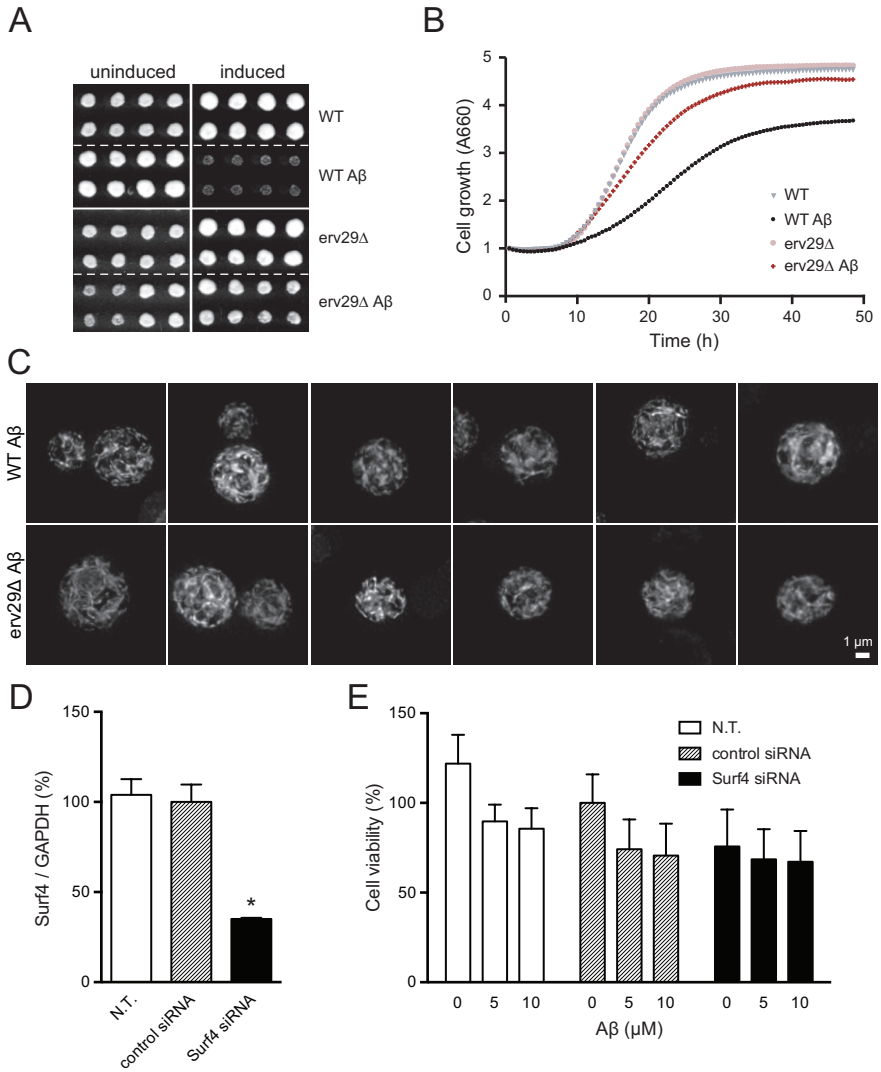

Figure 5. (A) Revalidation assay of WT strain transformed with an empty vector (WT) or Aβ (WT Aβ) compared to the erv29Δ strain expressing an empty vector (erv29Δ) or Aβ (erv29Δ Aβ). Strains were grown on uninducing or inducing medium for 4 days at 30 ºC. (B) Growth curve representation of the WT and the erv29Δ strains grown in inducing medium previously transformed with an empty vector or the Aβ construct. Growth was standarized to the initial OD660 value of each condition. (C) Z-projections of super-resolution microscopy images showing the subcellular localization of Aβ-GFP in the WT and erv29Δ strains transformed with the Aβ construct and grown in inducing medium for 15 h at 30 ºC. (D) Surf4

mRNA expression relative to GAPDH expression, 48 h after transfection. Mean ± SEM of 3 independent experiments. * $p < 0.0001$ by one-way ANOVA using Dunnett's multiple comparisons test. (E) Cell viability assessed in the human neuroblastoma SH-SY5Y cell line. 48 h after transfection, neuroblasts were treated with 0, 5 or 10 µM oligomeric Aβ. Viability of cells transfected with the control siRNA and not treated with Aβ was taken as 100%. Mean ± SEM of 8 independent experiments.

Table 1. Modulators of Aβ toxicity related to genetic AD risk factors.

Strain	Aβ toxicity	Function	Localization	Ortholog
*npr1*Δ	enhancer	endocytosis	cytoplasm, plasma membrane	EPHA1
*vps9*Δ	enhancer	vesicle vacuolar transport	cytosol	RABGEF1
*yol075c*Δ	partial revertant	putative protein transport	membrane	ABCA7
*vth1*Δ	partial revertant	golgi-vacuolar transport	endosome, membrane	SORL1
*gbp2*Δ	partial revertant	nuclear mRNA surveillance	cytosol, nucleus	CELF1
*ade12*Δ	revertant	adenylsuccinate synthesis	cytoplasm	ADSSL1

Modulators of Aβ toxicity the human orthologs of which are proven or putative AD risk factors. Enhancer strains growth rate after 5 days of induction was 0, revertant strains growth rate after 4 days of induction was higher than 0.7 and partial revertant strains growth rates after 4 days of induction ranged from 0.3 to 0.6.

Table 2. Cellular pathways with wide presence of Aβ toxicity modulators.

Strain	Aβ toxicity	Function	Localization	Ortholog
Removal of superoxide radicals				
*sod2*Δ	enhancer	oxygen radical detoxification	mitochondrial matrix	SOD2
*cta1*Δ	enhancer	hydrogen peroxide detoxification	mitochondrial and peroxisomal matrix	CAT
*hyr1*Δ	enhancer	cellular response to oxidative stress	mitochondrial intermembrane, peroxisomal matrix	GPX
Mitochondrial respiratory chain				
*cbs1,2*Δ	enhancer	cytochrome b mRNA activator	mitochondrial inner membrane and ribososome	-
*cbp2*Δ, *cbp1*Δ	enhancer	cytochrome b mRNA processing	mitochondria	-
*cbp6*Δ	enhancer	cytochrome b mRNA synthesis	mitochondrial ribosome	-
*ccm1*Δ	enhancer	cytochrome b and c oxidase mRNA maturation	mitochondria	-
*cor1*Δ	enhancer	cytochrome b additional subunit	mitochondrial inner membrane	UQCRC1

Strain	Aβ toxicity	Function	Localization	Ortholog
*qcr7*Δ	enhancer	cytochrome b additional subunit	mitochondrial inner membrane	UQCRB
*bcs1*Δ	enhancer	cytochrome b-c1 complex assembly	mitochondrial inner membrane	BCS1L
*cyc3*Δ	enhancer	cytochrome c heme liase	mitochondrial intermembrane	HCCS
*cox6*Δ	enhancer	COX additional subunit	mitochondrial inner membrane	COX5A
*cox9*Δ	enhancer	COX additional subunit	mitochondrial inner membrane	-
*cox10*Δ	enhancer	Heme A: farnesyl-transferase	mitochondrial membrane	COX10
*cox12*Δ	enhancer	COX additional subunit	mitochondrial inner membrane	COX6B1, 2
*cox11*Δ, *cox18*Δ	enhancer	COX assembly	mitochondrial inner membrane	COX11, 18
*cox19*Δ	enhancer	COX assembly	cytosol, mitochondrial intermembrane	COX19
*coa1*Δ	revertant	COX assembly	mitochondrial inner membrane	-
*coa2,3*Δ	enhancer	COX assembly	mitochondrial inner membrane	-
*pet100, 117*Δ	enhancer	COX assembly	mitochondrial inner membrane	-

Strain	Aβ toxicity	Function	Localization	Ortholog
*cox23*Δ	enhancer	mitochondrial copper homeostasis	mitochondrial intermembrane	~CHCHD7
		Clathrin-mediated endocytosis		
*sla1*Δ	partial enhancer	cytoskeletal protein binding	ubiquitous	CIN85
*bzz1*Δ	revertant	actin polymerization	cytoplasm, plasma membrane	~PACSIN
*ent5*Δ	revertant	clathrin recruitment and traffic	endosome	EPN1
*apm2*Δ	enhancer	golgi to vacuole transport	membrane	-
*apm3*Δ	enhancer	golgi to vacuole transport	membrane, golgi	-
		Calcium homeostasis		
*cna1*Δ	revertant	Crz1 regulation	cytoplasm	PPP3CA, B, C
*crz1*Δ	revertant	stress resp genes transcript.	cytoplasm, nucleus	~SP3
*rcn1*Δ	partial revertant	calcineurin regulation	cytoplasm	RCAN
*pmr1*Δ	revertant	protein sorting and processing	golgi membrane	ATP2C1
*mms22*Δ	enhancer	actin cytoskeleton, trafficking	plasma membrane	-

Strain	Aβ toxicity	Function	Localization	Ortholog
*frt2*Δ	enhancer	trafficking	ER membrane	-
*ecm7*Δ	enhancer	calcium uptake	membrane	-
*cmk1*Δ	enhancer	stress response	cytoplasm	CAMK1, 2
*erv29*Δ	revertant	vesicle formation	ER-Golgi, membrane	SURF4

Enhancer strains growth rate after 5 days of induction was 0, revertant strains growth rate after 4 days of induction was more than 0.7. ~ symbol was used to denote controversy in the corresponding human ortholog. COX, Cytochrome c oxidase.

Table 3. Additional Aβ toxicity modulators identified by Aβ transformation.

Strain	Aβ toxicity	Function	Localization	Ortholog
*yak1*Δ	revertant	glucose-sensing system	cytoplasm and nucleus	DYRK1A and B
*por1*Δ	enhancer	mitochondrial osmotic stability	mitochondrial outer membrane	VDAC
*dnf2*Δ	revertant	endocytosis and cell polarity	plasma membrane	ATP8B1

Genes of interest with controversial phenotype observed in the screen were revalidated. Growth rate was calculated as described in Fig. 4. In this case, strains with increased growth compared to WT were considered revertants whereas strains with impaired growth compared to WT were identified as enhancers.

SUPPLEMENTARY INFORMATION

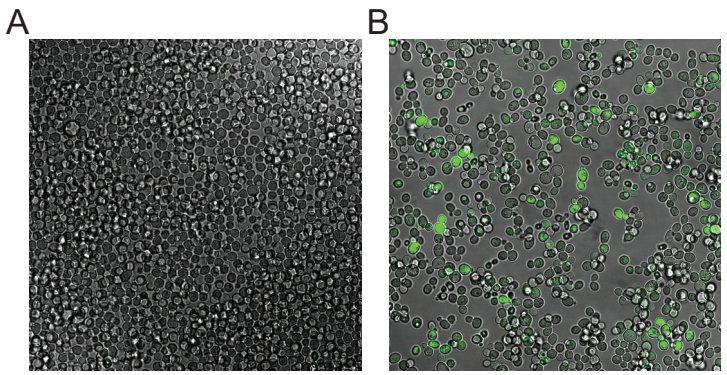

Figure S1. Representative confocal images of yeast transformed with the Aβ construct and cultured for 6 h at 30 ºC in non-inducing (A, glucose) and inducing (B, galactose) medium.

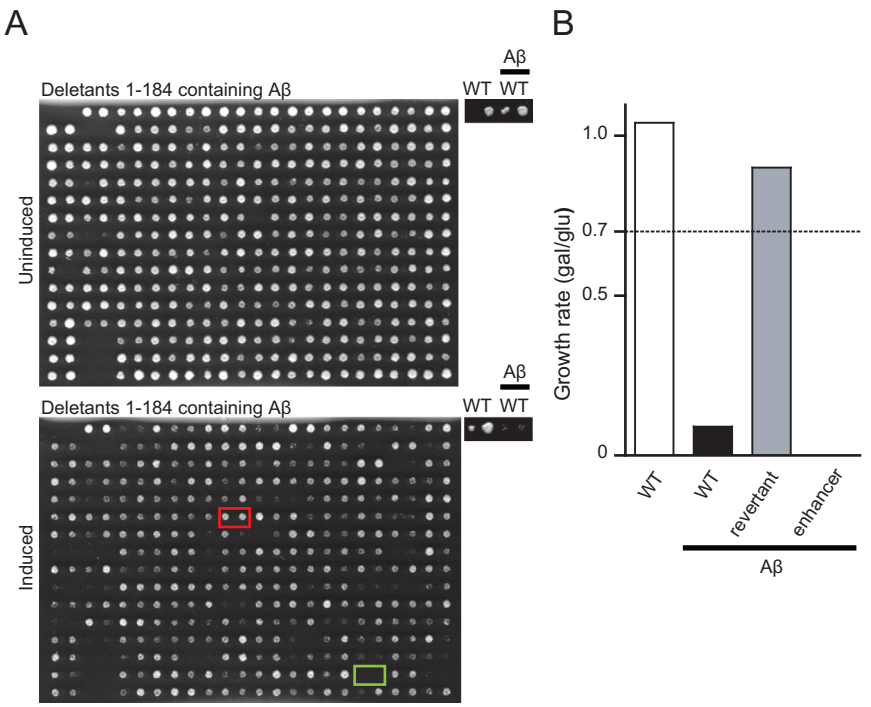

Figure S2. Screen plate for the first 185 knock-out strains assessed. (A) The strain containing Aβ construct was mated with a library with of ~5154 gene knock-out strains. Resultant haploid cells were spotted twice on medium containing galactose (induced) or glucose (uninduced) and cultured for

3, 4 and 5 days at 30 ºC. Image of the uninduced and the induced plate, at day 4, of the first 184 knock-outs containing the Aβ construct (left) and the plate of the WT strain containing or not the Aβ construct (right). An example of a knock-out that reverts Aβ toxicity (red) and a knock-out that enhances Aβ toxicity (green) is indicated. (B) Growth rate quantification of the WT strain containing or not Aβ and an example of a revertant and a enhancer knock-out strain of Aβ toxicity. The minimum growth rate set for the revertants is at 0.7 whereas the maximum growth rate set for the enhancers is at 0, as indicated.

Figure S3. Diagram of cellular compartments enriched in enhancer genes. Analysis of genes the knock-out strains of which are enhancers of Aβ toxicity (Table S1) was done with BiNGO plugin using cytoscape software. The list of mutants from the screen was used as background. Nodes (circles) sizes correspond to the number of genes from the dataset within the geneset. Colour of the nodes (circles) correspond to the significance based on the BiNGO p-value, darker blue indicates lower p-values.

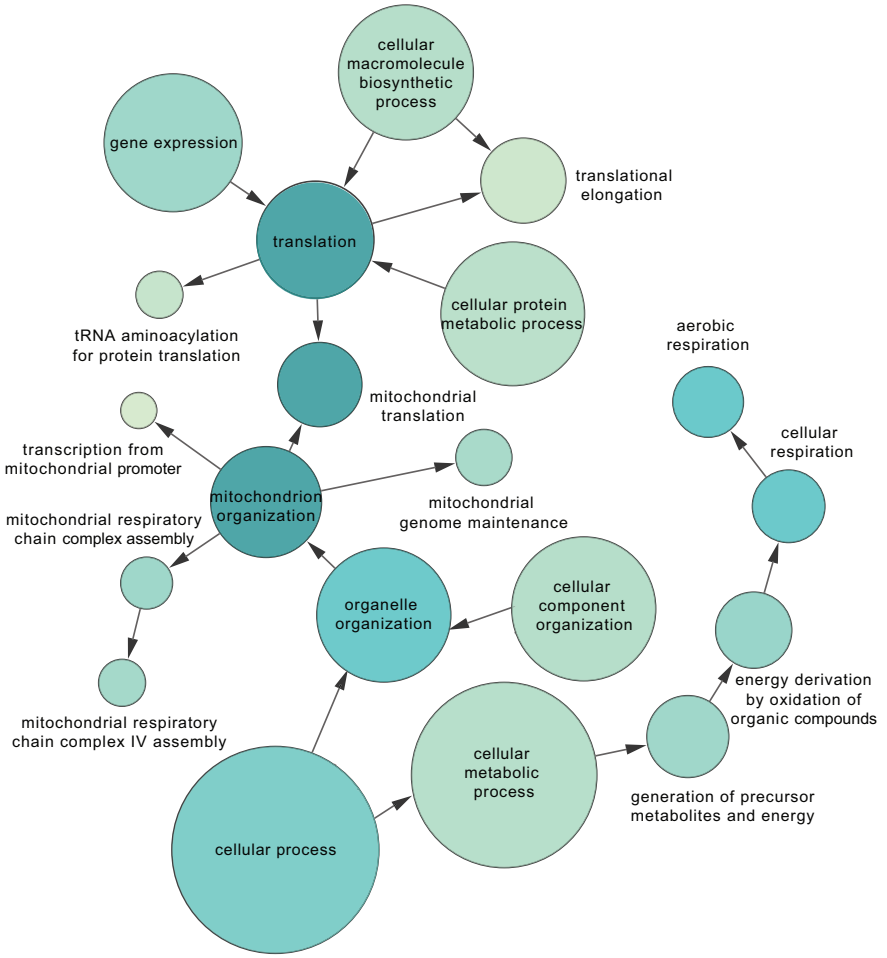

Figure S4. Diagram of biological processes enriched in enhancer genes. Analysis of genes the knock-out strains of which are enhancers of Aβ toxicity (Table S1) was done with BiNGO plugin using cytoscape software. The list of mutants from the screen was used as background. Nodes (circles) sizes correspond to the number of genes from the dataset within the geneset. Colour of the nodes (circles) correspond to the significance based on the BiNGO p-value, darker blue indicates lower p-values.

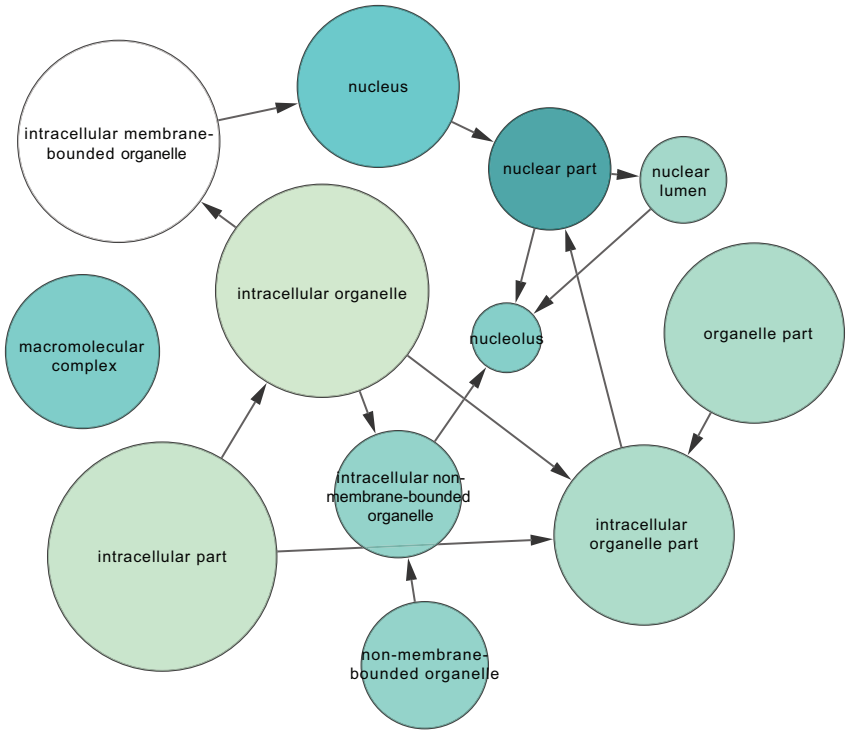

Figure S5. Diagram of cellular compartments enriched in revertant genes. Analysis of genes the knock-out strains of which are revertants of Aβ toxicity (Table S2) was done with BiNGO plugin using cytoscape software. The list of mutants from the screen was used as background. Nodes (circles) sizes correspond to the number of genes from the dataset within the geneset. Colour of the nodes (circles) correspond to the significance based on the BiNGO p-value, darker blue indicates lower p-values.

Figure S6. Diagram of biological processes enriched in revertant genes. Analysis of genes the knock-out strains of which are revertants of Aβ toxicity (Table S2) was done with BiNGO plugin using cytoscape software. The list of mutants from the screen was used as background. Nodes (circles) sizes correspond to the number of genes from the dataset within the geneset. Colour of the nodes (circles) correspond to the significance based on the BiNGO p-value, darker blue indicates lower p-values.

Table S1. Genes the deletion of which enhances Aβ toxicity.

