FEBS Letters
FEBS PRESS science publishing by scientists

Check for updates

REVIEW ARTICLE

The role of heparan sulfates in protein aggregation and their potential impact on neurodegeneration

Auriane Maïza, Sandrine Chantepie, Cecilia Vera, Alexandre Fifre, Minh Bao Huynh, Olivier Stettler, Mohand Ouidir Ouidja and Dulce Papy-Garcia

Cell Growth, Tissue Repair and Regeneration (CRRET), UPEC EA 4397/ERL CNRS 9215, Université Paris Est Créteil, Université Paris Est, Créteil, France

Correspondence
D. Papy-Garcia, Laboratoire CRRET, EA UPEC 4397/ERL CNRS 9215; 61, Av. du General de Gaulle, 94010 Créteil Cedex, France
Fax: +33145171816
Tel: +33 145177081
E-mail: papy@u-pec.fr

(Received 30 December 2017, revised 21 February 2018, accepted 22 February 2018, available online 14 June 2018)

doi:10.1002/1873-3468.13082

Edited by Sandro Sonnino

Neurodegenerative disorders, such as Alzheimer's, Parkinson's, and prion diseases, are directly linked to the formation and accumulation of protein aggregates in the brain. These aggregates, principally made of proteins or peptides that clamp together after acquisition of β-folded structures, also contain heparan sulfates. Several lines of evidence suggest that heparan sulfates centrally participate in the protein aggregation process. *In vitro*, they trigger misfolding, oligomerization, and fibrillation of amyloidogenic proteins, such as Aβ, tau, α-synuclein, prion protein, etc. They participate in the stabilization of protein aggregates, protect them from proteolysis, and act as cell-surface receptors for the cellular uptake of proteopathic seeds during their spreading. This review focuses attention on the importance of heparan sulfates in protein aggregation in brain disorders including Alzheimer's, Parkinson's, and prion diseases. The presence of these sulfated polysaccharides in protein inclusions *in vivo* and their capacity to trigger protein aggregation *in vitro* strongly suggest that they might play critical roles in the neurodegenerative process. Further advances in glyco-neurobiology will improve our understanding of the molecular and cellular mechanisms leading to protein aggregation and neurodegeneration.

Keywords: Alzheimer's disease; heparan sulfates; neurodegeneration; Parkinson's disease; prion diseases; protein aggregation

Protein aggregates deposition is a characteristic hallmark of several diseases known as protein misfolding diseases, amyloidosis, or 'proteinopathies' [1]. In these diseases, proteins acquire an abnormal β-sheet folded conformation, which induces a self-assemblage that confers to the aggregated protein a strong resistance to proteolysis, to denaturation, and to the general mechanisms of protein processing that operate in the cells [2]. This results in the protein deposition in form of filaments called amyloids, characteristic of proteinopathies. Proteinopathies include several systemic and neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, prion diseases, and others (Table 1). Although the exact mechanisms that lead to the *in vivo* protein misfolding and aggregation have not yet been completely elucidated, it is well-established that the deposed proteins are specific of the disease in where they accumulate. For instance, the

Abbreviations
CS, chondroitin sulfates; GAGs, glycosaminoglycans; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GlcA, glucuronic acid; HBP, heparin binding proteins; HS, heparan sulfates; HSPG, HS proteoglycans; HSSTs, HS sulfotransferases; IdoA, iduronic acid; KS, keratan sulfate; MAPT, microtubule associated protein tau; PAPS, 3′-phosphate 5′-phosphosulfate; SAA, serum amyloid A; STs, sulfotransferases; TSE, transmissible spongiform encephalopathies.

 © 2018 Federation of European Biochemical Societies
[The copyright line for this article was changed on 25 March 2019 after original publication].

Table 1. Diseases characterized by the accumulation of protein aggregates and the respective amyloidogenic proteins involved, their precursors, and the location of their protein deposits.

Protein precursor	Aggregated protein	Disease/syndrome	Location	References
Amyloid precursor protein (APP) and variants	Aβ	Alzheimer disease, cerebral amyloid angiopathy (CAA) or congophilic angiopathy	Brain	[43,90,91]
Tau protein	Tau	Alzheimer's disease, frontotemporal dementia, Pick's disease, progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD)	Brain	[6,47,91,92]
α-synuclein	α-Syn	Parkinson's disease (PD), dementia with Lewy bodies (DLB), Alzheimer's disease with Lewy bodies variant (LBVAD), multisystemic atrophy (MSA)	Brain	[73,74,78,93]
Prion protein (PrP) and variants	PrP^{SC}	Creutzfeldt-Jakob disease (CJD) Gerstmann-Straussler-Scheinker syndrome (GSS), Insomnia family lethal (FFI), Kuru	Brain	[36,81,86,87,94]
Huntingtin	HTT	Huntington's disease	Brain	[95]
Superoxide dismutase and others	SOD1	Amyotrophic lateral sclerosis (ALS)	Brain, spinal cord	[96]
Ataxin-1	Atxn-1	Spinocerebellar ataxia (SCA)	Brain, spinal cord	[97]
Atrophin-1 (DRPLA protein)	Atn-1	Dentatorubral-pallidoluysian atrophy (DRPLA)	Brain	[98]
Cystatin C	Cys	Hereditary cerebral amyloid angiopathy (HCCAA)	Brain	[99]
BriPP/DanPP	ABri/ADan	Familial British dementia and familial Danish dementia	Brain	[100]
Prolactin	Pro	Prolactinomas of the pituitary gland	Pituitary gland	[101]
Androgen receptor protein (AR)	AR	Bulbo spinal amyoatrophy (BSMA) or Kennedy disease	Brain, scrotal sac, dermis, kidney, heart, testicles, prostate, spinal cord	[102]
Apolipoprotein A-I	ApoAI	Systemic hereditary amyloidosis	Peripheral nervous system, heart, liver, kidney, testis, larynges, skin	[103]
Apolipoprotein A-IV	ApoAIV	Systemic secondary amyloidosis associated with inflammation	Systemic, kidney	[104]
Transthyretin variants	TTR	Familial amyloidotic polyneuropathy type I	Peripheral and autonomic nervous system, heart, eyes	[105]

β-amyloid peptide (Aβ) and the abnormally phosphorylated protein tau (P-tau) are deposed in Alzheimer's disease, α-synuclein in Parkinson's disease, and prion protein in an abnormal conformation (known as PrPsc) in prion diseases [3]. The lack of therapeutic solutions for the efficient treatment of these and other proteinopathies has led the biomedical research community to concentrate efforts on the exploration of the fine mechanisms priming and/or triggering protein aggregation in these pathologies.

Depending on the cell location of the protein building blocks, protein aggregates are formed either intra- or extracellularly [4,5]. Interestingly, this intra- or extracellular location also determines the probability of the building block interactions with aggregation-promoting macromolecules, such as arachidonic acid, RNA, or sulfated glycosaminoglycans [6–9]. Among glycosaminoglycans, heparan sulfates (HS) are of great interest due to their occurrence in most, if not all, intra- and extracellular protein aggregates that accumulate in neurodegenerative diseases, including those made of Aβ, tau, and α-synuclein [10,11]. Depending on their structures, HS can differentially interact with the soluble proteins, priming their initial misfolding into insoluble β-folded fibrils, and/or prompting their aggregation. This review presents an analysis of the literature focusing on the importance of HS, and HS structures, as possible key elements in the

physiopathologic mechanisms leading to protein misfolding and aggregation, particularly in Alzheimer's, Parkinson's, and prion diseases.

Heparan sulfates

Heparan sulfates and their great structural diversity

Heparan sulfates are linear polysaccharides belonging to the family of sulfated glycosaminoglycans (GAGs), which also includes chondroitin sulfates (CS) and keratan sulfate (KS) [12]. HS are the glycanic constituents of HS proteoglycans (HSPG), classically represented by the cell membrane-associated syndecans and glypicans, and by the secreted perlecan. Structurally, a HS chain is presented as the repetition of a disaccharide building block (Fig. 1), formed by an uronic acid, either glucuronic acid (GlcA) or iduronic acid (IdoA), and a glucosamine (GlcN). This disaccharide can carry sulfate groups at several of their hydroxyl groups, providing a variety of disaccharide sequences, which structures are finely dependent on the expression of the enzymes involved in the HS biosynthetic machinery. This machinery involves several glycosyl transferases (EXTs and EXTLs), several sulfotransferases (STs), and one epimerase [13]. While EXTs and EXTLs will determine the length of the forming HS chain, STs will integrate sulfate groups into the elongating sugar chain, giving high structural complexity to HS. In humans, STs include HS *N*-deacetyl sulfotransferases (NDST1-4) and HS sulfotransferases (HSSTs). Depending on the sugar position in which the sulfate group is transferred from the adenosine 3′-phosphate 5′-phosphosulfate (PAPS) sulfate donor. HSSTSs are regrouped in HS2ST1, HS3STs (HS3ST1, 2, 3A1, 3B1, 4, 5, and 6), and HS6STs (HS6ST1, 2varL, 2varS, 3). Thus, during the biosynthetic process, placement of sulfate groups on the elongating HS chain gives place to the formation of differently sulfated clusters among each single chain: NA domains stands for unsulfated sequences, NS domains for highly sulfated sequences, and NA/NS domains for sequences carrying intermediary levels of sulfation (Fig. 1). Among this, epimerase will additionally provoke an inversion of configuration at the carbon 5 (C5) of some GlcA units that will be converted into IdoA by the enzyme action. This will importantly affect the HS chain structure since the β 1–4 glycosidic linkage between GlcA and GlcN will become α in the IdoA containing disaccharides, concomitantly affecting recognition by heparinase, the enzyme that cleaves 1–4 glycosidic linkages during HS catabolism. Moreover, these stereochemical changes in uronic acids along the HS chain will strongly affect the polysaccharide flexibility, probably affecting the strength of interactions with amyloidogenic proteins. After biosynthesis, HSPGs are typically transported to the outer cell membrane or secreted into the ECM, where they exert their known biological functions, and where HS chains can be cleaved by heparanase, and/or 6-*O*-desulfated by HS 6-*O*-sulfatases (Sulf). Endocytosis afterward drives the HSPG to the lysosome pathway for degradation.

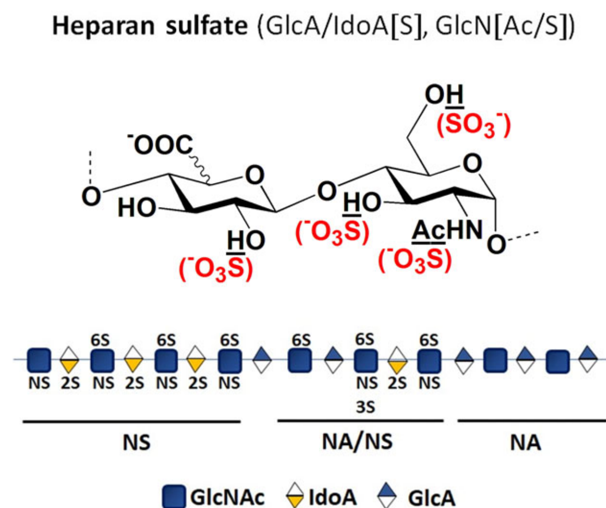

Fig. 1. Schematic representation of a heparan sulfate (HS) chain. The different HS domains are represented: NA (unsulfated domains), NA/NS (mixed *N*-sulfated and *N*-acetyled domains) and NS (rich *N*- and *O*-sulfated domains). The structure of a disaccharide characteristic of HS is represented to note the framed groups susceptible to carry sulfate groups.