Systematic	Gene	Description
YAL011W	SWC3	SWr Complex, protein of unknown function
YAL021C	CCR4	Carbon Catabolite Repression, component of the CCR4-NOT transcriptional complex
YAL040C	CLN3	CycLiN, g1 cyclin involved in cell cycle progression
YAL044C	GCV3	GlyCine cleaVage, H subunit of the mitochondrial glycine decarboxylase complex
YAL060W	BDH1	Butanediol DeHydrogenase, nAD-dependent
YBL071C-B		Putative protein of unknown function
YBR048W	RPS11B	Ribosomal Protein of the Small subunit.
YBR058C-A	TSC3	Temperature-sensitive Suppressors of Csg2 mutants, protein that stimulates the activity of serine palmitoyltransferase
YBR066C	NRG2	Negative Regulator of Glucose-controlled genes, transcriptional repressor
YBR181C	RPS6B	Ribosomal Protein of the Small subunit.
YBR186W	PCH2	Pachytene CHeckpoint, hexameric ring ATPase that remodels chromosome axis protein Hop1p
YBR206W		Dubious open reading frame
YBR214W	SDS24	Homolog of S. pombe SDS23, protein involved in cell separation during budding
YBR215W	HPC2	Histone Periodic Control, subunit of the HIR complex
YBR246W	RRT2	Regulator of rDNA Transcription, methylesterase in diphthamide biosynthesis

Systematic	Gene	Description
YBR266C	SLM6	Synthetic Lethal with Mss4, protein with a potential role in actin cytoskeleton organization
YBR267W	REI1	REquired for Isotropic bud growth, cytoplasmic pre-60S factor
YBR291C	CTP1	Citrate Transport Protein, mitochondrial inner membrane citrate transporter
YCR020W-B	HTL1	High-Temperature Lethal, component of the RSC chromatin remodeling complex
YCR061W		Protein of unknown function
YCR094W	CDC50	Cell Division Cycle, endosomal protein that interacts with phospholipid flippase Drs2p
YDL009C		Protein of unknown function
YDL063C	SYO1	SYnchronized impOrt or SYmpOrtin, transport adaptor or symportin
YDL075W	RPL31A	Ribosomal Protein of the Large subunit, ribosomal 60S subunit protein L31A
YDL080C	THI3	THIamine metabolism, regulatory protein that binds Pdc2p and Thi2p transcription factors
YDL101C	DUN1	DNA-damage UNinducible, cell-cycle checkpoint serine-threonine kinase
YDR123C	INO2	INOsitol requiring, transcription factor
YDR153C	ENT5	Epsin N-Terminal homology, protein containing an N-terminal epsin-like domain
YDR159W	SAC3	Suppressor of ACtin, mRNA export factor
YDR171W	HSP42	Heat Shock Protein, small heat shock protein (sHSP) with chaperone activity

Systematic	Gene	Description
YDR174W	HMO1	High MObility group family, chromatin associated high mobility group (HMG) family member
YDR186C		Putative protein of unknown function
YDR195W	REF2	RNA End Formation, rNA-binding protein
YDR214W	AHA1	Activator of HSP90 ATPase, co-chaperone that binds Hsp82p and activates its ATPase activity
YDR219C	MFB1	Mitochondria-associated F-Box protein
YDR225W	HTA1	Histone H Two A
YDR378C	LSM6	Like SM protein
YDR537C		Dubious open reading frame
YER016W	BIM1	BInding to Microtubules, microtubule plus end-tracking protein
YER066W	RRT13	Regulator of rDNA Transcription, putative protein of unknown function
YER068W	MOT2	Modulator Of Transcription, ubiquitin-protein ligase subunit of the CCR4-NOT complex
YER092W	IES5	Ino Eighty Subunit, non-essential INO80 chromatin remodeling complex subunit
YER119C-A		Dubious open reading frame
YER151C	UBP3	UBiquitin-specific Protease, ubiquitin-specific protease involved in transport and osmotic response
YFR034C	PHO4	PHOsphate metabolism, basic helix-loop-helix (bHLH) transcription factor of the myc-family
YFR036W	CDC26	Cell Division Cycle, subunit of the Anaphase-Promoting Complex/Cyclosome (APC/C)
YGL007C-A		Putative protein of unknown function

Systematic	Gene	Description
YGL041C-B		Putative protein of unknown function
YGL168W	HUR1	HydroxyUrea Resistance, protein of unknown function
YGL222C	EDC1	Enhancer of mRNA DeCapping, rNA-binding protein that activates mRNA decapping directly
YGL241W	KAP114	KAryoPherin, karyopherin
YGL253W	HXK2	HeXoKinase, hexokinase isoenzyme 2
YGR006W	PRP18	Pre-mRNA Processing, splicing factor and component of snRNP U5
YGR015C		Putative protein of unknown function
YGR081C	SLX9	Protein required for pre-rRNA processing
YGR133W	PEX4	PEroXin, peroxisomal ubiquitin conjugating enzyme
YGR226C		Dubious open reading frame
YGR271C-A	EFG1	Exit From G1, essential protein required for maturation of 18S rRNA
YGR284C	ERV29	ER Vesicle, protein localized to COPII-coated vesicles
YHR021C	RPS27B	Ribosomal Protein of the Small subunit.
YHR025W	THR1	THReonine requiring, homoserine kinase
YHR064C	SSZ1	Hsp70 protein that interacts with Zuo1p (a DnaJ homolog)
YIL154C	IMP2'	Independent of Mitochondrial Particle, transcriptional activator involved in maintenance of ion homeostasis
YIL156W	UBP7	UBiquitin-specific Protease, ubiquitin-specific protease that cleaves ubiquitin-protein fusions
YIL157C	COA1	Cytochrome Oxidase Assembly, mitochondrial inner membrane protein

Systematic	Gene	Description
YJL012C-A		Merged open reading frame
YJL027C		Putative protein of unknown function
YJL080C	SCP160	S. cerevisiae protein involved in the Control of Ploidy, essential RNA-binding G protein effector of mating response pathway
YJL123C	MTC1	Maintenance of Telomere Capping, protein of unknown function that may interact with ribosomes
YJL124C	LSM1	Like SM, lsm (Like Sm) protein
YJL141C	YAK1	Yet Another Kinase, serine-threonine protein kinase
YJL176C	SWI3	SWItching deficient, subunit of the SWI/SNF chromatin remodeling complex
YJL188C	BUD19	BUD site selection, dubious open reading frame
YKL009W	MRT4	mRNA Turnover 4, protein involved in mRNA turnover and ribosome assembly
YKL096C-B		Putative protein of unknown function
YKL139W	CTK1	Carboxy-Terminal domain Kinase, catalytic (alpha) subunit of C-terminal domain kinase I (CTDK-I)
YKL163W	PIR3	Protein containing Internal Repeats, o-glycosylated covalently-bound cell wall protein
YKR024C	DBP7	Dead Box Protein, putative ATP-dependent RNA helicase of the DEAD-box family
YKR095W	MLP1	Myosin-Like Protein, myosin-like protein associated with the nuclear envelope
YLL002W	RTT109	Regulator of Ty1 Transposition, histone acetyltransferase

Systematic	Gene	Description
YLL013C	PUF3	PUmilio-homology domain Family, protein of the mitochondrial outer surface
YLL024C	SSA2	Stress-Seventy subfamily A, aTP-binding protein
YLL043W	FPS1	Fdp1 Suppressor, aquaglyceroporin
YLL046C	RNP1	Ribonucleoprotein that contains two RNA recognition motifs (RRM)
YLR015W	BRE2	BREfeldin A sensitivity, subunit of COMPASS (Set1C) complex
YLR046C		Putative membrane protein
YLR047C	FRE8	Protein with sequence similarity to iron/copper reductases
YLR048W	RPS0B	Ribosomal Protein of the Small subunit.
YLR057W	MNL2	MaNnosidase-Like protein, putative mannosidase involved in ER-associated protein degradation
YLR061W	RPL22A	Ribosomal Protein of the Large subunit, ribosomal 60S subunit protein L22A
YLR062C	BUD28	BUD site selection, dubious open reading frame
YLR063W	BMT6	Base Methyltransferase of Twenty five S rRNA 6, methyltransferase required for m3U2843 methylation of the 25S rRNA
YLR072W		Lipid transfer protein Anchored at Membrane contact site, protein of unknown function
YLR079W	SIC1	Substrate/Subunit Inhibitor of Cyclin-dependent protein kinase, cyclin-dependent kinase inhibitor
YLR182W	SWI6	SWItching deficient, transcription cofactor
YLR322W	VPS65	Vacuolar Protein Sorting, dubious open reading frame

Systematic	Gene	Description
YLR369W	SSQ1	Stress-Seventy subfamily Q, mitochondrial hsp70-type molecular chaperone
YLR418C	CDC73	Cell Division Cycle, component of the Paf1p complex
YLR433C	CNA1	CalciNeurin A, calcineurin A
YML009W-B		Dubious open reading frame
YML012C-A		Dubious open reading frame
YML014W	TRM9	TRna Methyltransferase, tRNA methyltransferase
YML024W	RPS17A	Ribosomal Protein of the Small subunit, ribosomal protein 51 (rp51) of the small (40s) subunit
YML050W	AIM32	Altered Inheritance rate of Mitochondria, putative protein of unknown function
YML061C	PIF1	Petite Integration Frequency, dNA helicase
YMR018W		Putative protein of unknown function with similarity to human PEX5Rp
YMR116C	ASC1	Absence of growth Suppressor of Cyp1, g-protein _ subunit and guanine dissociation inhibitor for Gpa2p
YMR179W	SPT21	SuPpressor of Ty, protein with a role in transcriptional silencing
YMR198W	CIK1	Chromosome Instability and Karyogamy, kinesin-associated protein
YMR222C	FSH2	Family of Serine Hydrolases, putative serine hydrolase that localizes to the cytoplasm
YMR242W-A		Putative protein of unknown function
YNL022C	RCM1	rRNA m5C methyltransferase
YNL027W	CRZ1	Calcineurin-Responsive Zinc finger, transcription factor

Systematic	Gene	Description
YNL076W	MKS1	Multicopy Kinase Suppressor, pleiotropic negative transcriptional regulator
YNL138W	SRV2	Suppressor of RasVal19, cyclase-associated protein
YNL146W		Putative protein of unknown function
YNL220W	ADE12	ADEnine requiring, catalyzes the first step in synthesis of adenosine monophosphate
YNR052C	POP2	PGK promoter directed OverProduction, rNase of the DEDD superfamily
YOL045W	PSK2	Pas domain-containing Serine/threonine protein Kinase, pAS-domain containing serine/threonine protein kinase
YOL046C		Dubious open reading frame
YOL054W	PSH1	Pob3/Spt16 Histone associated, e3 ubiquitin ligase targeting centromere-binding protein Cse4p
YOL085C		Dubious open reading frame
YOR113W	AZF1	Asparagine-rich Zinc-Finger, transcription factor
YOR120W	GCY1	Galactose-inducible Crystallin-like Yeast protein, glycerol dehydrogenase
YOR141C	ARP8	Actin-Related Protein, nuclear actin-related protein involved in chromatin remodeling
YOR196C	LIP5	LIPoic acid, protein involved in biosynthesis of the coenzyme lipoic acid
YOR301W	RAX1	Protein involved in bud site selection during bipolar budding
YOR309C		Dubious open reading frame

Systematic	Gene	Description
YOR312C	RPL20B	Ribosomal Protein of the Large subunit, ribosomal 60S subunit protein L20B
YPL008W	CHL1	CHromosome Loss, probable DNA helicase
YPL098C	MGR2	Mitochondrial Genome Required, subunit of the TIM23 translocase complex
YPL106C	SSE1	ATPase component of heat shock protein Hsp90 chaperone complex
YPL145C	KES1	KrE11-1 Suppressor, one of seven members of the yeast oxysterol binding protein family
YPL174C	NIP100	Nuclear ImPort, large subunit of the dynactin complex
YPL178W	CBC2	Cap Binding Complex, small subunit of the heterodimeric cap binding complex with Sto1p
YPL240C	HSP82	Heat Shock Protein, hsp90 chaperone
YPR043W	RPL43A	Ribosomal Protein of the Large subunit, ribosomal 60S subunit protein L43A
YPR057W	BRR1	Bad Response to Refrigeration, snRNP protein component of spliceosomal snRNPs
YPR096C		Protein of unknown function
YPR159C-A		Protein of unknown function
YPR159W	KRE6	Killer toxin REsistant, type II integral membrane protein

Aβ toxicity revertant genes. List of genes the knock-out strain of which is considered revertant of Aβ toxicity since its growth rate after 4 days of induction is minimum 0.7 compared to 0.09 in the WT strain. A minimum

growth in uninduced medium at day 3 is set. In red, genes mentioned in Table 2.

Table S2. Genes the deletion of which enhances Aβ toxicity.

Systematic	Gene	Description
YAL039C	CYC3	CYtochrome C heme lyase, attaches heme to apo-cytochrome c.
YBL007C	SLA1	Synthetic Lethal with ABP1, cytoskeletal protein binding protein.
YBL022C	PIM1	Proteolysis In Mitochondria, ATP-dependent Lon protease.
YBL024W	NCL1	NuCLear protein, s-adenosyl-L-methionine-dependent tRNA: m5C-methyltransferase.
YBL038W	MRPL16	Mitochondrial Ribosomal Protein, Large subunit.
YBL045C	COR1	CORe protein of QH2 cytochrome c reductase.
YBL051C	PIN4	Psi+ INducibility, involved in G2/M phase progression and response to DNA damage.
YBL052C	SAS3	Something About Silencing, histone acetyltransferase catalytic subunit of NuA3 complex.
YBL080C	PET112	PETite colonies, subunit of the trimeric GatFAB AmidoTransferase(AdT) complex.
YBL090W	MRP21	Mitochondrial Ribosomal Protein, small subunit.
YBL098W	BNA4	Biosynthesis of Nicotinic Acid, kynurenine 3-monooxygenase.
YBL106C	SRO77	Suppressor of rho3, protein with roles in exocytosis and cation homeostasis.
YBR021W	FUR4	5-FlUoRouridine sensitivity, plasma membrane localized uracil permease.
YBR107C	IML3	Increased Minichromosome Loss, outer kinetochore protein and component of the Ctf19 complex.

Systematic	Gene	Description
YBR113W		Dubious open reading frame.
YBR114W	RAD16	RADiation sensitive, nucleotide excision repair (NER) protein.
YBR120C	CBP6	Cytochrome B Protein synthesis, mitochondrial protein required for translation of the COB mRNA.
YBR131W	CCZ1	Calcium Caffeine Zinc sensitivity, subunit of a heterodimeric guanine nucleotide exchange factor.
YBR163W	EXO5	EXOnuclease V, mitochondrial 5'-3' exonuclease and sliding exonuclease.
YBR241C		Putative transporter.
YBR280C	SAF1	SCF Associated Factor, f-Box protein involved in proteasome-dependent degradation of Aah1p.
YBR282W	MRPL27	Mitochondrial Ribosomal Protein, large subunit.
YBR283C	SSH1	Sec Sixty-one Homolog, subunit of the Ssh1 translocon complex.
YBR288C	APM3	Clathrin Adaptor Protein complex Medium chain, mu3-like subunit of the clathrin associated protein complex (AP-3).
YBR296C	PHO89	PHOsphate metabolism, plasma membrane Na+/Pi cotransporter.
YCR003W	MRPL32	Mitochondrial Ribosomal Protein, large subunit.
YCR028C-A	RIM1	Replication In Mitochondria, ssDNA-binding protein essential for mitochondrial genome maintenance.
YCR046C	IMG1	Integrity of Mitochondrial Genome, mitochondrial ribosomal protein of the large subunit.
YCR053W	THR4	THReonine requiring, threonine synthase.

Systematic	Gene	Description
YCR071C	IMG2	Integrity of Mitochondrial Genome, mitochondrial ribosomal protein of the large subunit.
YCR073W-A	SOL2	Suppressor Of Los1-1, protein with a possible role in tRNA export.
YDL044C	MTF2	Mitochondrial Transcription Factor, protein that interacts with mitochondrial RNA polymerase.
YDL067C	COX9	Cytochrome c OXidase, subunit VIIa of COX (Complex IV).
YDL069C	CBS1	Cytochrome B Synthesis, mitochondrial translational activator of the COB mRNA.
YDL104C	QRI7	Protein involved in threonylcarbamoyl adenosine biosynthesis.
YDL174C	DLD1	D-Lactate Dehydrogenase, d-lactate dehydrogenase.
YDL190C	UFD2	Ubiquitin Fusion Degradation, ubiquitin chain assembly factor (E4).
YDR028C	REG1	REsistance to Glucose repression, regulatory subunit of type 1 protein phosphatase Glc7p.
YDR029W		Dubious open reading frame.
YDR035W	ARO3	AROmatic amino acid requiring, 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase.
YDR069C	DOA4	Degradation Of Alpha, ubiquitin hydrolase.
YDR077W	SED1	Suppression of Exponential Defect, major stress-induced structural GPI-cell wall glycoprotein.
YDR079W	PET100	PETite colonies, chaperone that facilitates the assembly of COX.
YDR114C		Putative protein of unknown function.

Systematic	Gene	Description
YDR115W		Mitochondrial organization of gene expression.
YDR128W	MTC5	Maintenance of Telomere Capping, subunit of the SEA (Seh1-associated) complex.
YDR194C	MSS116	Mitochondrial Splicing System, mitochondrial transcription elongation factor.
YDR197W	CBS2	Cytochrome B Synthesis, mitochondrial translational activator of the COB mRNA.
YDR230W		Dubious open reading frame.
YDR256C	CTA1	CaTalase A, catalase A.
YDR268W	MSW1	Mitochondrial aminoacyl-tRNA Synthetase.
YDR296W	MHR1	Mitochondrial Homologous Recombination, protein involved mitochondrial homologous recombination.
YDR298C	ATP5	ATP synthase, subunit 5 of the stator stalk of mitochondrial F1Fo ATP synthase.
YDR347W	MRP1	Mitochondrial Ribosomal Protein, small subunit.
YDR375C	BCS1	Ubiquinol-cytochrome c reductase (bc1) Synthesis, protein translocase and chaperone required for Complex III assembly.
YDR377W	ATP17	ATP synthase, subunit f of the Fo sector of mitochondrial F1Fo ATP synthase.
YDR405W	MRP20	Mitochondrial Ribosomal Protein, large subunit
YDR421W	ARO80	AROmatic amino acid requiring, zinc finger transcriptional activator of the Zn2Cys6 family.
YDR442W		Dubious open reading frame.
YDR443C	SSN2	Suppressor of SNf1, subunit of the RNA polymerase II mediator complex.

Systematic	Gene	Description
YDR529C	QCR7	UbiQuinol-cytochrome C oxidoReductase, subunit 7 of ubiquinol cytochrome-c reductase (Complex III).
YEL071W	DLD3	D-Lactate Dehydrogenase, d-lactate dehydrogenase.
YER005W	YND1	Yeast Nucleoside Diphosphatase, apyrase with wide substrate specificity.
YER017C	AFG3	ATPase Family Gene, mitochondrial inner membrane m-AAA protease component.
YER035W	EDC2	Enhancer of mRNA DeCapping, rNA-binding protein that directly activates mRNA decapping.
YER042W	MXR1	Peptide Methionine sulfoXide Reductase, methionine-S-sulfoxide reductase.
YER050C	RSM18	Ribosomal Small subunit of Mitochondria.
YER055C	HIS1	HIStidine, aTP phosphoribosyltransferase.
YER058W	PET117	PETite colonies, protein required for assembly of COX.
YER060W-A	FCY22	FluoroCYtosine resistance, putative purine-cytosine permease.
YER084W		Protein of unknown function.
YER087W	AIM10	Altered Inheritance rate of Mitochondria, protein with similarity to tRNA synthetases.
YER108C		Merged ORF.
YER134C		Magnesium-dependent acid phosphatase.
YER153C	PET122	PETite colonies, mitochondrial translational activator specific for the COX3 mRNA.
YER154W	OXA1	Cytochrome OXidase Activity, mitochondrial inner membrane insertase.

Systematic	Gene	Description
YFL030W	AGX1	Alanine:Glyoxylate aminotrans(X)ferase, alanine:glyoxylate aminotransferase (AGT).
YFL036W	RPO41	RNA POlymerase, mitochondrial RNA polymerase.
YFR024C		Deleted ORF.
YGL003C	CDH1	CDC20 Homolog, activator of anaphase-promoting complex/cyclosome (APC/C).
YGL034C		Dubious open reading frame.
YGL042C		Dubious open reading frame.
YGL064C	MRH4	Mitochondrial RNA Helicase, mitochondrial ATP-dependent RNA helicase of the DEAD-box family.
YGL107C	RMD9	Required for Meiotic nuclear Division, mitochondrial protein required for respiratory growth.
YGL119W	COQ8	COenzyme Q, protein required for ubiquinone biosynthesis and respiratory growth.
YGL124C	MON1	MONensin sensitivity, subunit of a heterodimeric guanine nucleotide exchange factor (GEF).
YGL125W	MET13	METhionine requiring, major isozyme of methylenetetrahydrofolate reductase.
YGL126W	SCS3	Suppressor of Choline Sensitivity, protein required for inositol prototrophy.
YGL129C	RSM23	Ribosomal Small subunit of Mitochondria.
YGL143C	MRF1	Mitochondrial peptide chain Release Factor, mitochondrial translation release factor.
YGL148W	ARO2	AROmatic amino acid requiring, bifunctional chorismate synthase and flavin reductase.
YGL186C	TPN1	Transport of PyridoxiNe, plasma membrane pyridoxine (vitamin B6) transporter.

Systematic	Gene	Description
YGL205W	POX1	Fatty-acyl coenzyme A oxidase.
YGL208W	SIP2	SNF1-Interacting Protein, one of three beta subunits of the Snf1 kinase complex.
YGL215W	CLG1	Cyclin-Like Gene, cyclin-like protein that interacts with Pho85p.
YGL255W	ZRT1	Zinc-Regulated Transporter, high-affinity zinc transporter of the plasma membrane.
YGR034W	RPL26B	Ribosomal Protein of the Large subunit, ribosomal 60S subunit protein L26B.
YGR035C		Putative protein of unknown function.
YGR043C	NQM1	Non-Quiescent Mutant, transaldolase.
YGR062C	COX18	Cytochrome c OXidase, protein required for membrane insertion of C-terminus of Cox2p.
YGR070W	ROM1	RhO1 Multicopy suppressor, gDP/GTP exchange protein (GEP) for Rho1p.
YGR093W	DRN1	Debranching enzyme-associated RiboNuclease, splicing factor that modulates turnover of branched RNAs by Dbr1p.
YGR102C	GTF1	Glutaminyl Transamidase subunit F, subunit of the trimeric GatFAB AmidoTransferase(AdT) complex.
YGR112W	SHY1	SURF Homolog of Yeast, mitochondrial inner membrane protein required for complex IV assembly.
YGR129W	SYF2	SYnthetic lethal with cdc40 (Forty), member of the NineTeen Complex (NTC).
YGR138C	TPO2	Transporter of POlyamines, polyamine transporter of the major facilitator superfamily.

Systematic	Gene	Description
YGR150C	CCM1	COB and COX1 mRNA maturation, mitochondrial 15S rRNA-binding protein.
YGR165W	MRPS35	Mitochondrial Ribosomal Protein, small subunit.
YGR171C	MSM1	Mitochondrial aminoacyl-tRNA Synthetase, Methionine.
YGR204W	ADE3	ADEnine requiring, cytoplasmic trifunctional enzyme C1-tetrahydrofolate synthase.
YGR215W	RSM27	Ribosomal Small subunit of Mitochondria.
YGR220C	MRPL9	Mitochondrial Ribosomal Protein, large subunit.
YGR222W	PET54	PETite colonies, mitochondrial inner membrane protein.
YGR255C	COQ6	COenzyme Q, putative flavin-dependent monooxygenase.
YGR257C	MTM1	Manganese Trafficking factor for Mitochondrial SOD2, protein of the mitochondrial carrier family
YHL006C	SHU1	Suppressor of HU sensitivity, component of Shu complex (aka PCSS complex).
YHL008C		Putative protein of unknown function.
YHL010C	ETP1	Ethanol Tolerance Protein, putative protein of unknown function required for growth on ethanol.
YHL019C	APM2	Clathrin Adaptor Protein complex Medium chain.
YHL034C	SBP1	Protein that binds eIF4G and has a role in repression of translation.
YHL035C	VMR1	Vacuolar Multidrug Resistance, vacuolar membrane protein.
YHL038C	CBP2	Cytochrome B mRNA Processing, required for splicing of group I intron bI5 of the COB pre-mRNA.