Thus, depending on the expression of the HS metabolic machinery in each particular cell type or tissue, HS chains can carry different sulfate signatures (Fig. 1), which can vary as a consequence of aging, tissue injury or disease [14–16]. Accordingly, HS can interact with a large number of proteins or peptides, known under the generic name of heparin binding proteins (HBP) [13,17,18]. HBP include growth factors, cytokines, and most, if not all, proteins able to form amyloids, such as the Aβ peptide, tau, α-synuclein, huntingtin, superoxide dismutase, prion protein, etc. It has largely been reported that by interacting with growth factors and cytokines at the cell surface, HS participate to modulation of cell signaling [12,19], and that in the ECM, HS protect HBP proteins from proteolytic degradation, increasing their bioavailability [20,21]. By analogy, HS can interact with amyloidogenic proteins, increasing their bioavailability, but also by prompting their aggregation. The importance of the

HS biosynthetic machinery in the protein aggregation process has been suggested by the inhibition of amyloid deposition, which resulted from the inhibition of HS biosynthesis in cellular and animal models of neurodegeneration [22,23].

Protein aggregation

Amyloidosis

Protein aggregation is a process in which misfolded proteins clump together to form well-structured fibrils that form filaments, known as amyloids. Accumulation of amyloids in the brain tissue correlates with a wide variety of neurodegenerative disease including Alzheimer's disease, Parkinson's disease, Huntington disease, prion disease, and others [3,4,11]. Although all amyloids show considerable diversity in their constitutive protein sequences, they all share common characteristics [2]. The amyloids extracted from pathologic tissues are typically composed of fibrils assembled from two or three unbranched filaments (protofilaments) twisted around each other. These filaments are rich in β-sheet structures that form cross-β fibrils in which individual filaments are organized perpendicular to the long axis of the fibrils [2,24]. In solution, fibril formation generally occurs in a two-phase process (Fig. 2). The first phase is a nucleation phase, also called the lag phase. The second phase, in which the fibril is formed, is known as the polymerization or extension phase (growth phase). During the lag phase, a slow and reversible association of monomers forms nuclei structures. This process is thermodynamically unfavorable and constitutes the limiting step determining the speed of the fibrillation process. Once a nucleus has been formed, addition of monomers to the nucleus becomes thermodynamically favored, resulting in a rapid extension and formation of the amyloid fibers [25]. In this nucleated conversion model, the spherical oligomeric particles slowly become protofibrils by mediating a transition of the native protein or peptide into a β-sheet conformation [26]. This mechanism is at the basis of most known models of amyloid formation and has been proposed for several human amyloidogenic proteins and peptides [27,28]. Another proposed mechanism for the formation of amyloid fibrils involves a monomer-directed conversion in which the transition from a protein native state to a prefibrillar state directly influences other native monomers to undergo the same transition; this results in the formation of an intermediate fibrillar structure that can then grow to form fibrils [29]. Here, the trajectory of the formation of the fibrils begins with a prefibrillar kinetic precursor, represented by protofibrils or by their intermediate soluble oligomers [30]. Recently, the interest in the prefibrillar intermediates has grown, since they have been associated to a cytotoxicity higher than that of the mature filaments [31]. This has also led to the idea that the molecular bases of amyloid pathologies are centrally related to the formation and activity of prefibrillar protein aggregates. However, the specific mechanisms by which these species are generated *in vivo* and how they exert their toxic effects are not yet fully understood. Numerous studies suggest that HS participate to the different phases of amyloids formation, or that they are involved in alternative fibril growth pathways proposed to compete in the different stages of the protein aggregation process [10,11,19,20,32].

Heparan sulfates and protein aggregation

Several studies have shown that the kinetics of protein aggregation is catalysed, accelerated, or potentiated, by polyanionic molecules, including GAGs, particularly HS and heparin [19,32]. Heparin, the prototype of highly sulfated sequences in HS, has shown to stabilize the aggregated state of acyl phosphatase, a classic model for the study of amyloidosis [33]. Similarly, HS highly promoted the capacity of production of amyloids by the serum amyloid A (SAA) protein [34]. Indeed, several studies have shown that HS can be involved in the protein aggregation process by favoring a faster unfolding and induction of a fibrillogenic parallel path [32]. Accordingly, the analysis of tissues affected by amyloid disorders in brain and in peripheral organs, including Alzheimer's disease, type II diabetes, light chain amyloidosis, and prion diseases, among others, has revealed the presence of a significant amount of HS in the amyloid fibril deposits [35–38]. Besides, numerous other evidences indicate that these polysaccharides play an active role favoring the formation and stabilization of amyloid fibers in brain [11,39], and that they could act through a mechanism substantially different from that occurring in solution [40,41]. Available data suggest that HS can: (a) promote the folding of proteins or peptides to conformations allowing the formation of preamyloid structures [18,19,32,34]; (b) act as molecular platforms for monomeric protein or peptides self-assembly, thus increasing the formation and the density of nucleation seeds [33,40,42]; (c) act as molecular platforms for oligomers assembly, increasing the formation and stability of amyloid fibrils [43–45]; and (d) act as platforms allowing post-translational modifications that can then support subsequent amyloid formation [5]. In the later

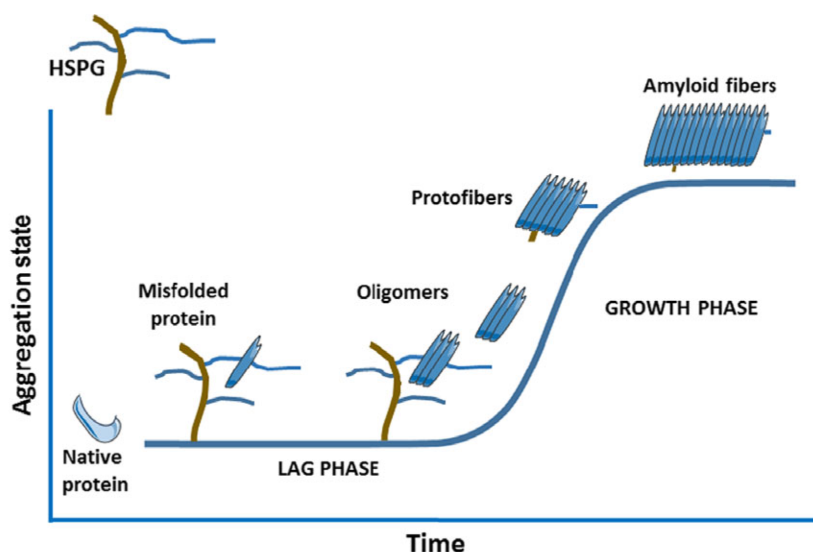

Fig. 2. Schematic representation of the kinetics of an amyloid fiber formation involving HS favored nucleation. This model involves the initial formation of oligomeric complexes that slowly evolve into fibrils followed by amyloid formation. HS chains can participate in both the nucleation or latency phase ('lag phase'), and in the polymerization phase (growth phase). In the first stage, they allow the formation of a nucleus of monomers, and in the second phase they promote a rapid extension of protofibrils and formation of amyloid fibrils [15,42,89].