Systematic	Gene	Description
YHL045W		Putative protein of unknown function.
YHL046C	PAU13	SeriPAUperin, protein of unknown function.
YHL047C	ARN2	AFT1 ReguloN, transporter.
YHR011W	DIA4	Digs Into Agar, probable mitochondrial seryl-tRNA synthetase.
YHR012W	VPS29	Vacuolar Protein Sorting, subunit of the membrane-associated retromer complex.
YHR015W	MIP6	Mex67-Interacting Protein, putative RNA-binding protein.
YHR037W	PUT2	Proline UTilization, delta-1-pyrroline-5-carboxylate dehydrogenase.
YHR050W-A		Protein of unknown function.
YHR051W	COX6	Cytochrome c OXidase, subunit VI of COX (Complex IV).
YHR116W	COX23	Cytochrome OXidase, protein that functions in mitochondrial copper homeostasis.
YHR147C	MRPL6	Mitochondrial Ribosomal Protein, large subunit.
YHR151C	MTC6	Maintenance of Telomere Capping, protein of unknown function.
YHR155W	YSP1	Yeast Suicide Protein, mitochondrial protein.
YHR168W	MTG2	MiTochondrial Gtpase 2, putative GTPase.
YHR198C	AIM18	Altered Inheritance rate of Mitochondria, putative protein of unknown function.
YIL002C	INP51	INositol polyphosphate 5-Phosphatase, phosphatidylinositol 4.
YIL006W	YIA6	Mitochondrial NAD+ transporter.

Systematic	Gene	Description
YIL036W	CST6	Chromosome STability, basic leucine zipper (bZIP) transcription factor.
YIL039W	TED1	Trafficking of Emp24p/Erv25p-dependent cargo Disrupted, gPI-glycan remodelase.
YIL041W	GVP36	Golgi Vesicle Protein, bAR domain protein that localizes to early and late Golgi vesicles.
YIL049W	DFG10	Defective for Filamentous Growth, probable polyprenol reductase.
YIL050W	PCL7	Pho85 CycLin, pho85p cyclin of the Pho80p subfamily.
YIL053W	GPP1	Glycerol-3-Phosphate Phosphatase, constitutively expressed DL-glycerol-3-phosphate phosphatase.
YIL064W	EFM4	Elongation Factor Methyltransferase, lysine methyltransferase.
YIL071C	PCI8	Proteasome-COP9 signalosome (CSN)-eIF3, possible shared subunit of Cop9 signalosome (CSN) and eIF3.
YIL073C	SPO22	SPOrulation, meiosis-specific protein essential for chromosome synapsis.
YIL077C		Putative protein of unknown function.
YIL092W		Putative protein of unknown function.
YIL114C	POR2	PORin, putative mitochondrial porin (voltage-dependent anion channel).
YIL135C	VHS2	Viable in a Hal3 Sit4 background, regulator of septin dynamics.
YIL152W		Putative protein of unknown function.
YIR016W		Putative protein of unknown function.
YIR029W	DAL2	Degradation of Allantoin, allantoicase.

Systematic	Gene	Description
YJL007C		Dubious open reading frame.
YJL023C	PET130	PETite colonies, protein required for respiratory growth.
YJL055W		Putative protein of unknown function.
YJL062W-A	COA3	Cytochrome Oxidase Assembly, mitochondrial protein required for COX assembly.
YJL095W	BCK1	Bypass of C Kinase, mAPKKK acting in the protein kinase C signaling pathway.
YJL096W	MRPL49	Mitochondrial Ribosomal Protein, large subunit.
YJL102W	MEF2	Mitochondrial Elongation Factor, mitochondrial elongation factor involved in translational elongation.
YJL116C	NCA3	Nuclear Control of ATPase, protein involved in mitochondrion organization.
YJL120W		Dubious open reading frame.
YJL147C		Mitochondrial organization of gene expression, mitochondrial protein of unknown function.
YJL149W	DAS1	Dst1-delta 6-Azauracil Sensitivity, putative SCF ubiquitin ligase F-box protein.
YJL163C		Putative protein of unknown function.
YJL192C	SOP4	Suppressor Of Pma1-7, eR-membrane protein.
YJL193W		Putative protein of unknown function.
YJL209W	CBP1	Cytochrome B mRNA Processing, mitochondrial protein.
YJR030C		Putative protein of unknown function.

Systematic	Gene	Description
YJR031C	GEA1	Guanine nucleotide Exchange on ARF, guanine nucleotide exchange factor for ADP ribosylation factors (ARFs).
YJR101W	RSM26	Ribosomal Small subunit of Mitochondria.
YJR113C	RSM7	Ribosomal Small subunit of Mitochondria.
YJR137C	MET5	METhionine requiring, sulfite reductase beta subunit.
YJR144W	MGM101	Mitochondrial Genome Maintenance, protein with a role in mitochondrial DNA recombinational repair.
YJR146W		Protein of unknown function.
YKL002W	DID4	Doa4-Independent Degradation, class E Vps protein of the ESCRT-III complex.
YKL003C	MRP17	Mitochondrial Ribosomal Protein, small subunit.
YKL073W	LHS1	Lumenal Hsp Seventy, molecular chaperone of the endoplasmic reticulum lumen.
YKL097C		Dubious open reading frame.
YKL137W	CMC1	Cx9C Mitochondrial protein necessary for full assembly of Cytochrome c oxidase, copper-binding protein of the mitochondrial intermembrane space.
YKL169C		Dubious open reading frame.
YKL170W	MRPL38	Mitochondrial Ribosomal Protein, large subunit.
YKL174C	TPO5	Transporter of POlyamines, protein involved in excretion of putrescine and spermidine.
YKL187C	FAT3	FATty acid transporter 3, protein required for fatty acid uptake.
YKL202W		Dubious open reading frame.

Systematic	Gene	Description
YKR035W-A	DID2	Doa4-Independent Degradation, class E protein of the vacuolar protein-sorting (Vps) pathway.
YKR046C	PET10	PETite colonies, protein of unknown function that localizes to lipid particles.
YKR085C	MRPL20	Mitochondrial Ribosomal Protein, large subunit.
YKR088C	TVP38	Tlg2-Vesicle Protein, integral membrane protein.
YKR093W	PTR2	Peptide TRansport, integral membrane peptide transporter.
YKR097W	PCK1	Phosphoenolpyruvate CarboxyKinase.
YLL018C-A	COX19	Cytochrome c OXidase, protein required for COX assembly.
YLL033W	IRC19	Increased Recombination Centers, putative protein of unknown function.
YLL039C	UBI4	Ubiquitin.
YLL047W		Dubious open reading frame.
YLL054C		Putative protein of unknown function with similarity to Pip2p.
YLR025W	SNF7	Sucrose NonFermenting, one of four subunits of the ESCRT-III complex.
YLR038C	COX12	Cytochrome c OXidase, subunit VIb of COX.
YLR055C	SPT8	SuPpressor of Ty, subunit of the SAGA transcriptional regulatory complex.
YLR056W	ERG3	ERGosterol biosynthesis, c-5 sterol desaturase.
YLR067C	PET309	PETite colonies, specific translational activator for the COX1 mRNA.

Systematic	Gene	Description
YLR069C	MEF1	Mitochondrial Elongation Factor, mitochondrial elongation factor involved in translational elongation.
YLR091W	GEP5	GEnetic interactors of Prohibitins, protein of unknown function.
YLR096W	KIN2	KINase, serine/threonine protein kinase involved in regulation of exocytosis
YLR098C	CHA4	Catabolism of Hydroxy Amino acids, dNA binding transcriptional activator.
YLR137W	RKM5	Ribosomal lysine (K) Methyltransferase 5, protein lysine methyltransferase.
YLR139C	SLS1	Synthetic Lethal with SSM4, mitochondrial membrane protein.
YLR148W	PEP3	carboxyPEPtidase Y-deficient, component of CORVET membrane tethering complex.
YLR150W	STM1	Suppressor of ToM1, protein required for optimal translation under nutrient stress.
YLR151C	PCD1	Peroxisomal Coenzyme A Diphosphatase, 8-oxo-dGTP diphosphatase.
YLR183C	TOS4	Target Of Sbf, putative transcription factor.
YLR201C	COQ9	COenzyme Q, protein required for ubiquinone biosynthesis and respiratory growth.
YLR202C		Dubious open reading frame.
YLR203C	MSS51	Mitochondrial Splicing Suppressor, specific translational activator for the mitochondrial COX1 mRNA.
YLR209C	PNP1	Purine nucleoside phosphorylase.
YLR282C		Dubious open reading frame.

Systematic	Gene	Description
YLR285W	NNT1	Nicotinamide N-methylTransferase, s-adenosylmethionine-dependent methyltransferase.
YLR337C	VRP1	VeRProlin, proline-rich actin-associated protein.
YLR363C	NMD4	Nonsense-Mediated mRNA Decay, protein that may be involved in nonsense-mediated mRNA decay.
YLR382C	NAM2	Nuclear Accommodation of Mitochondria, mitochondrial leucyl-tRNA synthetase.
YLR396C	VPS33	Vacuolar Protein Sorting, aTP-binding protein that is a subunit of the HOPS and CORVET complexes.
YLR429W	CRN1	CoRoNin, cortical actin cytoskeletal component
YLR431C	ATG23	AuTophaGy related, peripheral membrane protein required for autophagy and CVT.
YLR439W	MRPL4	Mitochondrial Ribosomal Protein, large subunit.
YLR446W		Putative hexokinase.
YML008C	ERG6	ERGosterol biosynthesis, delta(24)-sterol C-methyltransferase.
YML108W		Protein of unknown function.
YML121W	GTR1	GTp binding protein Resemblance, cytoplasmic GTPase.
YMR024W	MRPL3	Mitochondrial Ribosomal Protein, large subunit.
YMR025W	CSI1	Cop9 Signalosome Interactor, subunit of the Cop9 signalosome.
YMR030W	RSF1	ReSpiration Factor, protein required for respiratory growth.
YMR032W	HOF1	Homolog Of cdc Fifteen, protein that regulates actin cytoskeleton organization.

Systematic	Gene	Description
YMR035W	IMP2	Inner Membrane Protease, catalytic subunit of mitochondrial inner membrane peptidase complex.
YMR040W	YET2	Yeast Endoplasmic reticulum Transmembrane protein, protein of unknown function that may interact with ribosomes.
YMR044W	IOC4	Iswi One Complex, member of a complex (Isw1b) with Isw1p and Ioc2p.
YMR064W	AEP1	ATPase ExPression, protein required for expression of the mitochondrial OLI1 gene.
YMR072W	ABF2	ARS-Binding Factor, mitochondrial DNA-binding protein.
YMR086W	SEG1	Stability of Eisosomes Guaranteed, component of eisosome required for proper eisosome assembly.
YMR089C	YTA12	Yeast Tat-binding Analog, mitochondrial inner membrane m-AAA protease component.
YMR111C		Protein of unknown function.
YMR119W	ASI1	Amino acid Sensor-Independent, subunit of the nuclear inner membrane Asi ubiquitin ligase complex.
YMR122C		Dubious open reading frame.
YMR150C	IMP1	Inner Membrane Protease, catalytic subunit of mitochondrial inner membrane peptidase complex.
YMR158W	MRPS8	Mitochondrial Ribosomal Protein, small subunit.
YMR159C	ATG16	AuTophaGy related, protein involved in autophagy.
YMR174C	PAI3	Proteinase A Inhibitor, cytoplasmic proteinase A (Pep4p) inhibitor.
YMR228W	MTF1	Mitochondrial Transcription Factor, mitochondrial RNA polymerase specificity factor.

Systematic	Gene	Description
YNL068C	FKH2	ForK head Homolog, transcription factor.
YNL074C	MLF3	Multicopy suppressor of LeFlunomide sensitivity, serine-rich protein of unknown function.
YNL081C	SWS2	Sick Without Securin, putative mitochondrial ribosomal protein of the small subunit.
YNL093W	YPT53	Yeast Protein Two, stress-induced Rab family GTPase.
YNL095C		Putative protein of unknown function.
YNL097C	PHO23	PHOsphate metabolism, component of the Rpd3L histone deacetylase complex.
YNL122C		Putative protein of unknown function.
YNL143C		Protein of unknown function.
YNL177C	MRPL22	Mitochondrial Ribosomal Protein, large subunit.
YNL183C	NPR1	Nitrogen Permease Reactivator, protein kinase.
YNL184C		Protein of unknown function.
YNL190W		Hydrophilin essential in desiccation-rehydration process.
YNL194C		Integral membrane protein.
YNL196C	SLZ1	Sporulation-specific protein with a leucine zipper motif.
YNL201C	PSY2	Platinum SensitivitY, subunit of protein phosphatase PP4 complex.
YNL208W		Protein of unknown function.
YNL213C	RRG9	Required for Respiratory Growth, protein of unknown function.
YNL219C	ALG9	Asparagine-Linked Glycosylation, mannosyltransferase.

Systematic	Gene	Description
YNL225C	CNM67	Chaotic Nuclear Migration, component of the spindle pole body outer plaque.
YNL252C	MRPL17	Mitochondrial Ribosomal Protein, large subunit.
YNR036C	MRPS12	Mitochondrial Ribosomal Protein, small subunit.
YNR041C	COQ2	COenzyme Q, para hydroxybenzoate polyprenyl transferase.
YNR074C	AIF1	Apoptosis-Inducing Factor, mitochondrial cell death effector.
YNR075W	COS10	COnserved Sequence, protein of unknown function.
YOL004W	SIN3	Switch INdependent, component of both the Rpd3S and Rpd3L histone deacetylase complexes.
YOL007C	CSI2	Chitin Synthesis Involved, protein of unknown function.
YOL009C	MDM12	Mitochondrial Distribution and Morphology, mitochondrial outer membrane protein.
YOL013W-A		Putative protein of unknown function.
YOL033W	MSE1	Mitochondrial aminoacyl-tRNA Synthetase, Glutamate (E).
YOL104C	NDJ1	NonDisJunction, meiosis-specific telomere protein.
YOL105C	WSC3	Cell Wall integrity and Stress response Component, sensor-transducer of stress-activated PKC1-MPK1.
YOL129W	VPS68	Vacuolar Protein Sorting, vacuolar membrane protein of unknown function.
YOR125C	CAT5	CATabolite repression, protein required for ubiquinone (Coenzyme Q) biosynthesis.
YOR175C	ALE1	Acyltransferase for Lyso-phosphatidylEthanolamine, broad-specificity lysophospholipid acyltransferase.

Systematic	Gene	Description
YOR187W	TUF1	Mitochondrial translation elongation factor Tu.
YOR253W	NAT5	N-terminal AcetylTransferase, subunit of protein N-terminal acetyltransferase NatA.
YOR269W	PAC1	Perish in the Absence of Cin8p, involved in nuclear migration.
YOR277C		Dubious open reading frame.
YOR285W	RDL1	RhoDanese-Like protein, thiosulfate sulfurtransferase.
YOR298C-A	MBF1	Multiprotein Bridging Factor, transcriptional coactivator.
YOR300W		Dubious open reading frame.
YOR330C	MIP1	MItochondrial DNA Polymerase, mitochondrial DNA polymerase gamma subunit.
YOR344C	TYE7	Ty1-mediated Expression, serine-rich protein that contains a bHLH DNA binding motif.
YOR346W	REV1	REVersionless, deoxycytidyl transferase.
YOR360C	PDE2	PhosphoDiEsterase, high-affinity cyclic AMP phosphodiesterase.
YOR364W		Dubious open reading frame.
YOR378W	AMF1	AMmonium Facilitator, low affinity NH4+ transporter.
YPL005W	AEP3	ATPase ExPression, peripheral mitochondrial inner membrane protein.
YPL013C	MRPS16	Mitochondrial Ribosomal Protein, small subunit.
YPL065W	VPS28	Vacuolar Protein Sorting, component of the ESCRT-I complex.
YPL078C	ATP4	ATP synthase, subunit b of the stator stalk of mitochondrial F1Fo ATP synthase.

Systematic	Gene	Description
YPL104W	MSD1	Mitochondrial aminoacyl-tRNA Synthetase, Aspartate (D).
YPL132W	COX11	Cytochrome c OXidase, protein required for delivery of copper to Cox1p.
YPL189C-A	COA2	Cytochrome Oxidase Assembly, cytochrome oxidase assembly factor.
YPL216W		Putative protein of unknown function.
YPL247C		Putative protein of unknown function..
YPR023C	EAF3	Esa1p-Associated Factor, component of the Rpd3S histone deacetylase complex.
YPR036W	VMA13	Subunit H of the V1 peripheral membrane domain of V-ATPase.
YPR047W	MSF1	Mitochondrial aminoacyl-tRNA Synthetase, Phenylalanine (F).
YPR060C	ARO7	AROmatic amino acid requiring, chorismate mutase
YPR100W	MRPL51	Mitochondrial Ribosomal Protein, large subunit.
YPR155C	NCA2	Nuclear Control of ATPase, protein that regulates expression of Fo-F1 ATP synthase subunits.
YPR172W		Putative pyridoxal 5'-phosphate synthase.

List of genes the knock-out strain of which is considered enhancer of Aβ toxicity since its growth rate after 5 days of induction is 0 compared to 0.39 in the WT strain. A minimum growth in uninduced medium at day 3 is set. In red, genes mentioned in Table 2.

Table S3. Primers used for Aβ construct sequenciation.

FW1	GGGTGAACGTTACAGAAAAGC	RV1	GCGTATTACTGAAAGTTCCAAAGA
FW2	CACAAACCTTCAAATGAACGAA	RV2	GGAAAGAATATTTAGAGAAAAGAAGAA
FW3	GCATTAGCTGCTCCAGTCAA	RV3	TGTCGGAATTCTGCATCCAT
FW4	GCTGAAGTCAAGTTTGAAGGTG	RV4	RCAAGACTGGACCATCACCAA
FW5	TTTCCCATTCGATATTTCTATGTTC	RV5	GACTGGAAAGCGGGCAGT
FW6	TCAAAAGGCCTCTAGGTTCC	RV6	CCATGGAGGGCACAGTTAAG
FW7	GAGAGGCGGTTTGCGTATT	RV7	ATCTTAGATCACACTGCCTTTGC
FW8	GCGAGAGCGCTAATTTTTCA	RV8	CTCTCGGGATGCATTTTTGT
FW9	AGAGGTCGAGTTTAGATGCAAGTT	RV9	TCTAGAAAGTATAGGAACTTCAGAGCG
FW10	CTTATCGCTCCAATTTCCCA	RV10	ATTCTGAACCAGTCCTAAAACGA
FW11	GACACGACTTATCGCCACTG	RV11	TTTTCTGCGCGTAATCTGCT
FW12	TTCGCCAGTTAATAGTTTGC	RV12	CGGATGGCATGACAGTAAGA
FW13	TCAAGGATCTTACCGCTGTTGAGAT		

Part IV

DISCUSSION

1 Aβ NITROTYROSINATION AS A FACTOR IMPACTING ON AD PATHOPHYSIOLOGY

Nitro-oxidative stress plays a central role in the pathophysiology of AD. There are numerous reports evidencing the presence of oxidative and nitrative processes, affecting lipids, proteins and nucleic acids, in the brains of AD and MCI patients [454]. ROS and RNS deleterious effects accumulate with ageing, which is the main risk factor for AD. The augmented nitro-oxidative stress appears to be an early event in the pathology of AD, as it precedes the deposition of AP and the cognitive impairment in both human patients [455] and animal models of the disease [456].

The increase of ROS and RNS can be explained, at least partially, due to the presence of Aβ, which is simultaneously a cause and a consequence of nitro-oxidative stress (figure 16). Aβ directly produces H_2O_2 by means of a Fenton-type chemistry that involves the reduction of transition metals [322], something that has also been proven in neuronal cultures [307]. Conversely, ROS and RNS production lead to an increase of BACE1 expression and activity, as well as modifications in γ-secretase complex assembly, which increases the $A\beta_{42}$/ $A\beta_{40}$ ratio [302, 384, 385].

Another factor contributing to the increased nitro-oxidative stress present in AD is mitochondrial damage. In a similar manner to Aβ, mitochondria contribute to the raise of ROS and at the same time mitochondrial impairments are worsened

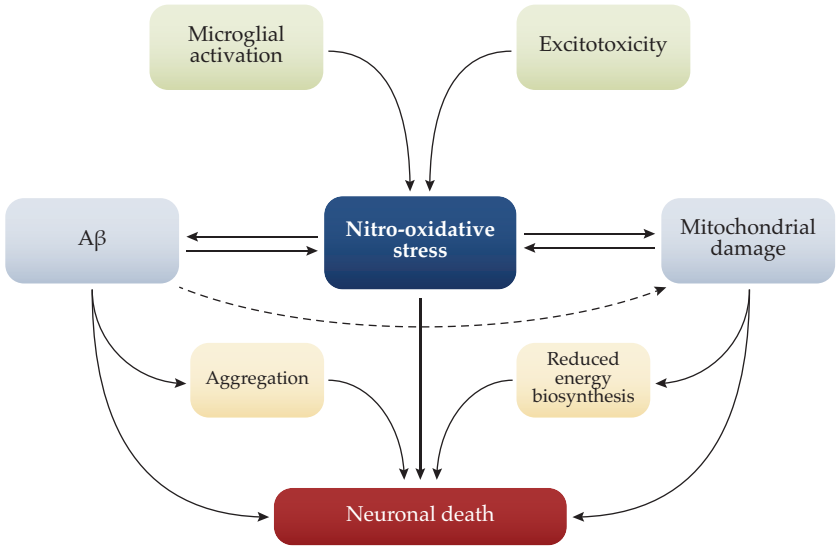

Figure 16: Nitro-oxidative stress in AD

Nitro-oxidative stress is a key factor in the pathology of AD. Excitotoxicity and microglial activation promote the formation of ROS and RNS. Aβ and mitochondrial damage are both a cause and a consequence of the increased nitro-oxidative stress present in AD. The increase of Aβ and especially the raise of the $A\beta_{42}$/ $A\beta_{40}$ ratio leads to the increased aggregation of the peptide, highly toxic to neurons. Mitochondrial damage and the action of Aβ trigger a reduced energy biosynthesis, tightly related with a decreased glucose metabolism. All these events lead directly or indirectly to neuronal death.

by nitro-oxidative stress, constituting a vicious circle. The fact that defective brain glucose metabolism is an acknowledged hallmark of AD [457] together with the link that has been repeatedly established between AD pathology and some glycolytic proteins, such as the enzyme triosephosphate isomerase (TPI) [305], is added proof to this relationship. In addition, Aβ itself can alter the activity of mitochondrial enzymes and trigger the release of ROS [458].

Additionally, inflammatory processes have been extensively related to the pathophysiology of AD. In particular, Aβ can activate microglia *in vitro* [459], which results in an increased

ROS and RNS production as well as in a potential damage to the neighbouring neurons [460]. At last, excitotoxicity also contributes to the formation of ROS and cell death by altering Ca^{2+} homeostasis upon the activation of NMDARs by high concentrations of glutamate [461].

Our results show that Aβ can be nitrated at Y10 *in vitro* with the peroxynitrite donor SIN-1 in a concentration dependent manner, as we confirmed with dot blot and Western blot. The concentration of 100 µM of SIN-1 used in the study allowed us to successfully nitrate the peptide and has proved to be sufficient to significantly alter the biological properties of Aβ. Notably, the use of a synthetically 100% nitrotyrosinated Aβ ($Aβ_{NTyr}$) to further confirm some of the results using a different approach had an exacerbated effect in protein aggregation and neuronal toxicity compared to SIN-1-treated Aβ, reaffirming our findings. However, to our consideration the observations made with SIN-1-treated Aβ add value to the study, as it resembles more to the actual scenario in the brain, where Aβ is not nitrotyrosinated in its totality.