stages of the amyloidosis, HS could also catalyse the lateral aggregation of small fibers, promoting their insolubility and resistance against proteolysis [19,20,42]. These and other observations suggest that HS can play a central role in the pathologic amyloidogenic processes *in vivo*. The various HS possible roles in protein aggregation are summarized in Fig. 3. Moreover, HS could also be protective thanks to their involvement in the conversion of soluble proteotoxic particles in less toxic amyloid fibers [10].

Although by definition HS are located in the extracellular space where they exert their known biological roles, it is important to remark that, in the disease tissues, these polysaccharides are present in amyloid deposits not only outside [36,39,46], but also inside cells [5,6,38,47]. This suggests that, *in vivo*, HS can be centrally involved in the protein aggregation processes occurring outside and inside cells. However, further research is required to investigate whether the presence of HS in protein aggregates *in vivo* is the result of protective or deleterious processes operating during disease, particularly in pathologies as Alzheimer's, Parkinson, and Prion diseases.

Heparan sulfates in Alzheimer's disease

Alzheimer's disease hallmarks

Alzheimer's disease is a slowly evolving brain disease recognized as the main cause of dementia in the world [48]. Alzheimer's disease is characterized by two types of brain lesions, extracellular accumulation of amyloid plaques made of Aβ peptides, and intraneuronal accumulation of neurofibrillary tangles made of the abnormally phosphorylated protein tau (P-tau) [48]. Since it has been recognized that both the accumulation of Aβ peptides in the extracellular matrix and of P-tau inside neurons are critical events in the development and evolution of Alzheimer's disease, and because HS have been found in these protein aggregates in the disease brain [5,36,45], one can consider that HS could play a critical role in the mechanisms leading to protein aggregation in Alzheimer's disease.

Heparan sulfates and Aβ pathology in Alzheimer's disease

The implication of HS in the formation of Aβ deposits in Alzheimer's disease was originally suggested by Snow *et al.* [47], who found the sulfated polysaccharide in amyloid plaques of Alzheimer's disease brains. It has been largely reported that HS can efficiently interact with Aβ peptides, inducing their aggregation [43,45]. Aβ peptides are 40 (Aβ40) or 42 (Aβ42) amino acids fragments derived from the sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases, enzymatic complexes that contain presenilins 1 (PSN1) and 2 (PSN2). The presence of autosomal dominant mutations in the genes coding for APP, PSN1, or PSN2 are known to cause hereditary Alzheimer's disease (<5% of cases). Interestingly, the Aβ42

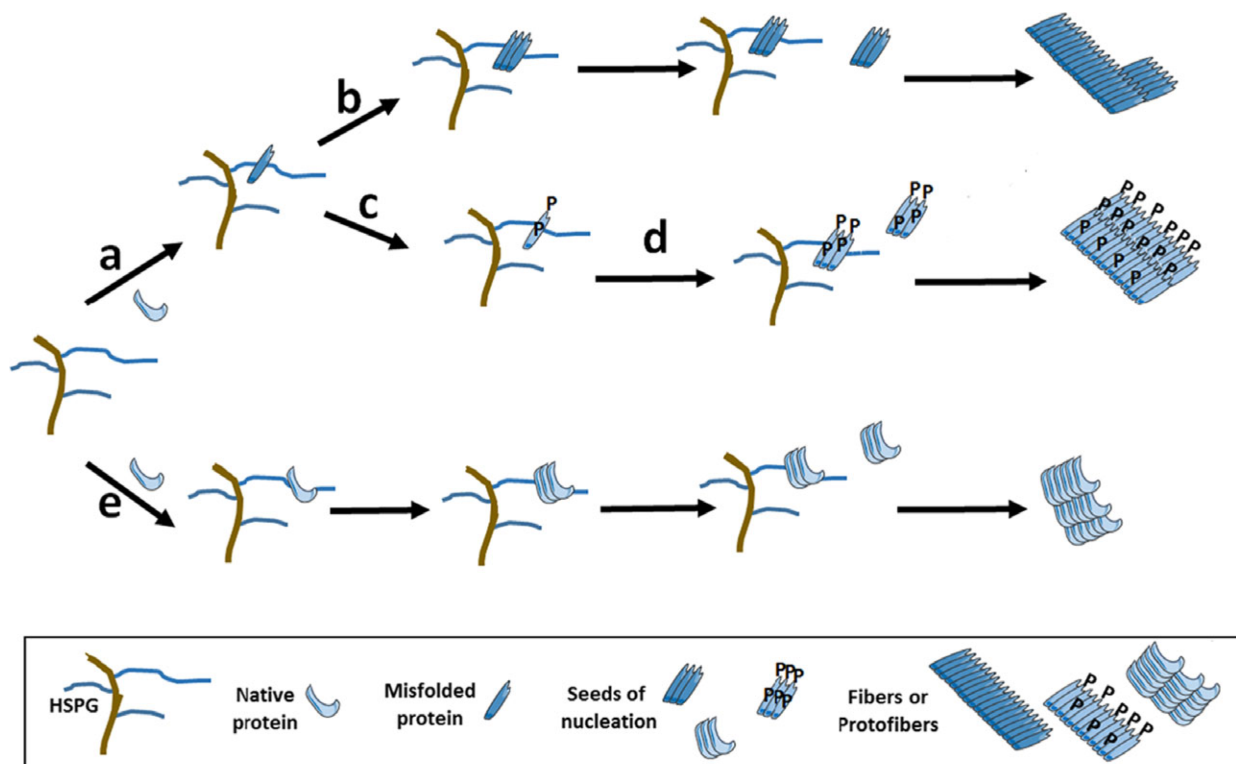

Fig. 3. Putative mechanisms involving HS in the *in vivo* formation of amyloid fibrils. HS can induce misfolding of the native protein (a) resulting in its nucleation and aggregation (b). HS can function as platforms for protein conformational changes allowing attack by kinases, as those responsible for abnormal phosphorylation (c), and promote the phosphorylated protein aggregation (d). HS could directly favor the nucleation process of already conveniently conformed proteins or peptides (e) resulting in the formation of preamyloid structures and thus increasing the number of nucleation seeds that can act as structural platforms for self-assembly.

peptide shows higher aggregation kinetics and higher toxicity than Aβ40 [49]. Accordingly, an increased Aβ42/Aβ40 ratio in Alzheimer's disease brain was associated with genetic forms of the disease [50]. The importance of HS in the formation of Aβ amyloids *in vivo* and *in vitro* has been highlighted by several studies. For instance, transgenic mice overexpressing heparanase showed a decreased number of Aβ amyloid plaques, without alteration on the production and proportion of Aβ40 and Aβ42 peptides [51]. Although residues 12–18 (VHHQKLV) in Aβ40 and Aβ42 are reported as the site of interaction with HS [52], the anionic bridge between lysine 28 and alanine 42, only present in the Aβ42, was broken by HS [44]. In the absence of HS, this bridge stabilizes an *S*-shaped structure formed by three folded β sheets. Upon interactions with HS, this *S*-shaped structure is altered, causing the acceleration of the aggregation process [53]. This would not occur with the Aβ40 peptide, whose loop-like structure is stabilized only by two folded β sheets, thus less susceptible to the aggregation process induced by HS. This can justify the different aggregation kinetics of the two peptides. Likewise, it has been shown that highly sulfated HS accumulate in both Aβ40 and Aβ42 amyloids, while lowly sulfated HS only accumulate in Aβ40 amyloids [53]. This suggests that high sulfation of HS could be required for prompting the aggregation of the Aβ42 peptide in the Alzheimer's brain. Accordingly, the interaction of HS with Aβ amyloid fibrils essentially requires *N*- and 2-*O*-sulfation, while the interaction of HS with the Aβ monomers additionally requires 6-*O*-sulfate groups [54]. Moreover, it has been shown that the content and position of the sulfate groups, as well as the length of the sulfated sequences in the HS chain, can directly affect the secondary structure of Aβ peptides [55], comforting the hypothesis that HS could be involved in the Aβ aggregation process in the disease brain. Although the exact mechanism by which HS could drive the Aβ aggregation *in vivo* remains to be established, some possibilities proposed in Fig. 3 could be considered. Interestingly, the neuroprotective effect of HS and of HS analogues against Aβ oligomers toxicity in neuronal cell cultures has been demonstrated

[56–58]. However, any effect with this kind of molecules has been observed in the clinical evolution of the pathology. Indeed, this is also true for any other strategy aiming to inhibit the formation and accumulation of Aβ oligomers and/or fibrils in the disease brain [59], questioning the hypothesis of the central role of Aβ in Alzheimer's disease pathogenesis. Thus, other events, as those involving tau protein aggregation and spreading are currently being considered as critical factors in the disease.

Heparan sulfates and tau pathology

The microtubule associated protein tau (MAPT), or tau, is a protein that participates in microtubule and neuronal cytoskeleton stabilization and in axonal transport [60]. Under physiological conditions, tau is a highly soluble protein that shows no tendency to aggregate. However, in the brain of patients affected by Alzheimer's disease, this protein is found aggregated in an abnormally phosphorylated form (P-tau) prone to aggregate. In the pathological brain, P-tau forms paired helical filaments that accumulate inside neurons and grow into neurofibrillary tangles, characteristic of Alzheimer's disease and other tauopathies [61,62]. Interestingly, *in vitro*, the tau aggregation is not possible without the incorporation of polyanionic molecules, such as heparin or HS, suggesting that HS might be involved in the mechanism leading to tau protein aggregation *in vivo*. This assumption is reinforced by the study of Snow *et al*., showing that highly sulfated HS accumulate with neurofibrillary tangles in the affected neurons of the disease brain [47]. Moreover, the kinetic constants characterizing the formation of tau fibrils in the presence or absence of heparin agrees with a central role of HS in the tau amyloidogenic process. Accordingly, *in vitro*, heparin can interact with two tau molecules forming a dimer able to form fine short fibrils [63]. However, an excess of heparin can also delay the tau aggregation lag phase, suggesting a modulatory role during the nucleation phase [64]. Interestingly, protein aggregates in the Alzheimer's brain principally contains P-tau, rather than normal tau, suggesting that *in vivo* the aggregation process mainly implicates the phosphorylated protein. This should be considered in current and future models and hypothesis considering tau aggregation mechanisms *in vivo*. In the disease brain, abnormal phosphorylation of tau results from the action of several kinases, which generate the characteristic Alzheimer's disease P-tau epitopes [65]. Interestingly, these P-tau epitopes cannot be obtained *in vitro* unless heparin is added to the kinase phosphorylation reaction mixture [5,66]. This suggests that in the Alzheimer's brain, HS can participate not only to the tau aggregation processes, but also to its abnormal phosphorylation, which might precede or concomitantly occurs with its aggregation (Fig. 3; pathway a-c). Moreover, beyond the potential role that HS seems to play in the processes of tau phosphorylation and aggregation, these polysaccharides have also shown to play a central role in the propagation of tau proteopathic particles (or proteopathic seeds) from one cell to another, a phenomenon known as spreading [67]. Indeed, in early Alzheimer's disease, tauopathy is detected in certain brain regions while, as the disease progresses, tauopathy appears in other regions [68]. Although it is not yet known how the transfer of proteopathic seeds occurs between cells and brain regions, the hypothesis according to which the propagation mechanism would be comparable to the one operating during the propagation of prions has been proposed [69]. Interestingly, the tau proteopathic seeds uptake by healthy neurons has been shown to be mediated by the interaction of these particles with cell membrane-associated HSPG [67]. Altogether, these works suggest that HS are involved in the cellular mechanisms leading to tau misfolding, phosphorylation, aggregation, and spreading in the Alzheimer's brain.