1.1 NITRO-Aβ AGGREGATION

Human and mouse APP have a 97% sequence identity. Three of the amino acid substitutions found in mice are within the Aβ region of APP, namely R5G, Y10F and H13R (figure 3). Considering that murine Aβ shows impaired aggregation and that mice do not naturally develop AD unless human APP is overexpressed [334], it seems plausible that these amino acids might have a relevant role in amyloid aggregation and toxicity. Interestingly, the degu (*O. degus*), an endemic chilean rodent

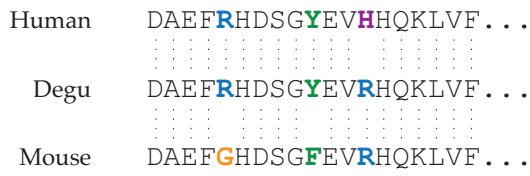

Figure 17: Differences between human, degu and mouse Aβ sequences
Amino acid alignment between human and two rodent sequences of Aβ. Amino acid changes appear coloured.

that only has the H13R substitution compared to the human Aβ sequence, is considered the first wild-type rodent model for neurodegenerative processes associated to AD, because it naturally develops AD-like pathology [462].

The importance of R5 has not yet been described, but H13 has been associated to Cu^{2+} binding via the formation of a intermolecular histidine bridge [463] and Y10 has been related to oxidation-related modifications, such as nitrotyrosination and dityrosine cross-linking [310].

Nitrotyrosination of proteins has been repeatedly proven to alter protein function. Notably, protein nitrotyrosination is characteristic in AD brains [464] and has been reported to affect many proteins, such as presenilins [302], albumin [303], triosephosphate isomerase (TPI) [304], α-enolase [295], VDAC1 [296] and tau [309].

We found nitrotyrosine-positive immunostaining in AD patients AP, in accordance with the results of Kummer an colleagues, who found nitrotyrosinated Aβ in the core of amyloid plaques of AD patients and a triple transgenic mouse model [310].

In contrast with the results from Kummer et al, who claim that Aβ nitrotyrosination leads to an increase in its aggregation

propensity, our findings demonstrate that nitrotyrosination severely impairs the aggregative capacity of Aβ, being virtually nonexistent in the case of synthetically nitrated Aβ ($A\beta_{NTyr}$). We show that this inability to form fibres favours the stabilization of soluble oligomers, which are acknowledged as the most toxic form of Aβ. This would support the controversial notion that the deposition of Aβ as AP is a protective mechanism rather than a detrimental factor. Although Aβ fibres are far from innocuous, from a stoichiometrically point of view, the formation of fibres and AP leaves less molecules available for interaction thus making them less biologically active.

Even though it may seem contradictory, we claim that Aβ aggregation is impaired upon nitrotyrosination in spite of having found nitrated Aβ in AP. A possible explanation to this paradox is that, although nitro-Aβ is not able to form fibrils itself, it may act as a seed for nucleation and elongation of unmodified Aβ fibrils, as it happens with $A\beta_{42}$ and $A\beta_{40}$.

Differences in the results from both studies might be partially due to the use of different nitrating agents. Kummer *et al* use peroxynitrite, which we find unreliable due to its high instability and its short half life ($\approx$1 s). Instead, we used the peroxynitrite donor SIN-1, which was proven effective to nitrotyrosinate Aβ in a concentration-dependent manner. To our favour, the results obtained with $A\beta_{NTyr}$ also point towards an impairment of aggregation. Additionally, their hypothesis is based on the results obtained by ThT measurements and Western blot, which can often be misleading unless supported by direct visualization techniques, such as transmission electron microscopy or atomic force microscopy, as we did.

Kummer and colleagues claim that the biological effects exerted by nitro-Aβ in their *in vitro* and *in vivo* and studies are triggered by high molecular weight oligomers instead of mature fibrils. Nevertheless, given that the most toxic and neuroactive forms of Aβ are low molecular weight oligomeric species [160, 224, 387], our findings support the observed increase of small soluble oligomers, rather than higher-order aggregates, as the more likely explanation for the impact on toxicity and memory observed by Kummer and colleagues.

Among the factors altering the aggregation of Aβ, possibly the most acknowledged is its length: The two last amino acids form Aβ_{42} confer substantially different aggregation properties to the peptide when compared to Aβ_{40}. The supramolecular structure of their fibres differs and it has been published that they aggregate through different mechanisms [213]. Although Aβ_{40} is much more abundant, it is thought that Aβ_{42} acts as a seed for Aβ_{40} aggregation, thus having a substantial contribution to the overall pathology. In the same direction, the relative amount of Aβ_{42} compared to Aβ_{40} dramatically increases in AD brains.

In addition, some post-translational modifications have been described for Aβ, directly affecting its aggregation properties (reviewed in 218). Pyroglutamation of E3 and D11 [465], phosphorilation of S8 [466], racemization of S26 [467] and isomerization of D1, D7 and D23 [468, 469] have been shown to increase aggregation, whereas oxidation of M35 [470] and phosphorilation of S26 [471] have been reported as aggregation inhibitors, stabilizing oligomeric assembly.

Because we pursued a molecular explanation to our observations regarding fibril formation, we developed a bioinformatic

model addressing the impact that Aβ nitrotyrosination has in its structure. We hypothesized that the addition of a nitro group to Y10 prevents its interaction with S26 of the adjacent protofibril (figure 18). Mutations directed to S26 and Y10 had already been shown to impair the formation of amyloid fibrils [220, 472], which is in good agreement with our hypothesis. To further confirm this, we addressed the problem with a different experimental approach: We speculated that by mutating H14 to E14, the positive charge would provoke a rearrangement of the side-chains in the N-terminal fragment, distancing Y10-S26 and preventing their interaction. Our results confirmed that, indeed, not only the E14 mutant mimicked the behaviour of nitro-Aβ in terms of aggregation impairment but also regarding neurotoxicity.

To our knowledge, we are the first group reporting the H14E Aβ ($A\beta_{E14}$) used in this study that, although not a naturally occurring mutation, provides some insights into the understanding of how Aβ fibrils are formed and stabilized. Until now, the region considered to determine Aβ aggregation was the one comprising from L17 to S26, which forms the first antiparallel beta sheet from the structure (β1). All the aggregation-enhancing FAD mutations (English, Tottori, Italian, Arctic, Osaka, Dutch and Iowa) as well as the synthetic V18A mutation, which inhibits Aβ aggregation, are located within this region (see figure 11). Interestingly, the $A\beta_{E14}$ mutation caracterized by us is located in the N-terminal part of the peptide, putting this fragment into the spotlight and providing a whole new possible role for this region.

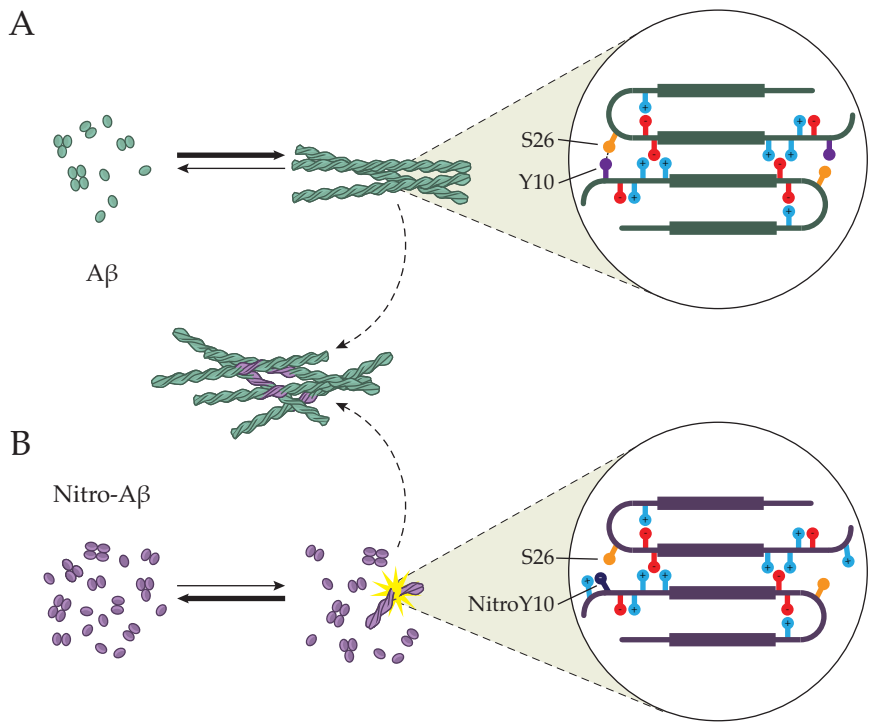

Figure 18: Model explaining the impaired nitro-Aβ aggregation
(A) Aβ aggregation results in the formation of oligomers and fibrils. The interaction between Tyr10 and Ser26 of adjacent protofibrils facilitates the stabilization and elongation of the fibril. (B) Nitro-Aβ is unable to form fibrils due to inability of NitroTyr10 and Ser26 to interact. The breakage of Aβ fibrils and their inefficacy to elongate promotes the formation of oligomers and their stabilization. We hypothesize that these oligomers may act as seeds for unmodified Aβ, enabling secondary nucleation and elongation in AD brains.

1.2 NITRO-Aβ NEUROTOXICITY

Once we concluded that Aβ nitrotyrosination stabilizes oligomers at the expense of fibril formation, we wanted to assess the impact that these oligomers have on neuronal viability. Oligomers have been widely reported to be the most neuroactive and synaptotoxic form of Aβ. Many oligomeric species, such as dimers, trimers, tetramers, dodecamers and ADDLs have been suggested as the molecular determinant of Aβ toxicity. Taking

into account that (a) oligomeric species are relatively dynamic structures, with heterogeneity in their molecular weight and structure, that coexist in the AD brain and (b) finding the exact molecular responsible for Aβ toxicity was beyond the scope of this thesis, we performed all toxicity experiments using high acute doses of a heterogeneous oligomer preparation, which we consider to have a greater physiological relevance. One might argue that the concentrations used in our work are far from physiological, considering that Aβ concentration is estimated to be at sub-nanomolar concentrations in the AD brain [473]. However, the possibility that at precise moments Aβ concentration reaches the micromolar range in certain brain areas should not be overlooked. In addition to that, the damage caused by decades of oligomer exposure is hardly reproducible under laboratory conditions.

Many mechanisms have been proposed to mediate the synaptic dysfunction carried out by Aβ oligomers. Since Aβ is mostly located at the extracellular milieu, it seems reasonable that oligomers exert their toxic effects by interacting with certain proteins at the plasmatic membrane, triggering a series of downstream pathways that will eventually lead to synaptic disruption and neuronal loss. Some of the proposed Aβ-binding partners include, but are not limited to, NMDARs [229, 474], nAChRs [225], metabotropic glutamate receptor 5 (mGluR5) [475], cellular prion protein (PrP^c) [238] and glutamate transporters [387].

Oligomers alter NMDAR function, either directly or indirectly, via two main mechanisms (figure 19):

1. By inducing synaptic depression: LTP and LTD induction requires the activation of NMDARs, which triggers a

signalling cascade that recruits or endocytes AMPARs, respectively. Multiple studies have shown that Aβ oligomers impair LTP *in vivo* and *ex vivo* through a mechanism that is dependent on NMDAR downstream pathways. nitro-Aβ-induced AMPAR endocytosis is dependent on the activation of calcineurin/PP2B and requires the downregulation of CaMKII. In a similar way, Aβ can also mediate the endocytosis of NMDARs, via the GluN2B dephosphorylation by the tyrosine phosphatase STEP.

2. By leading to the pathological activation of extrasynaptic NMDARs: It has been reported that Aβ oligomers impair glutamate uptake and trigger aberrant glutamate release by astrocytes. This glutamate accumulation causes a spillage to the extrasynaptic space, thereby activating extrasynaptic NMDARs and initiating a cascade of events that lead to synaptic damage. These pathways include an increase of intracellular Ca^{2+} and the hyperactivation of nNOS, which is involved with the pathological generation of ROS and RNS.

Additionally, Aβ oligomers have been reported to bind α-7-nAChRs with high affinity [225]. Aβ oligomers have dual effects on these receptors, depending on its concentration and the type of cells used [476]. It is well known that cholinergic dysfunction precedes glutamatergic dysfunction during the early stages of AD. This initial cholinergic damage has been demonstrated in the nucleus basalis of Meynert, a cholinergic structure with widespread connexions to the striatum, the brain cortex and the hippocampus [477].

In our cytosolic calcium measurements, Aβ exposure itself did not cause the activation of NMDARs, like other groups have

reported [478]. These differences are likely due to the different experimental models used: Ferreira and colleagues use cortical rat neurons whereas we use mouse hippocampal neurons. Although they share many features, these two rodent neuronal cultures exhibit very unalike behaviours [479]. Some authors argue that many pivotal processes such as neurogenesis are different between mice and rats and that most of the controversies in the field arise from incorrectly comparing results obtained with these two species [480].

However, subsequent stimulation with the synthetic agonist NMDA led to increased intracellular Ca^{2+} concentrations upon Aβ treatment, and even larger responses in the case of nitro-Aβ. This effect appeared to be specific, although perhaps not exclusive, to NMDAR, since we did not observe differences when we stimulated the cells with a physiological stimulus. Nitro-Aβ-triggered NMDAR overactivation seemed particularly relevant, since excessive glutamate stimulation can result in neuron dysfunction and death, through a process known as excitotoxicity [388]. In addition, the activation of nNOS induced by cytosolic Ca^{2+} increases could imply a positive feedback to this pathological sequence of events, leading to increased ROS / RNS production and further protein nitrotyrosination.

We hypothesized that the exposure of neurons to nitro-Aβ may enhance the vulnerability to excitotoxicity, as we experimentally confirmed. The NMDAR blocker MK-801 partially prevented the decrease in cell viability in hippocampal neurons exposed to nitro-Aβ and Aβ. From these experiments, three main conclusions can be drawn: First, 5 min of exposure to Aβ oligomers were sufficient to trigger neuronal death, which is consistent with the notion that Aβ neurotoxicity is started

at the plasma membrane. Second, there is a proportion of cell viability that cannot be recovered with MK-801, indicating that NMDAR-mediated excitotoxicity is not the only factor involved in Aβ-induced neuronal death. Third, the co-treatment with nitro-Aβ and MK-801 restored cell viability to the levels of Aβ and MK-801 co-treated cells, suggesting that the difference in cell viability between Aβ and nitro-Aβ oligomers is entirely NMDAR-dependent.

Our results indicate that nitro-Aβ oligomers bind more to dendritic spines, and specially to NMDAR receptors. Nevertheless, we cannot rule out complementary explanations to the observed NMDAR-mediated biological effects caused by nitro-Aβ oligomers. An increase of surface-NMDARs, changes in their synaptic / extrasynaptic localization or alterations in their gating mechanism could also be processes explaining the increase in the cytosolic Ca^{2+} concentration as well as the enhanced vulnerability to excitotoxicity.

Regarding why we have obtained that nitro-Aβ oligomers are especially toxic to cultured hippocampal neurons *in vitro*, there are mainly two non-exclusive explanations. First, quantitatively: It could be merely a matter of biologically active concentrations, where oligomers are more stable and hence more abundant in nitro-Aβ preparations compared to those of Aβ. Second, qualitatively: It could be that upon nitrotyrosination some particularly toxic oligomer conformations are favoured, thus triggering certain toxicity pathways that are exceptionally harmful to neurons. Because we lack experimental support addressing this issue, it is fair to assume that our observations are due to a combination of both.

The neuronal toxicity present in AD is most certainly caused by the synergy of many pathological pathways. Glutamate-derived toxicity has been proposed to play a central role in the synaptic dysfunction and neuronal death caused by Aβ oligomers. NMDAR is one of the many membrane proteins proposed to interact with Aβ oligomers, mediating their toxicity. Unfortunately, we could not address the impact that nitro-Aβ oligomers have on other post-synaptic glutamate receptors, such as AMPARs or mGluRs.

Memantine, a low-to-moderate affinity uncompetitive NMDAR antagonist, preferentially blocks excessively activated channels, allowing physiological activity but not continued activation of the receptors. As the only NMDAR antagonist approved by the European Agency for the Evaluation of Medicinal Products (EMEA) and by the US Food and Drug Administration (FDA) for the treatment of moderate to severe AD, it currently represents the rationale for the glutamate hypothesis of the disease. The controversial effectiveness of memantine to treat AD may be partially due to the fact that activation of NMDARs by Aβ accumulation occurs at early stages of the disease. Nevertheless, it has been shown effective to prevent necrosis, disruption of axonal transport, DNA fragmentation, neurite retraction and tau hyperphosphorylation *in vitro* [481]. In the triple transgenic mouse model of AD, memantine has been related to an improvement of cognitive function and the reduction of Aβ accumulation [482]. At last, memantine has been reported to ameliorate cognition, language and memory in clinical trials [483].

Taken together, our results support the role of peroxynitrite as a factor with impact on AD pathophysiology at several levels.

We propose a mechanism by which nitro-Aβ oligomers could worsen synaptic dysfunction in the initial stages of AD and become a seed for Aβ aggregation. We also suggest an assembly model for Aβ fibrils, in which aggregation is severely impaired by the disruption of the interaction between Y10 and S26 of adjacent protofibrils, as it happens upon nitrotyrosination. Additionally, nitro-Aβ oligomers could contribute to the overall redox imbalance found in AD and aggravate Ca^{2+} signalling alterations that play a central role in Aβ toxicity. At last, we performed a screen using a library of *S. cerevisiae* knock-out strains that overexpressed Aβ, in order to deepen on the signalling pathways that mediate Aβ toxicity.

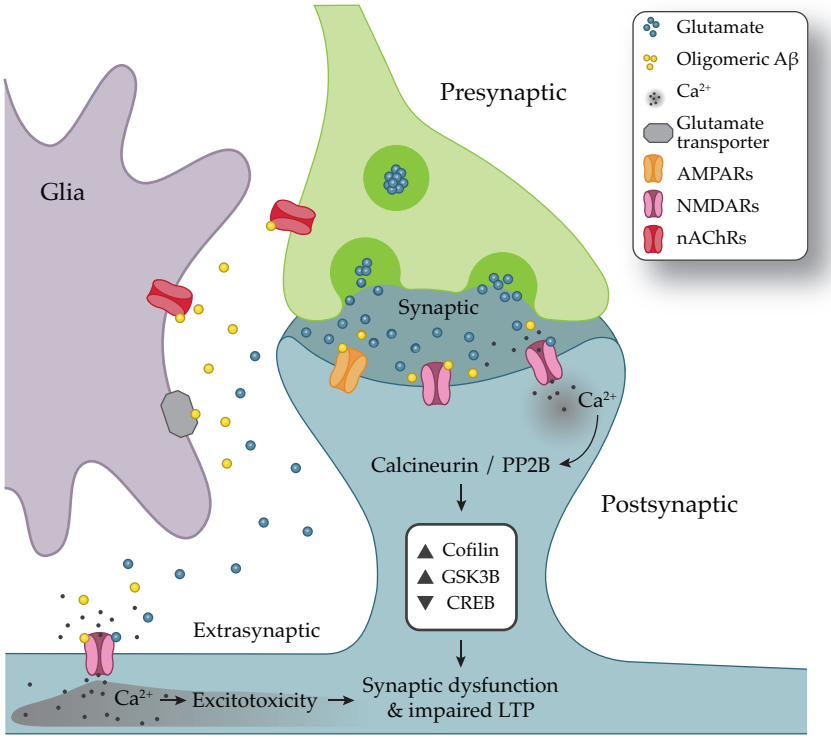

Figure 19: Aβ oligomers and glutamate-mediated transmission

Aβ oligomers interact with multiple astrocytic, microglial and neuronal synaptic proteins, including nAChRs, NMDARs, AMPARs and glutamate transporters, triggering a series of pathological events. Aberrant activation of NMDAR promotes the pathological modulation of proteins downstream of PP2B / calcineurin, such as an increase of cofilin and GSK3B activities as well as a decrease of CREB activity. On the other hand, the increase of glutamate concentration in the synaptic cleft causes a spillover that activates extrasynaptic NMDARs, leading to excitotoxicity.

2 PUTATIVE MODULATORS OF Aβ TOXICITY

The experiments with Arctic and Dutch Aβ variants allowed us to determine that Aβ toxicity is dependent on the aggregation state in yeast, as it happens in neurons. Then, we proceeded to identify putative mediators of wild-type (WT) Aβ toxicity by screening a library of ~5154 *S. cerevisiae* deletion mutants.

The knock-outs that showed differential growth compared to the WT strain were considered as Aβ toxicity modulators. Those genes the absence of which reverted Aβ toxicity were considered as essential genes for Aβ to mediate its toxic effects, *i.e.* genes that are involved in toxicity pathways. Conversely, those genes the absence of which enhanced Aβ toxicity were considered to take part in protective mechanisms against Aβ toxicity. In other words, the absence of these protective genes makes cells more susceptible to Aβ toxicity. Alternatively, they could also belong to pathways that are not protective themselves, but the impairment of which adds up to Aβ toxicity.

Recently, the publication of GWAS has allowed the identification of several genetic risk factors associated with LOAD. Nevertheless, as GWAS are mainly descriptive and point out association but not causation, a mechanistic approach is needed to address how certain molecular pathways are linked with Aβ toxicity. Some of the genes identified in our study, such as *EPHA1* and *ADSSL1*, are orthologues of genes that had been previously identified as genetic risk factors for AD. Our results

not only reaffirm their association with the disease, but also specifically involve them in Aβ toxicity.

Among our hits, there is a notable representation of genes related to clathrin-dependent endocytosis, Ca^{2+} homeostasis, mitochondrial related processes and anti-oxidant systems. All these cellular processes have already been linked, either directly or indirectly, to the pathophysiology of AD. However, some of the genes that we propose as modulators of Aβ toxicity have never been reported as such, as it happens with cytochrome b, which had never been linked to Aβ toxicity. On the other hand, the fact that some proteins that have been extensively related to Aβ and AD in the literature, such as COX [431, 484, 485], appear as hits in our screen provides further strength to our experimental model.

Our results point out at mitochondrial processes as one of the pathways especially involved in Aβ toxicity. Among the knock-out strains considered as enhancers, 43% belonged to mitochondrial genes and 22% to mitochondrial-related processes. Aβ peptide has been shown to impair mitochondrial functions. Damaged mitochondria are associated with Ca^{2+} leakage to the cytosol, reduced ATP levels and increased production of ROS, the accumulation of which induces oxidative stress. Hence, it was not completely unexpected to find this relationship between mitochondrial proteins and Aβ toxicity.