Recent studies aiming to better understand the importance of HS structures in Alzheimer's disease are currently advancing. For instance, we recently proposed the central implication of HS, and more particularly of 3-*O*-sulfated HS sequences, in the development of Alzheimer's disease-related tauopathy [5]. Moreover, more recent works have additionally shown that 6-*O*-sulfate groups in HS are centrally required to establish the interaction of HS with tau [70]. Interestingly, while 6-*O*-sulfation stands as one of the main modifications in the HS chains, 3-*O*-sulfation remains rare [71]. Thus, this is not surprising that avoiding 6-*O*-sulfation result in the highest loss of HS interactions with tau. Although these studies are already giving important insights into the complex structure of the HS sequences required for their interactions with tau in the Alzheimer's brain, it must be considered that the HS domain can carry sulfate at the different sugar positions, and that the sulfation pattern, rather than one unique sulfation position, can be of central importance in the HS interaction with tau. Moreover, the interaction domain in HS result from complex biosynthetic pathways involving several not only sulfotransferases, but also glycosyl transferases (EXTs and EXTLs), epimerase, heparanase and sulfatases (Sulfs), some of them could then play important roles in the production of the HS sequences

involved in tauopathy development and progression in the disease brain.

Glycosaminoglycans and Parkinson's disease

Parkinson's disease is a movement disorder in which dopaminergic neurons fail to produce dopamine due to their entry into a neuronal death process [72]. Some cases are genetic, but most are considered sporadic. Pathologically, Parkinson's disease is characterized by the accumulation of protein aggregates, called Lewy bodies, in neuronal cells. Lewy bodies are mainly formed by the fibrillated protein α-synuclein [73], characteristic of α-synucleinopathies. Although the physiological role of this protein has not yet been clarified, its central implication in the pathophysiology of Parkinson's disease is well-accepted [73,74]. Accordingly, Lewy bodies are observed in transgenic animals in which a mutation in the α-synuclein gene reproduces the dominant autosomal form observed in hereditary Parkinson's disease [73]. However, α-synuclein aggregates also accumulate in sporadic forms of the disease, in which no mutation has been identified. To date, the origin of the α-synuclein aggregation remains undetermined. Structurally, α-synuclein is a highly conserved 14 kDa protein abundant in distinct neurons and in the presynaptic compartment. In humans, three isoforms (112, 126, or 140 amino acids) produced by alternative splicing are known [75,76]. The central region of this protein, formed by residues 61–95, comprises an area highly prone to aggregation, while the C-terminal domain (residues 96–140) protects the protein from aggregation [75]. The great structural plasticity of α-synuclein allows it to adopt various conformations and gives to the protein an important tendency to unfold and to form profibrillar oligomers and amyloid fibrils [76]. Interestingly, although the main constituent of Lewy bodies is α-synuclein [73], other molecules such as HS are present in the protein aggregates, suggesting that the sulfated polysaccharides can play roles in the aggregation process [77]. Depending on their level of sulfation and on the position of the sulfate groups in the polysaccharidic chain, HS have shown to differentially stimulate the formation of α-synuclein fibrils through interactions with the *N*-terminal domain of the protein [77], which can possibly promote the protein aggregation by different pathways (Fig. 3). However, this is still controversial [78,79], possibly because of the current lack of widely available tools allowing the study of HS in biological contexts. In the other hand, indirect interactions between HS and proteins that are not directly involved in the neurodegenerative process, but that can indirectly influence it, have been reported. For instance, the interaction of α-synuclein with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) oligomers formed in contact with heparin has been proposed as a protective mechanism against α-synuclein oligomers toxicity [80]. Moreover, as other GAGs, HS can readily potentiate the aggregation kinetics of α-synuclein *in vitro*. Although these observations suggest the possible participation of HS in the process of intracellular aggregation of α-synuclein in Parkinson's disease, research in this area is still limited and the number of groups able to combine glycobiologic approaches to the study of the general mechanisms that operate in the disease remains scarce. Advances in this direction will further improve our understanding of the role of these sulfated polysaccharides in this pathology.

Heparan sulfates and prion diseases

The cellular prion protein (PrP^C) is a GPI-cell membrane anchored glycoprotein, which can acquire an abnormal conformation responsible of transmissible spongiform encephalopathies (TSE), known as prion diseases [81]. These fatal neurodegenerative diseases include scrapie, bovine spongiform encephalopathy, Gerstmann-Sträussler-Scheinker syndrome, Creutzfeldt-Jakob disease, and its humans variant, among others. The key event in prion diseases is the conversion of PrP^C into the abnormally conformed form called PrP^{SC}. The change in conformation of PrP^C in PrP^{SC} causes the aggregation of the misfolded form and its pathological accumulation as PrP^{SC} amyloid fibrils called 'prions' [81]. A number of studies have shown several PrP strains, multiple states of PrP^{SC} aggregation (oligomeric, prefibrillar, and fibrillar), and different capacities of the aggregates to deposit in different brain regions. Interestingly, it has been demonstrated that the conversion of PrP^C into PrP^{SC} occurs in a cell microenvironment requiring the presence of HSPG at the outer cellular membrane. HSPG probably act as platforms allowing the conformational change from PrP^C into PrP^{SC} (Fig. 3; pathway a-b) [40]. *In vivo*, HS are present in the prion amyloid plaques [36], in accord with their putative role in the transformation of PrP^C to PrP^{SC} and with their participation to the subsequent PrP^{SC} aggregation (Fig. 3; pathway a-b). Accordingly, certain sulfated polyanions mimicking HS have shown prophylactic effects in TSE cellular and animal models [82,83]. Interestingly, some of these studies have shown a concentration dependent contradictory polyanion effect [84,85], suggesting that the HS effect in amyloid formation might also be dependent on the polyanion

concentration present in the biological environment where protein aggregation takes place. Moreover, since some studies have shown that the various PrP^{SC} strains can differently be deposed in the different brain regions [86,87], further investigations are required to explore the effect of the particular HS structures, produced at the different regions, in the formation and accumulation of the various PrP^{SC} strains. This hypothesis is supported by the differential expression of the HS biosynthetic machinery in the different brain regions [88]. This glycobiology-based domain of research has not yet been explored and remains an open question in neurodegeneration.