On the other hand, revertants of Aβ toxicity were found to be particularly enriched in the nuclear compartment, taking part in processes related to gene transcription and protein translation. These results suggest that Aβ toxicity partially depends on protein synthesis, probably involving pathways the ulterior effectors of which are transcription factors or proteins

similarly involved in gene expression. The fact that not many other cellular compartments or biological processes appeared in the gene ontology analysis depicts the heterogeneity of the pathways involved in Aβ toxicity, evidencing that it is probably carried out on multiple fronts.

It is worth mentioning that the candidates we considered most promising were further confirmed with a revalidation study, in which we assessed the knock-out strains using a different approach: Instead of mating an Aβ overexpressing strain with the knock-outs, the selected gene deletant strains were directly transformed with the Aβ construct. As expected, the results obtained reproduced quite accurately what we had observed in the screen. Additionally, some genes with an unclear role based on the results of the screen were added to the revalidation assay. This allowed us to identify some molecular candidates, such as the yeast ortholog of *VDAC*, that otherwise would have been gone unnoticed.

As it was one of the candidates showing a greater toxicity reversion, we wanted to assess whether the absence of the *ERV29* human ortholog, *SURF4*, had a similar effect in human neuroblastoma cells. Although we successfully knocked down its expression, the results regarding Aβ toxicity were unclear. There are at least three possible explanations to this observation. First, considering that *SURF4* is a housekeeping gene, its absence may be prejudicial to the cells, masking its effect on Aβ toxicity. Second, the presence of functional NMDAR receptors in undifferentiated neuroblastoma cells is controversial. If Surf4 happened to mediate Aβ toxicity through a mechanism dependent on NMDAR, it could be possible that we are not able to observe its effects in our model. This hypothesis could

be ruled out by performing the experiment on mature mouse hippocampal primary cultures or by differentiating the neuroblasts. Third, it could be due to the pronounced variability that exists when evaluating Aβ toxicity in cultured mammalian cells. Thus, more experiments should be performed in order to clarify this issue.

It is certainly needed to explore the role of the hits of our study with more depth, providing a mechanistic explanation to their involvement in Aβ toxicity. Studying them not only can provide a deeper understanding of the pathophysiology of AD, but they may also represent potential targets for therapeutic approaches.

Part V

CONCLUSIONS

1. Aβ nitrotyrosination with peroxynitrite impairs fibril formation and stabilizes low molecular weight oligomers.
2. The inability of nitro-Aβ to form fibres is due to the disruption of the interprotofibrillar interaction between Y10 and S26.
3. The mutant H14E mimics the behaviour of nitro-Aβ and is unable to form fibrils.
4. Nitro-Aβ species are present within amyloid plaques of AD patients.
5. Nitro-Aβ oligomers affect calcium homeostasis specifically increasing the cytosolic calcium concentration upon NMDAR activation.
6. Nitro-Aβ oligomers exert NMDA-dependent toxicity that can be reverted with the NMDAR pharmacological blocker MK-801.
7. Nitro-Aβ oligomers bind more to dendritic spines and NMDARs.
8. The toxicity of Aβ in *S. cerevisiae* depends on its cellular localization, aggregative propensity and concentration.
9. Several orthologs of human AD risk factors are involved in the modulation of Aβ toxicity in yeast.
10. Many putative modulators of Aβ toxicity are associated with crucial biological processes such as Ca^{2+} homeostasis and the mitochondrial respiratory chain.
11. *Erv29*Δ is a great revertant of Aβ toxicity in yeast but the effect of the human ortholog Surf4 in mammalian cells is not conclusive.

Part VI

BIBLIOGRAPHY

BIBLIOGRAPHY

[1] Ramón y Cajal S. Estructura de los centros nerviosos de las aves; 1888.

[2] Gray EG. Electron Microscopy of Synaptic Contacts on Dendrite Spines of the Cerebral Cortex. Nature. 1959 jun;183(4675):1592–1593.

[3] Ramón y Cajal S. El azul de metileno en los centros nerviosos; 1896.

[4] Shepherd GM. The dendritic spine: a multifunctional integrative unit. J Neurophysiol. 1996 jun;75(6):2197–210.

[5] Ramon y Cajal S. Textura del Sistema Nervioso del Hombre y de los Vertebrados. Madrid, Moya; 1899.

[6] Swindale NV. Dendritic spines only connect. Trends Neurosci. 1981;4:240–241.

[7] Ziv NE, Smith SJ. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron. 1996 jul;17(1):91–102.

[8] Stepanyants A, Hof PR, Chklovskii DB. Geometry and Structural Plasticity of Synaptic Connectivity. Neuron. 2002 apr;34(2):275–288.

[9] Yuste R. Dendritic spines and distributed circuits. Neuron. 2011 sep;71(5):772–81.

[10] Cash S, Yuste R. Input summation by cultured pyramidal neurons is linear and position-independent. J Neurosci. 1998 jan;18(1):10–5.

[11] Cash S, Yuste R. Linear summation of excitatory inputs by CA1 pyramidal neurons. Neuron. 1999 feb;22(2):383–94.

[12] Llinás RR, Hillman DE. Physiological and morphological organization of the cerebellar circuits in various vertebrates; 1969.

[13] Bloodgood BL, Sabatini BL. Neuronal activity regulates diffusion across the neck of dendritic spines. Science. 2005 nov;310(5749):866–9.

[14] Grunditz A, Holbro N, Tian L, Zuo Y, Oertner TG. Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. J Neurosci. 2008 dec;28(50):13457–66.

[15] Llinás RR. I of the vortex: From neurons to self. MIT press; 2002.

[16] Bourne JN, Harris KM. Balancing structure and function at hippocampal dendritic spines. Annu Rev Neurosci. 2008 jan;31:47–67.

[17] Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GCR, Kasai H. The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron. 2008 mar;57(5):719–29.

[18] Hotulainen P, Hoogenraad CC. Actin in dendritic spines: connecting dynamics to function. J Cell Biol. 2010 may;189(4):619–29.

[19] van Spronsen M, Hoogenraad CC. Synapse pathology in psychiatric and neurologic disease. Curr Neurol Neurosci Rep. 2010 may;10(3):207–14.

[20] Furukawa H, Singh SK, Mancusso R, Gouaux E. Subunit arrangement and function in NMDA receptors. Nature. 2005;438(7065):185–192.

[21] Mayer ML, Westbrook GL, Guthrie PB. Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature. jan;309(5965):261–3.

[22] Forsythe ID, Westbrook GL. Slow excitatory postsynaptic currents mediated by N-methyl-D-aspartate receptors on cultured mouse central neurones. J Physiol. 1988 feb;396:515–33.

[23] Vicini S, Wang JF, Li JH, Zhu WJ, Wang YH, Luo JH, et al. Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. J Neurophysiol. 1998 feb;79(2):555–66.

[24] Bassani S, Folci A, Zapata J, Passafaro M. AMPAR trafficking in synapse maturation and plasticity. Cell Mol Life Sci. 2013 mar;.

[25] Higley MJ, Sabatini BL. Calcium signaling in dendritic spines. Cold Spring Harb Perspect Biol. 2012 apr;4(4):a005686.

[26] Tamminga CA, Stan AD, Wagner AD. The Hippocampal Formation in Schizophrenia. Am J Psychiatry. 2010 oct;167(10):1178–1193.

[27] Lackner JR. Observations on the speech processing capabilities of an amnesic patient: several aspects of H.M.'s language function. Neuropsychologia. 1974 mar;12(2):199–207.

[28] Mavrogiorgou P, Gertz HJ, Ferszt R, Wolf R, Bär KJ, Juckel G. Are routine methods good enough to stain senile plaques and neurofibrillary tangles in different brain regions of demented patients? Psychiatr Danub. 2011 dec;23(4):334–9.

[29] Fifková E, Van Harreveld A. Long-lasting morphological changes in dendritic spines of dentate granular cells following stimulation of the entorhinal area. J Neurocytol. 1977;6:211–230.

[30] Bosch M, Hayashi Y. Structural plasticity of dendritic spines. Curr Opin Neurobiol. 2012 jun;22(3):383–8.

[31] Ultanir SK, Kim JE, Hall BJ, Deerinck T, Ellisman M, Ghosh A. Regulation of spine morphology and spine density by NMDA receptor signaling in vivo. Proc Natl Acad Sci U S A. 2007 dec;104(49):19553–8.

[32] Bliss T, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol. 1973;p. 331–356.

[33] Kaibara T, Leung LS. Basal versus apical dendritic long-term potentiation of commissural afferents to hippocampal CA1: a current-source density study. J Neurosci. 1993 jun;13(6):2391–404.

[34] Cooke SF. Plasticity in the human central nervous system. Brain. 2006 jul;129(7):1659–1673.

[35] Collingridge GL, Kehl SJ, McLennan H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol. 1983 jan;334(1):33–46.

[36] Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A. Magnesium gates glutamate-activated channels in mouse central neurones. Nature. 1984 feb;307(5950):462–465.

[37] Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature. 1983 oct;305(5936):719–721.

[38] Malenka RC, Kauer JA, Zucker RS, Nicoll RA. Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science. 1988 oct;242(4875):81–4.

[39] Borgdorff AJ, Choquet D. Regulation of AMPA receptor lateral movements. Nature. 2002 jun;417(6889):649–53.

[40] Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci. 2007 feb;8(2):101–13.

[41] Makino H, Malinow R. AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. Neuron. 2009 nov;64(3):381–90.

[42] Patterson MA, Szatmari EM, Yasuda R. AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. Proc Natl Acad Sci U S A. 2010;107(36):15951–6.

[43] Frank DA, Greenberg ME. CREB: a mediator of long-term memory from mollusks to mammals. Cell. 1994 oct;79(1):5–8.

[44] Stevens CF. CREB and memory consolidation. Neuron. 1994 oct;13(4):769–70.

[45] Nestler EJ. Cellular basis of memory for addiction. Dialogues Clin Neurosci. 2013 dec;15(4):431–43.

[46] Nishiyama M, Hong K, Mikoshiba K, Poo MM, Kato K. Calcium stores regulate the polarity and input specificity of synaptic modification. Nature. 2000 nov;408(6812):584–8.

[47] Morishita W, Marie H, Malenka RC. Distinct triggering and expression mechanisms underlie LTD of AMPA and NMDA synaptic responses. Nat Neurosci. 2005 aug;8(8):1043–50.

[48] Blitzer RD, Connor JH, Brown GP, Wong T, Shenolikar S, Iyengar R, et al. Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. Science. 1998 jun;280(5371):1940–2.

[49] Lu J, Helton TD, Blanpied Ta, Rácz B, Newpher TM, Weinberg RJ, et al. Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. Neuron. 2007 sep;55(6):874–89.

[50] Collingridge GL, Peineau S, Howland JG, Wang YT. Long-term depression in the CNS. Nat Rev Neurosci. 2010 jul;11(7):459–73.

[51] Blanpied TA, Ehlers MD. Microanatomy of dendritic spines: emerging principles of synaptic pathology in psychiatric and neurological disease. Biol Psychiatry. 2004 jun;55(12):1121–7.

[52] Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol. 1991 oct;30(4):572–80.

[53] Small DH, Mok SS, Bornstein JC. Alzheimer's disease and Aβ toxicity: from top to bottom [Editorial Material]. Nat Rev Neurosci. 2001 aug;2(8):595–598.

[54] Glantz LA, Lewis DA. Dendritic spine density in schizophrenia and depression. Arch Gen Psychiatry. 2001 feb;58(2):203.

[55] Penzes P, Cahill M, Jones K. Dendritic spine pathology in neuropsychiatric disorders. Nat .... 2011 mar;14(3):285–93.

[56] Swann JW, Al-Noori S, Jiang M, Lee CL. Spine loss and other dendritic abnormalities in epilepsy. Hippocampus. 2000 jan;10(5):617–25.

[57] Zhang W, Yamawaki R, Wen X, Uhl J, Diaz J, Prince Da, et al. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. J Neurosci. 2009 nov;29(45):14247–56.

[58] Ferhat L. Potential role of drebrin a, an f-actin binding protein, in reactive synaptic plasticity after pilocarpine-induced seizures: functional implications in epilepsy. Int J Cell Biol. 2012 jan;2012:474351.

[59] Waung MW, Pfeiffer BE, Nosyreva ED, Ronesi Ja, Huber KM. Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron. 2008 jul;59(1):84–97.

[60] Thomas AM, Bui N, Perkins JR, Yuva-Paylor La, Paylor R. Group I metabotropic glutamate receptor antagonists alter select behaviors in a mouse model for fragile X syndrome. Psychopharmacology (Berl). 2012 jan;219(1):47–58.

[61] Bangash MA, Park JM, Melnikova T, Wang D, Jeon SK, Lee D, et al. Enhanced polyubiquitination of Shank3 and NMDA receptor in a mouse model of autism. Cell. 2011 may;145(5):758–72.

[62] Robinson TE, Kolb B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci. 1999 may;11(5):1598–604.

[63] Norrholm SD, Bibb JA, Nestler EJ, Ouimet CC, Taylor JR, Greengard P. Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. Neuroscience. 2003 jan;116(1):19–22.

[64] Haas MA, Bell D, Slender A, Lana-Elola E, Watson-Scales S, Fisher EMC, et al. Alterations to dendritic spine morphology, but not dendrite patterning, of cortical projection neurons in Tc1 and Ts1Rhr mouse models of Down syndrome. PLoS One. 2013 jan;8(10):e78561.

[65] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002 oct;298(5594):789–91.

[66] Spires-Jones T, Knafo S. Spines, plasticity, and cognition in Alzheimer's model mice. Neural Plast. 2012;2012:319836.

[67] Alzheimer A. Uber eine eigenartige Erkrankung der Hirnrinde. Allg Zeitschrife Psychiatr. 1907;64:146–148.

[68] 2015 Alzheimer's disease facts and figures. Alzheimer's Dement J Alzheimer's Assoc. 2015 nov;11(3):332–384.

[69] Prince MJ, Wimo A, Guerchet MMM, Ali GC, Wu YT, Prina M, et al. World Alzheimer Report 2015 - The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends; 2015.

[70] Bekris LM, Yu CE, Bird TD, Tsuang DW. Review Article: Genetics of Alzheimer Disease. J Geriatr Psychiatry Neurol. 2010;23(4):213–227.

[71] Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, et al. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet. 1999 sep;65(3):664–70.

[72] Bird TD. Genetic aspects of Alzheimer disease. Genet Med. 2008 apr;10(4):231–239.

[73] Brickell KL, Steinbart EJ, Rumbaugh M, Payami H, Schellenberg GD, Van Deerlin V, et al. Early-onset Alzheimer disease in families with late-onset Alzheimer disease: a potential important subtype of familial Alzheimer disease. Arch Neurol. 2006 sep;63(9):1307–11.

[74] Bertram L, Tanzi RE. Alzheimer's disease: one disorder, too many genes? Hum Mol Genet. 2004 apr;13 Spec No:R135–41.

[75] Wilson JS, Mruthinti S, Buccafusco JJ, Schade RF, Mitchell MB, Harrell DU, et al. Anti-RAGE and Abeta immunoglobulin levels are related to dementia level and cognitive performance. J Gerontol A Biol Sci Med Sci. 2009 mar;64(2):264–71.

[76] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, et al. Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry. 2006 feb;63(2):168–74.

[77] Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. Hum Mutat. 2012 sep;33(9):1340–4.

[78] Goldman JS, Hahn SE, Catania JW, Larusse-Eckert S, Butson MB, Rumbaugh M, et al. Genetic counseling and testing for Alzheimer disease: Joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. Genet Med. 2011 jun;13(6):597–605.

[79] Bermejo-Pareja F, Benito-León J, Vega S, Medrano MJ, Román GC. Incidence and subtypes of dementia in three elderly populations of central Spain. J Neurol Sci. 2008 jan;264(1-2):63–72.

[80] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A. 1993;90(5):1977–81.

[81] Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol. 2013 feb;9(2):106–18.

[82] Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet. 1994 jun;7(2):180–4.

[83] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet. 2007 jan;39(1):17–23.

[84] Rönnemaa E, Zethelius B, Lannfelt L, Kilander L. Vascular Risk Factors and Dementia: 40-Year Follow-Up of a Population-Based Cohort. Dement Geriatr Cogn Disord. 2011;31(6):460–466.

[85] Loef M, Walach H. Midlife obesity and dementia: Meta-analysis and adjusted forecast of dementia prevalence in the united states and china. Obesity. 2013 jan;21(1):E51–E55.

[86] Anstey KJ, Cherbuin N, Budge M, Young J. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obes Rev. 2011 may;12(5):e426–e437.

[87] Gudala K, Bansal D, Schifano F, Bhansali A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. J Diabetes Investig. 2013 nov;4(6):640–650.

[88] Vagelatos NT, Eslick GD. Type 2 Diabetes as a Risk Factor for Alzheimer's Disease: The Confounders, Interactions, and Neuropathology Associated With This Relationship. Epidemiol Rev. 2013 jan;35(1):152–160.

[89] Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, et al. Midlife blood pressure and dementia: the Honolulu-Asia aging study. Neurobiol Aging. 2000 jan;21(1):49–55.

[90] Ninomiya T, Ohara T, Hirakawa Y, Yoshida D, Doi Y, Hata J, et al. Midlife and Late-Life Blood Pressure and Dementia in Japanese Elderly: The Hisayama Study. Hypertension. 2011 jul;58(1):22–28.

[91] Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology. 2011 aug;77(5):461–468.

[92] Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. Dement Geriatr Cogn Disord. 2009;28(1):75–80.

[93] Meng XF, Yu JT, Wang HF, Tan MS, Wang C, Tan CC, et al. Midlife vascular risk factors and the risk of Alzheimer's disease: a systematic review and meta-analysis. J Alzheimers Dis. 2014;42(4):1295–310.

[94] Flament S, Delacourte A, Mann DM. Phosphorylation of Tau proteins: a major event during the process of neurofibrillary degeneration. A comparative study between Alzheimer's disease and Down's syndrome. Brain Res. 1990 may;516(1):15–9.

[95] Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol. 1989 oct;24(3):173–82.

[96] Masliah E, Terry RD, Mallory M, Alford M, Hansen LA. Diffuse plaques do not accentuate synapse loss in Alzheimer's disease. Am J Pathol. 1990 dec;137(6):1293–7.

[97] Pike CJ, Cummings BJ, Cotman CW. Early association of reactive astrocytes with senile plaques in Alzheimer's disease. Exp Neurol. 1995 apr;132(2):172–9.

[98] Knowles RB, Wyart C, Buldyrev SV, Cruz L, Urbanc B, Hasselmo ME, et al. Plaque-induced neurite abnormalities: implications for disruption of neural networks in Alzheimer's disease. Proc Natl Acad Sci U S A. 1999 apr;96(9):5274–9.

[99] Urbanc B, Cruz L, Le R, Sanders J, Ashe KH, Duff K, et al. Neurotoxic effects of thioflavin S-positive amyloid deposits in transgenic mice and Alzheimer's disease. Proc Natl Acad Sci U S A. 2002 oct;99(22):13990–5.

[100] Vehmas AK, Kawas CH, Stewart WF, Troncoso JC. Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurobiol Aging. jan;24(2):321–31.

[101] National Institute on Aging. Alzheimer's Disease Fact Sheet. 2015;.

[102] Janssen JC. Alzheimer's disease due to an intronic presenilin-1 (PSEN1 intron 4) mutation: A clinicopathological study. Brain. 2000 may;123(5):894–907.

[103] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011 sep;1(1):a006189.

[104] Gonzalez-billault C, Borquez DA, Palacios-garcia I, Ferrer I, Luna-muñoz J, Charles R, et al. Understanding Alzheimer's Disease. InTech; 2013. p. 89–109.

[105] Ikezu T, Gendelman HE, Kong Q. Neuroimmune pharmacology. Gendelman HE, Ikezu T, editors. Boston, MA: Springer US; 2008.

[106] Prada CM, Garcia-Alloza M, Betensky RA, Zhang-Nunes SX, Greenberg SM, Bacskai BJ, et al. Antibody-mediated clearance of amyloid-beta peptide from cerebral amyloid angiopathy revealed by quantitative in vivo imaging. J Neurosci. 2007 feb;27(8):1973–80.

[107] Wiessner C, Wiederhold KH, Tissot AC, Frey P, Danner S, Jacobson LH, et al. The Second-Generation Active A Immunotherapy CAD106 Reduces Amyloid Accumulation in APP Transgenic Mice While Minimizing Potential Side Effects. J Neurosci. 2011 jun;31(25):9323–9331.

[108] Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med. 2000 aug;6(8):916–9.

[109] Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature. 1999 jul;400(6740):173–7.

[110] Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimer's Res & Ther. 2014 jul;6(4):37.

[111] Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov. 2010 nov;9(3):203–14.

[112] Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotechnol. 2014 jan;32(1):40–51.

[113] Tajes M, Ramos-Fernández E, Weng-Jiang X, Bosch-Morató M, Guivernau B, Eraso-Pichot A, et al. The blood-brain barrier: Structure, function and therapeutic approaches to cross it. Mol Membr Biol. 2014 aug;31(5):152–167.

[114] Quiroz YT, Schultz AP, Chen K, Protas HD, Brickhouse M, Fleisher AS, et al. Brain Imaging and Blood Biomarker Abnormalities in Children With Autosomal Dominant Alzheimer Disease. JAMA Neurol. 2015 aug;72(8):912.

[115] Hooper M, Grill JD, Rodriguez-Agudelo Y, Medina LD, Fox M, Alvarez-Retuerto AI, et al. The impact of the availability of prevention studies on the desire to undergo predictive testing in persons at risk for autosomal dominant Alzheimer's disease. Contemp Clin Trials. 2013 sep;36(1):256–262.

[116] Morris JC, Roe CM, Grant EA, Head D, Storandt M, Goate AM, et al. Pittsburgh Compound B Imaging and Prediction of Progression From Cognitive Normality to Symptomatic Alzheimer Disease. Arch Neurol. 2009 dec;66(12):1469–75.

[117] Sutphen CL, Jasielec MS, Shah AR, Macy EM, Xiong C, Vlassenko AG, et al. Longitudinal Cerebrospinal Fluid Biomarker Changes in Preclinical Alzheimer Disease During Middle Age. JAMA Neurol. 2015 sep;72(9):1029.

[118] Alcolea D, Martinez-Lage P, Sanchez-Juan P, Olazaran J, Antunez C, Izagirre A, et al. Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. Neurology. 2015 aug;85(7):626–633.

[119] Schindler SE, Fagan AM. Autosomal Dominant Alzheimer Disease: A Unique Resource to Study CSF Biomarker Changes in Preclinical AD. Front Neurol. 2015 jun;6:142.

[120] Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, et al. APP Processing and Synaptic Function. Neuron. 2003 mar;37(6):925–937.

[121] Plant LD, Boyle JP, Smith IF, Peers C, Pearson HA. The production of amyloid beta peptide is a critical requirement for the viability of central neurons. J Neurosci. 2003 jul;23(13):5531–5.

[122] Atwood CS, Obrenovich ME, Liu T, Chan H, Perry G, Smith MA, et al. Amyloid-beta: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-beta. Brain Res Brain Res Rev. 2003 sep;43(1):1–16.

[123] Pearson H, Peers C. Physiological roles for amyloid β peptides. J Physiol. 2006;575(Pt 1):5–10.

[124] Hardy Ja, Higgins Ga. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992 apr;256(5054):184–5.

[125] Selkoe DJ. The molecular pathology of Alzheimer's disease; 1991.

[126] Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease; 1991.

[127] Glenner GG. The proteins and genes of Alzheimer's disease. Biomed Pharmacother. 1988;42(9):579–584.

[128] Masters CL, Beyreuther KT. The pathology of the amyloid A4 precursor of Alzheimer's disease. Ann Med. 1989 jan;21(2):89–90.

[129] Bettens K, Sleegers K, Van Broeckhoven C. Genetic insights in Alzheimer's disease. Lancet Neurol. 2013 jan;12(1):92–104.

[130] Murrell J, Farlow M, Ghetti B, Benson MD. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science. 1991 oct;254(5028):97–9.

[131] Naruse S, Igarashi S, Kobayashi H, Aoki K, Inuzuka T, Kaneko K, et al. Mis-sense mutation Val—-Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. Lancet (London, England). 1991 apr;337(8747):978–9.

[132] van Duijn CM, Hendriks L, Cruts M, Hardy JA, Hofman A, Van Broeckhoven C. Amyloid precursor protein gene mutation in early-onset Alzheimer's disease. Lancet (London, England). 1991 apr;337(8747):978.

[133] Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature. 1991 feb;349(6311):704–6.

[134] Mann DM, Brown A, Prinja D, Davies CA, Landon M, Masters CL, et al. An analysis of the morphology of senile plaques in Down's syndrome patients of different ages using immunocytochemical and lectin histochemical techniques. Neuropathol Appl Neurobiol. jan;15(4):317–29.

[135] Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature. 2012 aug;488(7409):96–9.

[136] Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW. In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res. 1991 nov;563(1-2):311–4.

[137] Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, et al. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci U S A. 1993 oct;90(20):9649–53.

[138] Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. Lancet Neurol. 2011 mar;10(3):241–52.

[139] Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, et al. Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. Sci Transl Med. 2011 jun;3(89):89ra57.

[140] Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, Sartorius LJ, et al. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A. 2000 mar;97(6):2892–7.

[141] Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature. jan;408(6815):982–5.

[142] Morris GP, Clark Ia, Vissel B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol Commun. 2014;2:135.

[143] Drachman DA. The amyloid hypothesis, time to move on: Amyloid is the downstream result, not cause, of Alzheimer's disease. Alzheimers Dement. 2014 may;10(3):372–80.

[144] Lee HG, Casadesus G, Zhu X, Takeda A, Perry G, Smith MA. Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. Ann N Y Acad Sci. 2004 jun;1019(1):1–4.

[145] Herrup K. The case for rejecting the amyloid cascade hypothesis. Nat Neurosci. 2015 may;18(6):794–799.

[146] Villemagne VL, Pike KE, Chételat G, Ellis KA, Mulligan RS, Bourgeat P, et al. Longitudinal assessment of Aβ and cognition in aging and Alzheimer disease. Ann Neurol. 2011 jan;69(1):181–92.

[147] Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch Neurol. 2008 nov;65(11):1509–17.

[148] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002;297(July):353–356.

[149] Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. JAMA. jan;283(12):1571–7.

[150] Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, et al. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am J Pathol. 1999 sep;155(3):853–62.

[151] McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol. 1999 dec;46(6):860–6.

[152] Wang J, Dickson DW, Trojanowski JQ, Lee VM. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. Exp Neurol. 1999 aug;158(2):328–37.

[153] DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol. 1990 may;27(5):457–64.

[154] Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with

cognitive decline in Alzheimer disease. J Neuropathol Exp Neurol. 1997 aug;56(8):933–44.

[155] Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. J Neurosci. 2007 jan;27(4):796–807.

[156] Calabrese B, Shaked GM, Tabarean IV, Braga J, Koo EH, Halpain S. Rapid, concurrent alterations in pre- and postsynaptic structure induced by naturally-secreted amyloid-beta protein. Mol Cell Neurosci. 2007 jun;35(2):183–93.

[157] Evans NA, Facci L, Owen DE, Soden PE, Burbidge SA, Prinjha RK, et al. Abeta(1-42) reduces synapse number and inhibits neurite outgrowth in primary cortical and hippocampal neurons: a quantitative analysis. J Neurosci Methods. 2008 oct;175(1):96–103.

[158] Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J Neurosci. 2007 mar;27(11):2866–75.

[159] Shrestha BR, Vitolo OV, Joshi P, Lordkipanidze T, Shelanski M, Dunaevsky A. Amyloid beta peptide adversely affects spine number and motility in hippocampal neurons. Mol Cell Neurosci. 2006 nov;33(3):274–82.

[160] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008 aug;14(8):837–42.

[161] Jaunmuktane Z, Mead S, Ellis M, Wadsworth JDF, Nicoll AJ, Kenny J, et al. Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. Nature. 2015 sep;525(7568):247–50.

[162] Yoshikai S, Sasaki H, Doh-ura K, Furuya H, Sakaki Y. Genomic organization of the human amyloid beta-protein precursor gene. Gene. 1990 mar;87(2):257–63.

[163] Hayashi Y, Kashiwagi K, Ohta J, Nakajima M, Kawashima T, Yoshikawa K. Alzheimer amyloid protein precursor enhances proliferation of neural stem cells from fetal rat brain. Biochem Biophys Res Commun. 1994 nov;205(1):936–43.

[164] Freude KK, Penjwini M, Davis JL, LaFerla FM, Blurton-Jones M. Soluble amyloid precursor protein induces rapid neural differentiation of human embryonic stem cells. J Biol Chem. 2011 jul;286(27):24264–74.

[165] Soba P, Eggert S, Wagner K, Zentgraf H, Siehl K, Kreger S, et al. Homo- and heterodimerization of APP family members promotes intercellular adhesion. EMBO J. 2005 oct;24(20):3624–34.

[166] Priller C, Bauer T, Mitteregger G, Krebs B, Kretzschmar HA, Herms J. Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci. 2006 jul;26(27):7212–21.

[167] Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol. 2003 may;70(1):1–32.

[168] Duce JA, Tsatsanis A, Cater MA, James SA, Robb E, Wikhe K, et al. Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. Cell. 2010 sep;142(6):857–67.

[169] Allinson TMJ, Parkin ET, Turner AJ, Hooper NM. ADAMs family members as amyloid precursor protein alpha-secretases. J Neurosci Res. 2003 nov;74(3):342–52.

[170] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science. 1999 oct;286(5440):735–41.

[171] Edbauer D, Winkler E, Regula JT, Pesold B, Steiner H, Haass C. Reconstitution of gamma-secretase activity. Nat Cell Biol. 2003 may;5(5):486–8.

[172] Lammich S, Kojro E, Postina R, Gilbert S, Pfeiffer R, Jasionowski M, et al. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc Natl Acad Sci U S A. 1999 mar;96(7):3922–7.

[173] Haass C, Hung AY, Schlossmacher MG, Teplow DB, Selkoe DJ. beta-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. J Biol Chem. 1993 feb;268(5):3021–4.

[174] Chen AC, Kim S, Shepardson N, Patel S, Hong S, Selkoe DJ. Physical and functional interaction between the α- and γ-secretases : A new model of regulated intramembrane proteolysis. J Cell Biol. 2015;211(6):1157–1176.

[175] Citron M, Teplow DB, Selkoe DJ. Generation of amyloid beta protein from its precursor is sequence specific. Neuron. 1995 mar;14(3):661–70.

[176] Huse JT, Pijak DS, Leslie GJ, Lee VM, Doms RW. Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. J Biol Chem. 2000 oct;275(43):33729–37.

[177] Prabhu Y, Burgos PV, Schindler C, Farías GG, Magadán JG, Bonifacino JS. Adaptor protein 2-mediated endocytosis of the β-secretase BACE1 is dispensable for amyloid precursor protein processing. Mol Biol Cell. 2012 jul;23(12):2339–51.

[178] Chyung JH, Selkoe DJ. Inhibition of receptor-mediated endocytosis demonstrates generation of amyloid beta-protein at the cell surface. J Biol Chem. 2003 dec;278(51):51035–43.

[179] Potter R, Patterson BW, Elbert DL, Ovod V, Kasten T, Sigurdson W, et al. Increased in vivo amyloid-β42 production, exchange, and loss in presenilin mutation carriers. Sci Transl Med. 2013 jun;5(189):189ra77.

[180] Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science. 2010 dec;330(6012):1774.

[181] Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, et al. Clearance systems in the brain-implications for Alzheimer disease. Nat Rev Neurol. 2015;11(8):457–70.

[182] Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med. 2010 jan;362(4):329–44.

[183] Perez FP, Bose D, Maloney B, Nho K, Shah K, Lahiri DK. Late-onset Alzheimer's disease, heating up and foxed by several proteins: pathomolecular effects of the aging process. J Alzheimers Dis. 2014 jan;40(1):1–17.

[184] Nixon RA, Cataldo AM. Lysosomal system pathways: genes to neurodegeneration in Alzheimer's disease. J Alzheimers Dis. 2006 jan;9(3 Suppl):277–89.

[185] Cho MH, Cho K, Kang HJ, Jeon EY, Kim HS, Kwon HJ, et al. Autophagy in microglia degrades extracellular β-amyloid fibrils and regulates the NLRP3 inflammasome. Autophagy. 2014 oct;10(10):1761–75.

[186] Iwata N, Tsubuki S, Takaki Y, Shirotani K, Lu B, Gerard NP, et al. Metabolic regulation of brain Abeta by neprilysin. Science. 2001 may;292(5521):1550–2.

[187] Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. J Biol Chem. 1998 dec;273(49):32730–8.

[188] Miners JS, Baig S, Palmer J, Palmer LE, Kehoe PG, Love S. Abeta-degrading enzymes in Alzheimer's disease. Brain Pathol. 2008 apr;18(2):240–52.

[189] Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci. 2011 dec;12(December):723–739.

[190] Yoon SS, Jo SA. Mechanisms of Amyloid-β Peptide Clearance: Potential Therapeutic Targets for Alzheimer's Disease. Biomol Ther (Seoul). 2012 may;20(3):245–55.

[191] Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, et al. Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. J Clin Invest. 2000 dec;106(12):1489–99.

[192] Zlokovic BV, Martel CL, Matsubara E, McComb JG, Zheng G, McCluskey RT, et al. Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. Proc Natl Acad Sci U S A. 1996 apr;93(9):4229–34.

[193] Storck SE, Meister S, Nahrath J, Meißner JN, Schubert N, Di Spiezio A, et al. Endothelial LRP1 transports amyloid-β1-42 across the blood-brain barrier. J Clin Invest. 2016 jan;126(1):123–36.

[194] Fitz NF, Cronican AA, Saleem M, Fauq AH, Chapman R, Lefterov I, et al. Abca1 deficiency affects Alzheimer's disease-like phenotype in human ApoE4 but not in ApoE3-targeted replacement mice. J Neurosci. 2012 sep;32(38):13125–36.

[195] Ito S, Ohtsuki S, Murata S, Katsukura Y, Suzuki H, Funaki M, et al. Involvement of insulin-degrading enzyme in insulin- and atrial natriuretic peptide-sensitive internalization of amyloid-β peptide in mouse brain capillary endothelial cells. J Alzheimers Dis. 2014 jan;38(1):185–200.

[196] Hone E, Martins IJ, Fonte J, Martins RN. Apolipoprotein E influences amyloid-beta clearance from the murine periphery. J Alzheimers Dis. 2003 feb;5(1):1–8.

[197] Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med. 2012;4(147):147ra111.

[198] Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, et al. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med. 2003 jul;9(7):907–13.

[199] Webster S, Rogers J. Relative efficacies of amyloid beta peptide (A beta) binding proteins in A beta aggregation. J Neurosci Res. 1996 oct;46(1):58–66.

[200] Barrow CJ, Yasuda A, Kenny PT, Zagorski MG. Solution conformations and aggregational properties of synthetic amyloid beta-peptides of Alzheimer's disease. Analysis of circular dichroism spectra. J Mol Biol. 1992 jun;225(4):1075–93.

[201] Cohen SIA, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, et al. Proliferation of amyloid- 42 aggregates occurs through a secondary nucleation mechanism. Proc Natl Acad Sci. 2013 jun;110(24):9758–9763.

[202] Fezoui Y, Hartley DM, Harper JD, Khurana R, Walsh DM, Condron MM, et al. An improved method of preparing the amyloid beta-protein for fibrillogenesis and neurotoxicity experiments. Amyloid. 2000;7:166–178.

[203] Gessel MM, Bernstein S, Kemper M, Teplow DB, Bowers MT. Familial Alzheimer's disease mutations differentially alter amyloid β-protein oligomerization. ACS Chem Neurosci. 2012 nov;3(11):909–18.

[204] Janssen JC, Beck JA, Campbell TA, Dickinson A, Fox NC, Harvey RJ, et al. Early onset familial Alzheimer's disease: Mutation frequency in 31 families. Neurology. 2003 jan;60(2):235–9.

[205] Wakutani Y, Watanabe K, Adachi Y, Wada-Isoe K, Urakami K, Ninomiya H, et al. Novel amyloid precursor protein gene missense mutation (D678N) in probable familial Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2004 jul;75(7):1039–42.

[206] Bugiani O, Giaccone G, Rossi G, Mangieri M, Capobianco R, Morbin M, et al. Hereditary cerebral hemorrhage with amyloidosis associated with the E693K mutation of APP. Arch Neurol. 2010 aug;67(8):987–95.

[207] Kamino K, Orr HT, Payami H, Wijsman EM, Alonso ME, Pulst SM, et al. Linkage and mutational analysis of familial Alzheimer disease kindreds for the APP gene region. Am J Hum Genet. 1992 nov;51(5):998–1014.

[208] Tomiyama T, Nagata T, Shimada H, Teraoka R, Fukushima A, Kanemitsu H, et al. A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia. Ann Neurol. 2008 mar;63(3):377–87.

[209] Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, et al. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science. 1990 jun;248(4959):1124–6.

[210] Grabowski TJ, Cho HS, Vonsattel JP, Rebeck GW, Greenberg SM. Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. Ann Neurol. 2001 jun;49(6):697–705.

[211] Soto C, Castaño EM, Frangione B, Inestrosa NC. The alpha-helical to beta-strand transition in the amino-terminal fragment of the amyloid beta-peptide modulates amyloid formation. J Biol Chem. 1995 feb;270(7):3063–7.

[212] Haass C, Selkoe DJ. Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. Cell. 1993 dec;75(6):1039–42.

[213] Meisl G, Yang X, Hellstrand E, Frohm B, Kirkegaard JB, Cohen SIA, et al. Differences in nucleation behavior underlie the contrasting aggregation kinetics of the AÂ 40 and AÂ 42 peptides. Proc Natl Acad Sci. 2014 jun;111(26):9384–9389.

[214] Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, et al. Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. Arch Biochem Biophys. 1993 feb;301(1):41–52.

[215] Kumar-Singh S, Theuns J, Van Broeck B, Pirici D, Vennekens K, Corsmit E, et al. Mean age-of-onset of familial alzheimer disease caused by presenilin mutations correlates with both increased Abeta42 and decreased Abeta40. Hum Mutat. 2006 jul;27(7):686–95.

[216] Kuperstein I, Broersen K, Benilova I, Rozenski J, Jonckheere W, Debulpaep M, et al. Neurotoxicity of Alzheimer's disease Aβ peptides is induced by small changes in the Aβ42 to Aβ40 ratio. EMBO J. 2010;29(19):3408–3420.

[217] Kim J, Onstead L, Randle S, Price R, Smithson L, Zwizinski C, et al. Abeta40 inhibits amyloid deposition in vivo. J Neurosci. 2007 jan;27(3):627–33.

[218] Kummer MP, Heneka MT. Truncated and modified amyloid-beta species. Alzheimers Res Ther. 2014;6(3):28.

[219] Lührs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Döbeli H, et al. 3D structure of Alzheimer's amyloid-beta(1-42) fibrils. Proc Natl Acad Sci U S A. 2005;102:17342–17347.

[220] Rezaei-Ghaleh N, Amininasab M, Giller K, Kumar S, Stündl A, Schneider A, et al. Turn Plasticity Distinguishes Different Modes of Amyloid-β Aggregation. J Am Chem Soc. 2014 apr;136(13):4913–4919.

[221] Egnaczyk GF, Greis KD, Stimson ER, Maggio JE. Photoaffinity cross-linking of Alzheimer's disease amyloid fibrils reveals interstrand contact regions between assembled β-amyloid peptide subunits. Biochemistry. 2001;40:11706–11714.

[222] Zhang R, Hu X, Khant H, Ludtke SJ, Chiu W, Schmid MF, et al. Interprotofilament interactions between Alzheimer's Abeta1-42 peptides in amyloid fibrils revealed by cryoEM. Proc Natl Acad Sci U S A. 2009;106(12):4653–4658.

[223] Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, et al. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. Nat Neurosci. 2005 jan;8(1):79–84.

[224] Selkoe DJ. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res. 2008 sep;192(1):106–13.

[225] Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP, Reitz AB. beta-Amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J Biol Chem. 2000 feb;275(8):5626–32.

[226] Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al. Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci. 2005 aug;8(8):1051–8.

[227] Zhang Y, Kurup P, Xu J, Anderson GM, Greengard P, Nairn AC, et al. Reduced levels of the tyrosine phosphatase STEP block β amyloid-mediated GluA1/GluA2 receptor internalization. J Neurochem. 2011 nov;119(3):664–72.

[228] Ye C, Walsh DM, Selkoe DJ, Hartley DM. Amyloid beta-protein induced electrophysiological changes are dependent on aggregation state: N-methyl-D-aspartate (NMDA) versus non-NMDA receptor/channel activation. Neurosci Lett. 2004 aug;366(3):320–5.

[229] De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, et al. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem. 2007 apr;282(15):11590–601.

[230] Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE. beta-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci. 1992;12(2):376–389.

[231] Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, et al. Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. Nat Med. 1999 jan;5(1):101–6.

[232] Yatin SM, Aksenova M, Aksenov M, Markesbery WR, Aulick T, Butterfield DA. Temporal relations among amyloid beta-peptide-induced free-radical oxidative stress, neuronal toxicity, and neuronal defensive responses. J Mol Neurosci. 1998 dec;11(3):183–97.

[233] Takashima A, Noguchi K, Sato K, Hoshino T, Imahori K. Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. Proc Natl Acad Sci U S A. 1993 aug;90(16):7789–93.

[234] Yamamoto N, Matsubara E, Maeda S, Minagawa H, Takashima A, Maruyama W, et al. A ganglioside-induced toxic soluble Abeta assembly. Its enhanced formation from Abeta bearing the Arctic mutation. J Biol Chem. 2007 jan;282(4):2646–55.

[235] Hansma HG, Laney DE, Bezanilla M, Sinsheimer RL, Hansma PK. Applications for atomic force microscopy of DNA. Biophys J. 1995 may;68(5):1672–7.

[236] Zhao WQ, De Felice FG, Fernandez S, Chen H, Lambert MP, Quon MJ, et al. Amyloid beta oligomers induce impairment of neuronal insulin receptors. FASEB J. 2008 jan;22(1):246–60.

[237] Magdesian MH, Carvalho MMVF, Mendes FA, Saraiva LM, Juliano MA, Juliano L, et al. Amyloid-beta binds to the extracellular cysteine-rich domain of Frizzled and inhibits Wnt/beta-catenin signaling. J Biol Chem. 2008 apr;283(14):9359–68.

[238] Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. Nature. 2009 feb;457(7233):1128–1132.

[239] Kayed R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, et al. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. J Biol Chem. 2004 nov;279(45):46363–6.

[240] Clifford PM, Zarrabi S, Siu G, Kinsler KJ, Kosciuk MC, Venkataraman V, et al. Abeta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons. Brain Res. 2007 apr;1142:223–36.

[241] Yu C, Nwabuisi-Heath E, Laxton K, Ladu MJ. Endocytic pathways mediating oligomeric Abeta42 neurotoxicity. Mol Neurodegener. 2010 jan;5:19.

[242] Nagele RG, D'Andrea MR, Anderson WJ, Wang HY. Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease. Neuroscience. 2002 jan;110(2):199–211.

[243] Du H, Guo L, Yan SSS, Sosunov AA, McKhann GM, Yan SSS. Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. Proc Natl Acad Sci U S A. 2010 oct;107(43):18670–5.

[244] Sasaki N, Toki S, Chowei H, Saito T, Nakano N, Hayashi Y, et al. Immunohistochemical distribution of the receptor for advanced glycation end products in neurons and astrocytes in Alzheimer's disease. Brain Res. 2001 jan;888(2):256–262.

[245] Bu G, Cam J, Zerbinatti C. LRP in amyloid-beta production and metabolism. Ann N Y Acad Sci. 2006 nov;1086:35–53.

[246] Almeida CG, Takahashi RH, Gouras GK. Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. J Neurosci. 2006 apr;26(16):4277–88.

[247] Tseng BP, Green KN, Chan JL, Blurton-Jones M, LaFerla FM. Abeta inhibits the proteasome and enhances amyloid and tau accumulation. Neurobiol Aging. 2008 nov;29(11):1607–18.

[248] Stutzmann GE, Smith I, Caccamo A, Oddo S, Laferla FM, Parker I. Enhanced ryanodine receptor recruitment contributes to Ca2+ disruptions in young, adult, and aged Alzheimer's disease mice. J Neurosci. 2006 may;26(19):5180–9.

[249] Caspersen C, Wang N, Yao J, Sosunov A, Chen X, Lustbader JW, et al. Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. FASEB J. 2005 dec;19(14):2040–1.

[250] Wang X, Su B, Siedlak SL, Moreira PI, Fujioka H, Wang Y, et al. Amyloid-beta overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. Proc Natl Acad Sci U S A. 2008 dec;105(49):19318–23.

[251] Cha MY, Han SH, Son SM, Hong HS, Choi YJ, Byun J, et al. Mitochondria-specific accumulation of amyloid β induces mitochondrial dysfunction leading to apoptotic cell death. PLoS One. 2012 jan;7(4):e34929.

[252] Butterfield DA. Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. Free Radic Res. 2002 dec;36(12):1307–13.

[253] Uttara B, Singh A, Zamboni P, Mahajan R. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Curr Neuropharmacol. 2009 mar;7(1):65–74.

[254] Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress1,2 1Guest Editors: Mark A. Smith and George Perry 2This article is part of a ser. Free Radic Biol Med. 2002 jun;32(11):1050–1060.

[255] Sultana R, Butterfield DA. Role of oxidative stress in the progression of Alzheimer's disease. J Alzheimers Dis. 2010 jan;19(1):341–53.