Heparan sulfates in other neurodegenerative diseases

The molecular mechanisms leading to protein aggregation characteristic of proteinopathies are not yet well-understood. However, HS have been found in most, if not all, protein inclusions characterizing these diseases. Although this review has focused on Alzheimer's, Parkinson's, and prion diseases, HS also accumulate with protein deposits in other neurodegenerative diseases. Further and extensive studies will be necessary to investigate whether and how HS, or other sulfated GAGs, can be involved in the molecular mechanisms responsible of protein aggregation and deposition in the different neurodegenerative diseases (Table 1), and how GAGs could selectively be involved in the specific brain region vulnerability to amyloid deposition in those diseases.

Conclusion

Sulfated GAGs, and particularly HS, coaccumulate with protein inclusions characteristic of neurodegenerative diseases including Alzheimer's, Parkinson's, and prion diseases. Although previous and emerging data suggest a critical role of HS in the kinetics of aggregation of most, if not all, amyloidogenic proteins, in the modulation of their post-translational modifications, and in the promotion of proteopathic seeds cellular uptake and toxicity, it is not yet clear whether and how these complex polysaccharides can influence the pathways leading to protein deposition inside and outside cells. Identification of the HS biosynthetic pathways producing particular HS structures in each brain region, and how these structures could differently affect protein aggregation and deposition in each particular region, will allow a better understanding of the glycobiology-related mechanisms leading to proteinopathies in brain.

Acknowledgments

Authors are thankful the 'Fondation Vaincre Alzheimer' for supporting A. Maïza (No. FR-15055), to the European Union FET OPEN RIA H2020 program for supporting the ArrestAD project (grant number 737390) that studies the involvement of HS in Alzheimer's disease, and to GDR GAGoSciences (No. 3739) for supporting GAGs research in France. Authors thank the CRRET GAGs group for their strong involvement in Alzheimer's disease research. We acknowledge *Revista de Neurología* for giving its accord to publish part of the original work published in Spanish in Issue 65, pages 457-468 (PMID: 29130469).

References

1 Bayer TA (2015) Proteinopathies, a core concept for understanding and ultimately treating degenerative disorders? *Eur Neuropsychopharmacol* **25**, 713–724.
2 Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R and Eisenberg D (2005) Structure of the cross-beta spine of amyloid-like fibrils. *Nature* **435**, 773–778.
3 Dobson CM (2017) The amyloid phenomenon and its links with human disease. *Cold Spring Harb Perspect Biol* **9**, 6; pii: a023648.
4 Goedert M (2015) Neurodegeneration. Alzheimer's and Parkinson's diseases: the prion concept in relation to assembled Abeta, tau, and alpha-synuclein. *Science* **349** (6248), 1255555.
5 Sepulveda-Diaz JE, Alavi Naini SM, Huynh MB, Ouidja MO, Yanicostas C, Chantepie S, Villares J, Lamari F, Jospin E, van Kuppevelt TH *et al.* (2015) HS3ST2 expression is critical for the abnormal phosphorylation of tau in Alzheimer's disease-related tau pathology. *Brain* **138**, 1339–1354.
6 Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ and Crowther RA (1996) Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature* **383**, 550–553.
7 Hasegawa M, Crowther RA, Jakes R and Goedert M (1997) Alzheimer-like changes in microtubule-associated protein Tau induced by sulfated glycosaminoglycans. Inhibition of microtubule binding, stimulation of phosphorylation, and filament assembly depend on the degree of sulfation. *J Biol Chem* **272**, 33118–33124.
8 Kampers T, Friedhoff P, Biernat J, Mandelkow EM and Mandelkow E (1996) RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett* **399**, 344–349.