[256] Dasuri K, Zhang L, Keller JN. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. Free Radic Biol Med. 2013 sep;62:170–85.

[257] Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997 mar;82(2):291–5.

[258] Schulz E, Wenzel P, Münzel T, Daiber A. Mitochondrial redox signaling: Interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. Antioxid Redox Signal. 2014 jan;20(2):308–24.

[259] Butterfield DA, Castegna A, Lauderback CM, Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. Neurobiol Aging. 2002 sep;23(5):655–64.

[260] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979 jul;59(3):527–605.

[261] Raichle ME, Gusnard Da. Appraising the brain's energy budget. Proc Natl Acad Sci U S A. 2002;99(16):10237–10239.

[262] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol. 2003 oct;552(2):335–344.

[263] Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell. 2005 feb;120(4):483–95.

[264] Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci. 2000 oct;25(10):502–8.

[265] Muller F. The nature and mechanism of superoxide production by the electron transport chain: Its relevance to aging. J Am Aging Assoc. 2000 oct;23(4):227–53.

[266] Fridovich I. Biological effects of the superoxide radical. Arch Biochem Biophys. 1986 may;247(1):1–11.

[267] Deby C, Goutier R. New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. Biochem Pharmacol. 1990 feb;39(3):399–405.

[268] Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J. 1984 apr;219(1):1–14.

[269] Swomley AM, Förster S, Keeney JT, Triplett J, Zhang Z, Sultana R, et al. Abeta, oxidative stress in Alzheimer disease: Evidence based on proteomics studies. Biochim Biophys Acta - Mol Basis Dis. 2014 aug;1842(8):1248–1257.

[270] Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev. 2012 may;70(5):257–65.

[271] Guix FX, Uribesalgo I, Coma M, Muñoz FJ. The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol. 2005 jun;76(2):126–52.

[272] Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A. 1990 feb;87(4):1620–4.

[273] Ignarro LJ. Endothelium-derived nitric oxide: actions and properties. FASEB J. 1989 jan;3(1):31–6.

[274] Huie RE, Padmaja S. The reaction of no with superoxide. Free Radic Res Commun. 1993 jan;18(4):195–9.

[275] Cudd A, Fridovich I. Electrostatic interactions in the reaction mechanism of bovine erythrocyte superoxide dismutase. J Biol Chem. 1982 oct;257(19):11443–7.

[276] Saran M, Michel C, Bors W. Reaction of NO with O2-. implications for the action of endothelium-derived relaxing factor (EDRF). Free Radic Res Commun. 1990 jan;10(4-5):221–6.

[277] Souza JM, Daikhin E, Yudkoff M, Raman CS, Ischiropoulos H. Factors determining the selectivity of protein tyrosine nitration. Arch Biochem Biophys. 1999 nov;371(2):169–78.

[278] Ischiropoulos H. Biological selectivity and functional aspects of protein tyrosine nitration. Biochem Biophys Res Commun. 2003 jun;305(3):776–83.

[279] Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD, et al. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. Arch Biochem Biophys. 1992 nov;298(2):431–7.

[280] MacMillan-Crow LA, Crow JP, Kerby JD, Beckman JS, Thompson JA. Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. Proc Natl Acad Sci U S A. 1996 oct;93(21):11853–8.

[281] Aslan M, Ryan TM, Townes TM, Coward L, Kirk MC, Barnes S, et al. Nitric oxide-dependent generation of reactive species in sickle cell disease. Actin tyrosine induces defective cytoskeletal polymerization. J Biol Chem. 2003 feb;278(6):4194–204.

[282] Berlett BS, Friguet B, Yim MB, Chock PB, Stadtman ER. Peroxynitrite-mediated nitration of tyrosine residues in Escherichia coli glutamine synthetase mimics adenylylation: relevance to signal transduction. Proc Natl Acad Sci U S A. 1996 mar;93(5):1776–80.

[283] Kinobe R, Ji Y, Nakatsu K. Peroxynitrite-mediated inactivation of heme oxygenases. BMC Pharmacol. 2004 oct;4:26.

[284] Gonzalez D, Drapier JC, Bouton C. Endogenous nitration of iron regulatory protein-1 (IRP-1) in nitric oxide-producing murine macrophages: further insight into the mechanism of nitration in vivo and its impact on IRP-1 functions. J Biol Chem. 2004 oct;279(41):43345–51.

[285] Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. Biochem Biophys Res Commun. 2004 feb;315(1):240–5.

[286] Koeck T, Levison B, Hazen SL, Crabb JW, Stuehr DJ, Aulak KS. Tyrosine nitration impairs mammalian aldolase A activity. Mol Cell Proteomics. 2004 jun;3(6):548–57.

[287] Cobbs CS, Whisenhunt TR, Wesemann DR, Harkins LE, Van Meir EG, Samanta M. Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. Cancer Res. 2003 dec;63(24):8670–3.

[288] Hink U, Oelze M, Kolb P, Bachschmid M, Zou MH, Daiber A, et al. Role for peroxynitrite in the inhibition of prostacyclin synthase in nitrate tolerance. J Am Coll Cardiol. 2003 nov;42(10):1826–34.

[289] Knapp LT, Kanterewicz BI, Hayes EL, Klann E. Peroxynitrite-induced tyrosine nitration and inhibition of protein kinase C. Biochem Biophys Res Commun. 2001 aug;286(4):764–70.

[290] Cassina AM, Hodara R, Souza JM, Thomson L, Castro L, Ischiropoulos H, et al. Cytochrome c nitration by peroxynitrite. J Biol Chem. 2000 jul;275(28):21409–15.

[291] Vadseth C, Souza JM, Thomson L, Seagraves A, Nagaswami C, Scheiner T, et al. Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species. J Biol Chem. 2004 mar;279(10):8820–6.

[292] Ji Y, Bennett BM. Activation of microsomal glutathione s-transferase by peroxynitrite. Mol Pharmacol. 2003 jan;63(1):136–46.

[293] Go YM, Patel RP, Maland MC, Park H, Beckman JS, Darley-Usmar VM, et al. Evidence for peroxynitrite as a signaling molecule in flow-dependent activation of c-Jun NH(2)-terminal kinase. Am J Physiol. 1999 oct;277(4 Pt 2):H1647–53.

[294] Zhang J, Dawson VL, Dawson TM, Snyder SH. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. Science. 1994 feb;263(5147):687–9.

[295] Castegna A, Thongboonkerd V, Klein JB, Lynn B, Markesbery WR, Butterfield DA. Proteomic identification of nitrated proteins in Alzheimer's disease brain. J Neurochem. 2003 may;85(6):1394–1401.

[296] Sultana R, Poon HF, Cai J, Pierce WM, Merchant M, Klein JB, et al. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. Neurobiol Dis. 2006;22(1):76–87.

[297] Good PF, Werner P, Hsu A, Olanow CW, Perl DP. Evidence of neuronal oxidative damage in Alzheimer's disease. Am J Pathol. 1996 jul;149(1):21–8.

[298] Reynolds MR, Reyes JF, Fu Y, Bigio EH, Guillozet-Bongaarts AL, Berry RW, et al. Tau nitration occurs at tyrosine 29 in the fibrillar lesions of Alzheimer's disease and other tauopathies. J Neurosci. 2006 oct;26(42):10636–45.

[299] Butterfield DA, Reed TT, Perluigi M, De Marco C, Coccia R, Keller JN, et al. Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. Brain Res. 2007 may;1148:243–8.

[300] Good PF, Hsu A, Werner P, Perl DP, Olanow CW. Protein Nitration in ParkinsonÊŒs Disease. J Neuropathol Exp Neurol. 1998 apr;57(4):338–342.

[301] Reynolds MR, Berry RW, Binder LI. Nitration in neurodegeneration: deciphering the "Hows" "nYs". Biochemistry. 2007 jun;46(25):7325–36.

[302] Guix FX, Wahle T, Vennekens K, Snellinx A, Chávez-Gutiérrez L, Ill-Raga G, et al. Modification of γ-secretase by nitrosative stress links neuronal ageing to sporadic Alzheimer's disease. EMBO Mol Med. 2012 jul;4(7):660–73.

[303] Ramos-Fernández E, Tajes M, Palomer E, Ill-Raga G, Bosch-Morató M, Guivernau B, et al. Posttranslational Nitro-Glycative Modifications of Albumin in Alzheimer's Disease: Implications in Cytotoxicity and Amyloid-β Peptide Aggregation. J Alzheimers Dis. 2014 feb;p. 1–15.

[304] Guix FX, Ill-Raga G, Bravo R, Nakaya T, de Fabritiis G, Coma M, et al. Amyloid-dependent triosephosphate isomerase nitrotyrosination induces glycation and tau fibrillation. Brain. 2009 may;132(Pt 5):1335–45.

[305] Tajes M, Guivernau B, Ramos-Fernández E, Bosch-Morató M, Palomer E, Guix FX, et al. The pathophysiology of triose phosphate isomerase dysfunction in Alzheimer's disease. Histol Histopathol. 2013 jan;28(1):43–51.

[306] Tajes M, Eraso-Pichot A, Rubio-Moscardó F, Guivernau B, Ramos-Fernández E, Bosch-Morató M, et al. Methylglyoxal Produced by Amyloid-β Peptide-Induced Nitrotyrosination of Triosephosphate Isomerase Triggers Neuronal Death in Alzheimer's Disease. J Alzheimers Dis. 2014 mar;.

[307] Ill-Raga G, Ramos-Fernázndez E, Guix FX, Tajes M, Bosch-Morató M, Palomer E, et al. Amyloid-β peptide fibrils induce nitro-oxidative stress in neuronal cells. J Alzheimers Dis. 2010 jan;22(2):641–52.

[308] Reed TT, Pierce WM, Turner DM, Markesbery WR, Butterfield DA. Proteomic identification of nitrated brain proteins in early Alzheimer's disease inferior parietal lobule. J Cell Mol Med. 2009 aug;13(8B):2019–29.

[309] Reynolds MR, Berry RW, Binder LI. Site-specific nitration and oxidative dityrosine bridging of the tau protein by peroxynitrite: implications for Alzheimer's disease. Biochemistry. 2005 mar;44(5):1690–700.

[310] Kummer MP, Hermes M, Delekarte A, Hammerschmidt T, Kumar S, Terwel D, et al. Nitration of tyrosine 10 critically enhances amyloid β aggregation and plaque formation. Neuron. 2011 sep;71(5):833–44.

[311] Somerville MJ, Percy ME, Bergeron C, Yoong LK, Grima EA, McLachlan DR. Localization and quantitation of 68 kDa neurofilament and superoxide dismutase-1 mRNA in Alzheimer brains. Brain Res Mol Brain Res. 1991 jan;9(1-2):1–8.

[312] Lovell MA, Ehmann WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. Neurology. 1995 aug;45(8):1594–601.

[313] Markesbery WR, Lovell MA. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol Aging. 1998 jan;19(1):33–6.

[314] Smith MA, Taneda S, Richey PL, Miyata S, Yan SD, Stern D, et al. Advanced Maillard reaction end products are associated with Alzheimer disease pathology. Proc Natl Acad Sci U S A. 1994 jun;91(12):5710–4.

[315] Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, et al. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem. 1995 nov;65(5):2146–56.

[316] Aksenova MV, Aksenov MY, Payne RM, Trojanowski JQ, Schmidt ML, Carney JM, et al. Oxidation of cytosolic proteins and expression of creatine kinase BB in frontal lobe in different neurodegenerative disorders. Dement Geriatr Cogn Disord. 1999 jan;10(2):158–65.

[317] Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol. 1994 nov;36(5):747–51.

[318] Mecocci P, Beal MF, Cecchetti R, Polidori MC, Cherubini A, Chionne F, et al. Mitochondrial membrane fluidity and oxidative damage to mitochondrial DNA in aged and AD human brain. Mol Chem Neuropathol. 1997 may;31(1):53–64.

[319] Gabbita SP, Lovell MA, Markesbery WR. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. J Neurochem. 1998 nov;71(5):2034–40.

[320] Aksenov MY, Tucker HM, Nair P, Aksenova MV, Butterfield DA, Estus S, et al. The expression of key oxidative stress-handling genes in different brain regions in Alzheimer's disease. J Mol Neurosci. 1998;11(2):151–164.

[321] Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, et al. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci U S A. 1991;88(23):10540–3.

[322] Huang X, Atwood CS, Hartshorn MA, Multhaup G, Goldstein LE, Scarpa RC, et al. The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry. 1999 jun;38(24):7609–16.

[323] Varadarajan S, Yatin S, Kanski J, Jahanshahi F, Butterfield DA. Methionine residue 35 is important in amyloid β-peptide-associated free radical oxidative stress. Brain Res Bull. 1999 sep;50(2):133–141.

[324] Hensley K, Carney JM, Mattson MP, Aksenova M, Harris M, Wu JF, et al. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc Natl Acad Sci U S A. 1994 apr;91(8):3270–4.

[325] Harris ME, Hensley K, Butterfield DA, Leedle RA, Carney JM. Direct evidence of oxidative injury produced by the Alzheimer's beta-amyloid peptide (1-40) in cultured hippocampal neurons. Exp Neurol. 1995 feb;131(2):193–202.

[326] Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. J Neurochem. 1997 jan;68(1):255–64.

[327] Mark RJ, Fuson KS, May PC. Characterization of 8-epiprostaglandin F2alpha as a marker of amyloid beta-peptide-induced oxidative damage. J Neurochem. 1999 mar;72(3):1146–53.

[328] Lauderback CM, Hackett JM, Huang FF, Keller JN, Szweda LI, Markesbery WR, et al. The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Abeta1-42. J Neurochem. 2001 jul;78(2):413–6.

[329] Meda L, Cassatella MA, Szendrei GI, Otvos L, Baron P, Villalba M, et al. Activation of microglial cells by beta-amyloid protein and interferon-gamma. Nature. 1995 apr;374(6523):647–50.

[330] Rossi F, Bianchini E. Synergistic induction of nitric oxide by beta-amyloid and cytokines in astrocytes. Biochem Biophys Res Commun. 1996 aug;225(2):474–8.

[331] Berr C, Richard MJ, Gourlet V, Garrel C, Favier A. Enzymatic antioxidant balance and cognitive decline in aging–the EVA study. Eur J Epidemiol. 2004 jan;19(2):133–8.

[332] Bourdel-Marchasson I, Delmas-Beauvieux MC, Peuchant E, Richard-Harston S, Decamps A, Reignier B, et al. Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. Age Ageing. 2001 may;30(3):235–41.

[333] Rinaldi P, Polidori MC, Metastasio A, Mariani E, Mattioli P, Cherubini A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging. 2003 nov;24(7):915–9.

[334] Dyrks T, Dyrks E, Hartmann T, Masters C, Beyreuther K. Amyloidogenicity of beta A4 and beta A4-bearing amyloid protein precursor fragments by metal-catalyzed oxidation. J Biol Chem. 1992 sep;267(25):18210–7.

[335] Kaminsky YG, Kosenko Ea. Effects of amyloid-beta peptides on hydrogen peroxide-metabolizing enzymes in rat brain in vivo. Free Radic Res. 2008;42(6):564–573.

[336] Behl C. Hydrogen peroxide mediates amyloid β protein toxicity. Cell. 1994 jun;77(6):817–827.

[337] Calderón FH, Bonnefont A, Muñoz FJ, Fernández V, Videla LA, Inestrosa NC. PC12 and neuro 2a cells have different susceptibilities to acetylcholinesterase-amyloid complexes, amyloid25-35 fragment, glutamate, and hydrogen peroxide. J Neurosci Res. 1999 jun;56(6):620–31.

[338] Yatin SM, Varadarajan S, Butterfield DA. Vitamin E Prevents Alzheimer's Amyloid beta-Peptide (1-42)-Induced Neuronal Protein Oxidation and Reactive Oxygen Species Production. J Alzheimers Dis. 2000 jun;2(2):123–131.

[339] Koppal T, Subramaniam R, Drake J, Prasad MR, Dhillon H, Butterfield DA. Vitamin E protects against Alzheimer's amyloid peptide (25-35)-induced changes in neocortical synaptosomal membrane lipid structure and composition. Brain Res. 1998 mar;786(1-2):270–3.

[340] Behl C, Davis J, Cole GM, Schubert D. Vitamin E protects nerve cells from amyloid beta protein toxicity. Biochem Biophys Res Commun. 1992 jul;186(2):944–50.

[341] Muñoz FJ, Opazo C, Gil-Gómez G, Tapia G, Fernández V, Valverde Ma, et al. Vitamin E but not 17beta-estradiol protects against vascular toxicity induced by beta-amyloid wild type and the Dutch amyloid variant. J Neurosci. 2002 apr;22(8):3081–9.

[342] Karuppagounder SS, Pinto JT, Xu H, Chen HL, Beal MF, Gibson GE. Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. Neurochem Int. 2009 feb;54(2):111–8.

[343] Cole GM, Teter B, Frautschy SA. Neuroprotective effects of curcumin. Adv Exp Med Biol. 2007 jan;595:197–212.

[344] Frautschy SA, Cole GM. Why Pleiotropic Interventions are Needed for Alzheimer's Disease. Mol Neurobiol. 2010 may;41(2-3):392–409.

[345] Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. J Biol Chem. 2005 feb;280(7):5892–901.

[346] Ono K, Hasegawa K, Naiki H, Yamada M. Curcumin has potent anti-amyloidogenic effects for Alzheimer's beta-amyloid fibrils in vitro. J Neurosci Res. 2004 mar;75(6):742–50.

[347] Baum L, Ng A. Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. J Alzheimers Dis. 2004 aug;6(4):367–77; discussion 443–9.

[348] Kang SK, Cha SH, Jeon HG. Curcumin-induced histone hypoacetylation enhances caspase-3-dependent glioma cell death and neurogenesis of neural progenitor cells. Stem Cells Dev. 2006 apr;15(2):165–74.

[349] Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, et al. Life with 6000 genes. Science. 1996 oct;274(5287):546, 563–7.

[350] Winderickx J, Delay C, De Vos A, Klinger H, Pellens K, Vanhelmont T, et al. Protein folding diseases and neurodegeneration: lessons learned from yeast. Biochim Biophys Acta. 2008 jul;1783(7):1381–95.

[351] Braun RJ, Büttner S, Ring J, Kroemer G, Madeo F. Nervous yeast: modeling neurotoxic cell death. Trends Biochem Sci. 2010 mar;35(3):135–44.

[352] Steensels J, Snoek T, Meersman E, Nicolino MP, Voordeckers K, Verstrepen KJ. Improving industrial yeast strains: Exploiting natural and artificial diversity. FEMS Microbiol Rev. 2014;38(5):947–995.

[353] Neiman AM. Ascospore formation in the yeast Saccharomyces cerevisiae. Microbiol Mol Biol Rev. 2005 dec;69(4):565–84.

[354] Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S, et al. Functional profiling of the Saccharomyces cerevisiae genome. Nature. 2002 jul;418(6896):387–91.

[355] Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, et al. Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science. 1999 aug;285(5429):901–6.

[356] Tong AHY, Evangelista M, Parsons AB, Xu H, Bader GD, Pagé N, et al. Systematic Genetic Analysis with Ordered Arrays of Yeast Deletion Mutants. Science (80-). 2001 dec;294(5550):2364–2368.

[357] Davierwala AP, Haynes J, Li Z, Brost RL, Robinson MD, Yu L, et al. The synthetic genetic interaction spectrum of essential genes. Nat Genet. 2005 oct;37(10):1147–52.

[358] Boone C, Bussey H, Andrews BJ. Exploring genetic interactions and networks with yeast. TL - 8. Nat Rev Genet. 2007;8 VN - re(6):437–449.

[359] Foury F. Human genetic diseases: a cross-talk between man and yeast. Gene. 1997 aug;195(1):1–10.

[360] Bassett DE, Boguski MS, Hieter P. Yeast genes and human disease. Nature. 1996 feb;379(6566):589–90.

[361] Lüthi U, Schaerer-Brodbeck C, Tanner S, Middendorp O, Edler K, Barberis A. Human beta-secretase activity in yeast detected by a novel cellular growth selection system. Biochim Biophys Acta. 2003 mar;1620(1-3):167–78.

[362] Vandebroek T, Terwel D, Vanhelmont T, Gysemans M, Van Haesendonck C, Engelborghs Y, et al. Microtubule binding and clustering of human Tau-4R and Tau-P301L proteins isolated from yeast deficient in orthologues of glycogen synthase kinase-3beta or cdk5. J Biol Chem. 2006 sep;281(35):25388–97.

[363] Zhang H, Komano H, Fuller RS, Gandy SE, Frail DE. Proteolytic processing and secretion of human beta-amyloid precursor protein in yeast. Evidence for a yeast secretase activity. J Biol Chem. 1994 nov;269(45):27799–802.

[364] Caine J, Sankovich S, Antony H, Waddington L, Macreadie P, Varghese J, et al. Alzheimer's Abeta fused to green fluorescent protein induces growth stress and a heat shock response. FEMS Yeast Res. 2007 dec;7(8):1230–6.

[365] Morell M, de Groot NS, Vendrell J, Avilés FX, Ventura S. Linking amyloid protein aggregation and yeast survival. Mol Biosyst. 2011 apr;7(4):1121–8.

[366] López LC, Dos-Reis S, Espargaró A, Carrodeguas JA, Maddelein ML, Ventura S, et al. Discovery of novel inhibitors of amyloid β-peptide 1-42 aggregation. J Med Chem. 2012 nov;55(22):9521–30.

[367] Villar-Piqué A, Ventura S. Protein aggregation propensity is a crucial determinant of intracellular inclusion formation and quality control degradation. Biochim Biophys Acta. 2013 dec;1833(12):2714–24.

[368] Garai K, Frieden C. Quantitative analysis of the time course of Aβ oligomerization and subsequent growth steps using tetramethylrhodamine-labeled Aβ. Proc Natl Acad Sci U S A. 2013 feb;110(9):3321–6.

[369] D'Angelo F, Vignaud H, Di Martino J, Salin B, Devin A, Cullin C, et al. A yeast model for amyloid-β aggregation exemplifies the role of membrane trafficking and PICALM in cytotoxicity. Dis Model Mech. 2013 jan;6(1):206–16.

[370] Treusch S, Hamamichi S, Goodman JL, Matlack KES, Chung CY, Baru V, et al. Functional links between Aβ toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast. Science. 2011 dec;334(6060):1241–5.

[371] Miranda S, Opazo C, Larrondo LF, Muñoz FJ, Ruiz F, Leighton F, et al. The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer's disease. Prog Neurobiol. 2000 dec;62(6):633–48.

[372] Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A. 1986 jul;83(13):4913–7.

[373] Masters CL, Simms G, Weinman NA. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A. 1985;82(12):4245–4249.

[374] Xia W, Zhang J, Ostaszewski BL, Kimberly WT, Seubert P, Koo EH, et al. Presenilin 1 regulates the processing of beta-amyloid precursor protein C-terminal fragments and the generation of amyloid beta-protein in endoplasmic reticulum and Golgi. Biochemistry. 1998 nov;37(47):16465–71.

[375] Arimon M. Fine structure study of Abeta 1-42 fibrillogenesis with atomic force microscopy. FASEB J. 2005 jun;19(10):1344–6.

[376] Hsia AY, Masliah E, McConlogue L, Yu GQ, Tatsuno G, Hu K, et al. Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. Proc Natl Acad Sci. 1999 mar;96(6):3228–3233.

[377] Klein WL. Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. Neurochem Int. 2002 nov;41(5):345–52.

[378] Danysz W, Parsons CG. Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine–searching for the connections. Br J Pharmacol. 2012 sep;167(2):324–52.

[379] Esposito Z, Belli L, Toniolo S, Sancesario G, Bianconi C, Martorana A. Amyloid β, glutamate, excitotoxicity in Alzheimer's disease: are we on the right track? CNS Neurosci Ther. 2013 aug;19(8):549–55.

[380] Dawson VL, Dawson TM. Nitric oxide neurotoxicity. J Chem Neuroanat. 1996 jun;10(3-4):179–90.

[381] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol. 1996;271(73):C1424–C1437.

[382] Tajes M, Ill-Raga G, Palomer E, Ramos-Fernández E, Guix F, Bosch-Morató M, et al. Nitro-oxidative stress after neuronal ischemia induces protein nitrotyrosination and cell death. Oxid Med Cell Longev. 2013;2013.

[383] Cohen SIA, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, et al. Proliferation of amyloid-β42 aggregates occurs through a secondary nucleation mechanism. Proc Natl Acad Sci U S A. 2013 jun;110(24):9758–63.

[384] Tamagno E, Bardini P, Obbili A, Vitali A, Borghi R, Zaccheo D, et al. Oxidative stress increases expression and activity of BACE in NT2 neurons. Neurobiol Dis. 2002 aug;10(3):279–88.

[385] Coma M, Guix FX, Ill-Raga G, Uribesalgo I, Alameda F, Valverde MA, et al. Oxidative stress triggers the amyloidogenic pathway in human vascular smooth muscle cells. Neurobiol Aging. 2008 jul;29(7):969–80.

[386] Zhao J, Wang P, Li H, Gao Z. Nitration of Y10 in Aβ 1-40 : Is It a Compensatory Reaction against Oxidative/Nitrative Stress and Aβ Aggregation? Chem Res Toxicol. 2015 mar;28(3):401–407.

[387] Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D. Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron. 2009 jun;62(6):788–801.

[388] Choi DW. Glutamate neurotoxicity in cortical cell culture is calcium dependent. Neurosci Lett. 1985 aug;58(3):293–7.

[389] Berridge MJ. Calcium hypothesis of Alzheimer's disease. Pflugers Arch. 2010 feb;459(3):441–9.

[390] Fernàndez-Busquets X. Amyloid fibrils in neurodegenerative diseases: villains or heroes? Future Med Chem. 2013 oct;5(16):1903–1906.

[391] Bitan G, Teplow DB. Preparation of aggregate-free, low molecular weight amyloid-beta for assembly and toxicity assays. Methods Mol Biol. 2005 jan;299:3–9.

[392] Petkova AT, Yau WM, Tycko R. Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils. Biochemistry. 2006 jan;45(2):498–512.

[393] Berman HM. The Protein Data Bank. Nucleic Acids Res. 2000 jan;28(1):235–242.

[394] Webb B, Sali A. Protein structure modeling with MODELLER. Methods Mol Biol. 2014 jan;1137:1–15.

[395] Pierce B, Weng Z. ZRANK: reranking protein docking predictions with an optimized energy function. Proteins. 2007 jun;67(4):1078–86.

[396] Duhovny D, Nussinov R, Wolfson H. Efficient Unbound Docking of Rigid Molecules. In: Algorithms Bioinforma. SE - 14. vol. 2452 of Lecture Notes in Computer Science. Springer Berlin Heidelberg; 2002. p. 185–200.

[397] Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J. 1985;4(11):2757–2763.

[398] Frackowiak J, Zoltowska A, Wisniewski HM. Non-fibrillar beta-amyloid protein is associated with smooth muscle cells of vessel walls in Alzheimer disease. J Neuropathol Exp Neurol. 1994;53(6):637–45.

[399] Nogalska A, D'Agostino C, Engel WK, Klein WL, Askanas V. Novel demonstration of amyloid-β oligomers in sporadic inclusion-body myositis muscle fibers. Acta Neuropathol. 2010 nov;120(5):661–6.

[400] Askanas V, Engel WK, Alvarez RB. Enhanced detection of congo-red-positive amyloid deposits in muscle fibers of inclusion body myositis and brain of Alzheimer's disease using fluorescence technique. Neurology. 1993 jun;43(6):1265–7.

[401] De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature. 1998 jan;391(6665):387–90.

[402] Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, et al. Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am J Pathol. 2002 nov;161(5):1869–79.

[403] Koo EH, Squazzo SL. Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J Biol Chem. 1994 jul;269(26):17386–9.

[404] Bahr BA, Hoffman KB, Yang AJ, Hess US, Glabe CG, Lynch G. Amyloid beta protein is internalized selectively by hippocampal field CA1 and causes neurons to accumulate amyloidogenic carboxyterminal fragments of the amyloid precursor protein. J Comp Neurol. 1998 jul;397(1):139–47.

[405] Saavedra L, Mohamed A, Ma V, Kar S, de Chaves EP. Internalization of beta-amyloid peptide by primary neurons in the absence of apolipoprotein E. J Biol Chem. 2007 dec;282(49):35722–32.

[406] Supnet C, Bezprozvanny I. The dysregulation of intracellular calcium in Alzheimer disease. Cell Calcium. 2010 feb;47(2):183–9.

[407] Rubio-Moscardo F, Setó-Salvia N, Pera M, Bosch-Morató M, Plata C, Belbin O, et al. Rare variants in calcium homeostasis modulator 1 (CALHM1) found in early onset Alzheimer's disease patients alter calcium homeostasis. PLoS One. 2013 jan;8(9):e74203.

[408] LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci. 2002 nov;3(11):862–72.

[409] Magrané J, Smith RC, Walsh K, Querfurth HW. Heat shock protein 70 participates in the neuroprotective response to intracellularly expressed beta-amyloid in neurons. J Neurosci. 2004 feb;24(7):1700–6.

[410] Gregori L, Fuchs C, Figueiredo-Pereira ME, Van Nostrand WE, Goldgaber D. Amyloid beta-protein inhibits ubiquitin-dependent protein degradation in vitro. J Biol Chem. 1995 aug;270(34):19702–8.

[411] Agholme L, Hallbeck M, Benedikz E, Marcusson J, Kågedal K. Amyloid-β secretion, generation, and lysosomal sequestration in response to proteasome inhibition: involvement of autophagy. J Alzheimers Dis. 2012 jan;31(2):343–58.

[412] Guglielmotto M, Monteleone D, Piras A, Valsecchi V, Tropiano M, Ariano S, et al. Aβ1-42 monomers or oligomers have different effects on autophagy and apoptosis. Autophagy. 2014 oct;10(10):1827–43.

[413] Khurana V, Lindquist S. Modelling neurodegeneration in Saccharomyces cerevisiae: why cook with baker's yeast? Nat Rev Neurosci. 2010 jun;11(6):436–49.

[414] Bharadwaj P, Waddington L, Varghese J, Macreadie IG. A new method to measure cellular toxicity of non-fibrillar and fibrillar Alzheimer's Abeta using yeast. J Alzheimers Dis. 2008 mar;13(2):147–50.

[415] Kurjan J, Herskowitz I. Structure of a yeast pheromone gene (MF alpha): a putative alpha-factor precursor contains four tandem copies of mature alpha-factor. Cell. 1982 oct;30(3):933–43.

[416] Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, et al. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. Nat Neurosci. 2001 sep;4(9):887–93.

[417] Miravalle L, Tokuda T, Chiarle R, Giaccone G, Bugiani O, Tagliavini F, et al. Substitutions at codon 22 of Alzheimer's abeta peptide induce diverse conformational changes and apoptotic effects in human cerebral endothelial cells. J Biol Chem. 2000 sep;275(35):27110–6.

[418] Tong L, Thornton PL, Balazs R, Cotman CW. Beta -amyloid-(1-42) impairs activity-dependent cAMP-response element-binding protein signaling in neurons at concentrations in which cell survival Is not compromised. J Biol Chem. 2001 may;276(20):17301–6.

[419] Gibson GE, Shi Q. A mitocentric view of Alzheimer's disease suggests multi-faceted treatments. J Alzheimers Dis. 2010 jan;20 Suppl 2:S591–607.

[420] Challet C, Maechler P, Wollheim CB, Ruegg UT. Mitochondrial calcium oscillations in C2C12 myotubes. J Biol Chem. 2001 feb;276(6):3791–7.

[421] Nair S, Traini M, Dawes IW, Perrone GG. Genome-wide analysis of Saccharomyces cerevisiae identifies cellular processes affecting intracellular aggregation of Alzheimer's amyloid-β42: importance of lipid homeostasis. Mol Biol Cell. 2014 aug;25(15):2235–49.

[422] Lai KO, Ip NY. Synapse development and plasticity: roles of ephrin/Eph receptor signaling. Curr Opin Neurobiol. 2009 jun;19(3):275–83.

[423] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet. 2011 may;43(5):436–41.

[424] Hughes TM, Lopez OL, Evans RW, Kamboh MI, Williamson JD, Klunk WE, et al. Markers of cholesterol transport are associated with amyloid deposition in the brain. Neurobiol Aging. 2014 apr;35(4):802–7.

[425] Pfrieger FW. Cholesterol homeostasis and function in neurons of the central nervous system. Cell Mol Life Sci. 2003 jun;60(6):1158–71.

[426] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011 may;43(5):429–35.

[427] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013 dec;45(12):1452–8.

[428] Gallo JM, Spickett C. The role of CELF proteins in neurological disorders. RNA Biol. jan;7(4):474–9.

[429] Hinney A, Albayrak O, Antel J, Volckmar AL, Sims R, Chapman J, et al. Genetic variation at the CELF1 (CUGBP, elav-like family member 1 gene) locus is genome-wide associated with Alzheimer's disease and obesity. Am J Med Genet B Neuropsychiatr Genet. 2014 jun;165B(4):283–93.

[430] Bobba A, Amadoro G, Valenti D, Corsetti V, Lassandro R, Atlante A. Mitochondrial respiratory chain Complexes I and IV are impaired by β-amyloid via direct interaction and through Complex I-dependent ROS production, respectively. Mitochondrion. 2013 jul;13(4):298–311.

[431] Hernandez-Zimbron LF, Luna-Muñoz J, Mena R, Vazquez-Ramirez R, Kubli-Garfias C, Cribbs DH, et al. Amyloid-β peptide binds to cytochrome C oxidase subunit 1. PLoS One. 2012 jan;7(8):e42344.

[432] Francis BM, Yang J, Song BJ, Gupta S, Maj M, Bazinet RP, et al. Reduced levels of mitochondrial complex I subunit NDUFB8 and linked complex I + III oxidoreductase activity in the TgCRND8 mouse model of Alzheimer's disease. J Alzheimers Dis. 2014 jan;39(2):347–55.

[433] Schneider A, Rajendran L, Honsho M, Gralle M, Donnert G, Wouters F, et al. Flotillin-dependent clustering of the amyloid precursor protein regulates its endocytosis and amyloidogenic processing in neurons. J Neurosci. 2008 mar;28(11):2874–82.

[434] Kuboyama T, Lee YA, Nishiko H, Tohda C. Inhibition of clathrin-mediated endocytosis prevents amyloid β-induced axonal damage. Neurobiol Aging. 2015 may;36(5):1808–19.

[435] Dreses-Werringloer U, Lambert JC, Vingtdeux V, Zhao H, Vais H, Siebert A, et al. A polymorphism in CALHM1 influences Ca2+ homeostasis, Abeta levels, and Alzheimer's disease risk. Cell. 2008 jun;133(7):1149–61.

[436] Stathopoulos AM, Cyert MS. Calcineurin acts through the CRZ1/TCN1-encoded transcription factor to regulate gene expression in yeast. Genes Dev. 1997 dec;11(24):3432–44.

[437] Polizotto RS, Cyert MS. Calcineurin-dependent nuclear import of the transcription factor Crz1p requires Nmd5p. J Cell Biol. 2001 sep;154(5):951–60.

[438] Matheos DP, Kingsbury TJ, Ahsan US, Cunningham KW. Tcn1p/Crz1p, a calcineurin-dependent transcription factor that differentially regulates gene expression in Saccharomyces cerevisiae. Genes Dev. 1997 dec;11(24):3445–58.

[439] Kingsbury TJ, Cunningham KW. A conserved family of calcineurin regulators. Genes Dev. 2000 jul;14(13):1595–604.

[440] Rudolph HK, Antebi A, Fink GR, Buckley CM, Dorman TE, LeVitre J, et al. The yeast secretory pathway is perturbed by mutations in PMR1, a member of a Ca2+ ATPase family. Cell. 1989 jul;58(1):133–45.

[441] Antebi A, Fink GR. The yeast Ca(2+)-ATPase homologue, PMR1, is required for normal Golgi function and localizes in a novel Golgi-like distribution. Mol Biol Cell. 1992 jun;3(6):633–54.

[442] Heath VL, Shaw SL, Roy S, Cyert MS. Hph1p and Hph2p, novel components of calcineurin-mediated stress responses in Saccharomyces cerevisiae. Eukaryot Cell. 2004 jun;3(3):695–704.

[443] Bultynck G, Heath VL, Majeed AP, Galan JM, Haguenauer-Tsapis R, Cyert MS. Slm1 and slm2 are novel substrates of the calcineurin phosphatase required for heat stress-induced endocytosis of the yeast uracil permease. Mol Cell Biol. 2006 jun;26(12):4729–45.

[444] Fujii Y, Shiota M, Ohkawa Y, Baba A, Wanibuchi H, Kinashi T, et al. Surf4 modulates STIM1-dependent calcium entry. Biochem Biophys Res Commun. 2012 jun;422(4):615–20.

[445] Chen QS, Wei WZ, Shimahara T, Xie CW. Alzheimer amyloid beta-peptide inhibits the late phase of long-term potentiation through calcineurin-dependent mechanisms in the hippocampal dentate gyrus. Neurobiol Learn Mem. 2002 may;77(3):354–71.

[446] Ermak G, Morgan TE, Davies KJ. Chronic overexpression of the calcineurin inhibitory gene DSCR1 (Adapt78) is associated with Alzheimer's disease. J Biol Chem. 2001 oct;276(42):38787–94.

[447] Flynn JM, Melov S. SOD2 in mitochondrial dysfunction and neurodegeneration. Free Radic Biol Med. 2013 sep;62:4–12.

[448] Peng Q, Lao X, Huang X, Qin X, Li S, Zeng Z. The MTHFR C677T polymorphism contributes to increased risk of Alzheimer's disease: evidence based on 40 case-control studies. Neurosci Lett. 2015 jan;586:36–42.

[449] Kimura R, Kamino K, Yamamoto M, Nuripa A, Kida T, Kazui H, et al. The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between beta-amyloid production and tau phosphorylation in Alzheimer disease. Hum Mol Genet. 2007 jan;16(1):15–23.

[450] Duhig T, Ruhrberg C, Mor O, Fried M. The human Surfeit locus. Genomics. 1998 aug;52(1):72–8.

[451] Mitrovic S, Ben-Tekaya H, Koegler E, Gruenberg J, Hauri HP. The cargo receptors Surf4, endoplasmic reticulum-Golgi intermediate compartment (ERGIC)-53, and p25 are required to maintain the architecture of ERGIC and Golgi. Mol Biol Cell. 2008 may;19(5):1976–90.

[452] Gietz RD, Schiestl RH, Willems AR, Woods RA. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast. 1995 apr;11(4):355–60.

[453] Tong AHY, Boone C. Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods Mol Biol. 2006 jan;313:171–92.

[454] Mangialasche F, Polidori MC, Monastero R, Ercolani S, Camarda C, Cecchetti R, et al. Biomarkers of oxidative and nitrosative damage in Alzheimer's disease and mild cognitive impairment. Ageing Res Rev. 2009;8(4):285–305.

[455] Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, et al. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology. 2005 apr;64(7):1152–6.

[456] Praticò D, Uryu K, Leight S, Trojanoswki JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J Neurosci. 2001 jun;21(12):4183–7.

[457] Heiss WD, Szelies B, Kessler J, Herholz K. Abnormalities of energy metabolism in Alzheimer's disease studied with PET. Ann N Y Acad Sci. 1991 jan;640:65–71.

[458] Chen X, Yan SD. Mitochondrial Abeta: a potential cause of metabolic dysfunction in Alzheimer's disease. IUBMB Life. 2006 dec;58(12):686–94.

[459] Jekabsone A, Mander PK, Tickler A, Sharpe M, Brown GC. Fibrillar beta-amyloid peptide Abeta1-40 activates microglial proliferation via stimulating TNF-alpha release and H2O2 derived from NADPH oxidase: a cell culture study. J Neuroinflammation. 2006 jan;3:24.

[460] Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 2007 jan;8(1):57–69.

[461] Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1988 oct;1(8):623–34.

[462] Inestrosa NC, Reyes AE, Chacón MA, Cerpa W, Villalón A, Montiel J, et al. Human-like rodent amyloid-β-peptide determines Alzheimer pathology in aged wild-type Octodon degu. Neurobiol Aging. 2005;26(7):1023–1028.

[463] Smith DP, Smith DG, Curtain CC, Boas JF, Pilbrow JR, Ciccotosto GD, et al. Copper-mediated amyloid-beta toxicity is associated with an intermolecular histidine bridge. J Biol Chem. 2006 jun;281(22):15145–54.

[464] Smith MA, Richey Harris PL, Sayre LM, Beckman JS, Perry G. Widespread peroxynitrite-mediated damage in Alzheimer's disease. J Neurosci. 1997 apr;17(8):2653–7.

[465] Perez-Garmendia R, Gevorkian G. Pyroglutamate-Modified Amyloid Beta Peptides: Emerging Targets for AlzheimerÂŽs Disease Immunotherapy. Curr Neuropharmacol. 2013 sep;11(5):491–8.

[466] Kumar S, Rezaei-Ghaleh N, Terwel D, Thal DR, Richard M, Hoch M, et al. Extracellular phosphorylation of the amyloid β-peptide promotes formation

of toxic aggregates during the pathogenesis of Alzheimer's disease. EMBO J. 2011 jun;30(11):2255–65.

[467] Kubo T, Kumagae Y, Miller CA, Kaneko I. Beta-amyloid racemized at the Ser26 residue in the brains of patients with Alzheimer disease: implications in the pathogenesis of Alzheimer disease. J Neuropathol Exp Neurol. 2003 mar;62(3):248–59.

[468] Shimizu T, Fukuda H, Murayama S, Izumiyama N, Shirasawa T. Isoaspartate formation at position 23 of amyloid beta peptide enhanced fibril formation and deposited onto senile plaques and vascular amyloids in Alzheimer's disease. J Neurosci Res. 2002 nov;70(3):451–61.

[469] Fukuda H, Shimizu T, Nakajima M, Mori H, Shirasawa T. Synthesis, aggregation, and neurotoxicity of the Alzheimer's Abeta1-42 amyloid peptide and its isoaspartyl isomers. Bioorg Med Chem Lett. 1999 apr;9(7):953–6.

[470] Hou L, Shao H, Zhang Y, Li H, Menon NK, Neuhaus EB, et al. Solution NMR studies of the A beta(1-40) and A beta(1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. J Am Chem Soc. 2004 feb;126(7):1992–2005.

[471] Kumar S, Wirths O, Stüber K, Wunderlich P, Koch P, Theil S, et al. Phosphorylation of the amyloid β-peptide at Ser26 stabilizes oligomeric assembly and increases neurotoxicity. Acta Neuropathol. 2016 feb;131(4):525–37.

[472] Mazargui H, Lévêque C, Bartnik D, Fantini J, Gouget T, Melone MAB, et al. A synthetic amino acid substitution of Tyr10 in Aβ peptide sequence yields a dominant negative variant in amyloidogenesis. Aging Cell. 2012 jun;11(3):530–41.

[473] Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature. 2002 apr;416(6880):535–9.

[474] Decker H, Jürgensen S, Adrover MF, Brito-Moreira J, Bomfim TR, Klein WL, et al. N-methyl-D-aspartate receptors are required for synaptic targeting of Alzheimer's toxic amyloid-β peptide oligomers. J Neurochem. 2010 dec;115(6):1520–9.

[475] Renner M, Lacor PN, Velasco PT, Xu J, Contractor A, Klein WL, et al. Deleterious effects of amyloid beta oligomers acting as an extracellular scaffold for mGluR5. Neuron. 2010 jun;66(5):739–54.

[476] Sadigh-Eteghad S, Talebi M, Farhoudi M, Golzari SEJ, Sabermarouf B, Mahmoudi J. Beta-amyloid exhibits antagonistic effects on alpha 7 nicotinic acetylcholine receptors in orchestrated manner. J Med Hypotheses Ideas. 2014 jul;8(2):49–52.

[477] Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science. 1982 mar;215(4537):1237–9.

[478] Ferreira IL, Bajouco LM, Mota SI, Auberson YP, Oliveira CR, Rego AC. Amyloid beta peptide 1-42 disturbs intracellular calcium homeostasis through activation of GluN2B-containing N-methyl-d-aspartate receptors in cortical cultures. Cell Calcium. 2012;51(2):95–106.

[479] House SB, Thomas A, Kusano K, Gainer H. Stationary organotypic cultures of oxytocin and vasopressin magnocellular neurones from rat and mouse hypothalamus. J Neuroendocrinol. 1998 nov;10(11):849–61.

[480] Rosenzweig S, Luu P. Adult neurogenesis workshop report. Front Neurosci. 2010 jan;4.

[481] Song MS, Rauw G, Baker GB, Kar S. Memantine protects rat cortical cultured neurons against beta-amyloid-induced toxicity by attenuating tau phosphorylation. Eur J Neurosci. 2008 nov;28(10):1989–2002.

[482] Martinez-Coria H, Green KN, Billings LM, Kitazawa M, Albrecht M, Rammes G, et al. Memantine improves cognition and reduces Alzheimer's-like neuropathology in transgenic mice. Am J Pathol. 2010 feb;176(2):870–80.

[483] Wilkinson D. A review of the effects of memantine on clinical progression in Alzheimer's disease. Int J Geriatr Psychiatry. 2012 aug;27(8):769–76.

[484] Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. J Neurochem. 2002 jan;80(1):91–100.

[485] Canevari L, Clark JB, Bates TE. beta-Amyloid fragment 25-35 selectively decreases complex IV activity in isolated mitochondria. FEBS Lett. 1999 aug;457(1):131–4.