9 Wilson DM and Binder LI (1997) Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol* **150**, 2181–2195.
10 Iannuzzi C, Irace G and Sirangelo I (2015) The effect of glycosaminoglycans (GAGs) on amyloid aggregation and toxicity. *Molecules* **20**, 2510–2528.
11 Papy-Garcia D, Christophe M, Huynh MB, Fernando S, Ludmilla S, Sepulveda-Diaz JE and Raisman-Vozari R (2011) Glycosaminoglycans, protein aggregation and neurodegeneration. *Curr Protein Pept Sci* **12**, 258–268.
12 Gandhi NS and Mancera RL (2008) The structure of glycosaminoglycans and their interactions with proteins. *Chem Biol Drug Des* **72**, 455–482.
13 Li JP and Kusche-Gullberg M (2016) Heparan sulfate: biosynthesis, structure, and function. *Int Rev Cell Mol Biol* **325**, 215–273.
14 Huynh MB, Morin C, Carpentier G, Garcia-Filipe S, Talhas-Perret S, Barbier-Chassefiere V, van Kuppevelt TH, Martelly I, Albanese P and Papy-Garcia D (2012) Age-related changes in rat myocardium involve altered capacities of glycosaminoglycans to potentiate growth factor functions and heparan sulfate-altered sulfation. *J Biol Chem* **287**, 11363–11373.
15 Huynh MB, Villares J, Diaz JE, Christiaans S, Carpentier G, Ouidja MO, Sissoeff L, Raisman-Vozari R and Papy-Garcia D (2012) Glycosaminoglycans from aged human hippocampus have altered capacities to regulate trophic factors activities but not Abeta42 peptide toxicity. *Neurobiol Aging* **33**, 1005.e1011-1022.
16 Negroni E, Henault E, Chevalier F, Gilbert-Sirieix M, Van Kuppevelt TH, Papy-Garcia D, Uzan G and Albanese P (2014) Glycosaminoglycan modifications in Duchenne muscular dystrophy: specific remodeling of chondroitin sulfate/dermatan sulfate. *J Neuropathol Exp Neurol* **73**, 789–797.
17 Sarkar A and Desai UR (2015) A simple method for discovering druggable, specific glycosaminoglycan-protein systems. Elucidation of key principles from heparin/heparan sulfate-binding proteins. *PLoS ONE* **10**, e0141127.
18 Xu D and Esko JD (2014) Demystifying heparan sulfate-protein interactions. *Annu Rev Biochem* **83**, 129–157.
19 Schultz V, Suflita M, Liu X, Zhang X, Yu Y, Li L, Green DE, Xu Y, Zhang F, DeAngelis PL *et al.* (2017) Heparan sulfate domains required for fibroblast growth factor 1 and 2 signaling through fibroblast growth factor receptor 1c. *J Biol Chem* **292**, 2495–2509.
20 Ancsin JB (2003) Amyloidogenesis: historical and modern observations point to heparan sulfate proteoglycans as a major culprit. *Amyloid* **10**, 67–79.
21 Gupta-Bansal R, Frederickson RC and Brunden KR (1995) Proteoglycan-mediated inhibition of A beta proteolysis. A potential cause of senile plaque accumulation. *J Biol Chem* **270**, 18666–18671.
22 Kisilevsky R, Szarek WA, Ancsin JB, Elimova E, Marone S, Bhat S and Berkin A (2004) Inhibition of amyloid A amyloidogenesis in vivo and in tissue culture by 4-deoxy analogues of peracetylated 2-acetamido-2-deoxy-alpha- and beta-d-glucose: implications for the treatment of various amyloidoses. *Am J Pathol* **164**, 2127–2137.
23 Li JP, Galvis ML, Gong F, Zhang X, Zcharia E, Metzger S, Vlodavsky I, Kisilevsky R and Lindahl U (2005) In vivo fragmentation of heparan sulfate by heparanase overexpression renders mice resistant to amyloid protein A amyloidosis. *Proc Natl Acad Sci USA* **102**, 6473–6477.
24 Makin OS and Serpell LC (2002) Examining the structure of the mature amyloid fibril. *Biochem Soc Trans* **30**, 521–525.
25 Kelly JW (1998) The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. *Curr Opin Struct Biol* **8**, 101–106.
26 Serio TR, Cashikar AG, Kowal AS, Sawicki GJ, Moslehi JJ, Serpell L, Arnsdorf MF and Lindquist SL (2000) Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* **289**, 1317–1321.
27 Lee J, Culyba EK, Powers ET and Kelly JW (2011) Amyloid-beta forms fibrils by nucleated conformational conversion of oligomers. *Nat Chem Biol* **7**, 602–609.
28 Wei L, Jiang P, Xu W, Li H, Zhang H, Yan L, Chan-Park MB, Liu XW, Tang K, Mu Y *et al.* (2011) The molecular basis of distinct aggregation pathways of islet amyloid polypeptide. *J Biol Chem* **286**, 6291–6300.
29 Gibson TJ and Murphy RM (2006) Inhibition of insulin fibrillogenesis with targeted peptides. *Protein Sci* **15**, 1133–1141.
30 Bernacki JP and Murphy RM (2009) Model discrimination and mechanistic interpretation of kinetic data in protein aggregation studies. *Biophys J* **96**, 2871–2887.
31 Cecchi C and Stefani M (2013) The amyloid-cell membrane system. The interplay between the biophysical features of oligomers/fibrils and cell membrane defines amyloid toxicity. *Biophys Chem* **182**, 30–43.
32 Motamedi-Shad N, Monsellier E, Torrassa S, Relini A and Chiti F (2009) Kinetic analysis of amyloid formation in the presence of heparan sulfate: faster unfolding and change of pathway. *J Biol Chem* **284**, 29921–29934.
33 Motamedi-Shad N, Monsellier E and Chiti F (2009) Amyloid formation by the model protein muscle

acylphosphatase is accelerated by heparin and heparan sulphate through a scaffolding-based mechanism. *J Biochem* **146**, 805–814.

34 Elimova E, Kisilevsky R and Ancsin JB (2009) Heparan sulfate promotes the aggregation of HDL-associated serum amyloid A: evidence for a proamyloidogenic histidine molecular switch. *FASEB J* **23**, 3436–3448.

35 Naiki H and Nagai Y (2009) Molecular pathogenesis of protein misfolding diseases: pathological molecular environments versus quality control systems against misfolded proteins. *J Biochem* **146**, 751–756.

36 Snow AD, Wight TN, Nochlin D, Koike Y, Kimata K, DeArmond SJ and Prusiner SB (1990) Immunolocalization of heparan sulfate proteoglycans to the prion protein amyloid plaques of Gerstmann-Straussler syndrome, Creutzfeldt-Jakob disease and scrapie. *Lab Invest* **63**, 601–611.

37 Young ID, Ailles L, Narindrasorasak S, Tan R and Kisilevsky R (1992) Localization of the basement membrane heparan sulfate proteoglycan in islet amyloid deposits in type II diabetes mellitus. *Arch Pathol Lab Med* **116**, 951–954.

38 Hernandez F, Perez M, Lucas JJ and Avila J (2002) Sulfo-glycosaminoglycan content affects PHF-tau solubility and allows the identification of different types of PHFs. *Brain Res* **935**, 65–72.

39 Diaz-Nido J, Wandosell F and Avila J (2002) Glycosaminoglycans and beta-amyloid, prion and tau peptides in neurodegenerative diseases. *Peptides* **23**, 1323–1332.

40 Rouvinski A, Karniely S, Kounin M, Moussa S, Goldberg MD, Warburg G, Lyakhovetsky R, Papy-Garcia D, Kutzsche J, Korth C *et al.* (2014) Live imaging of prions reveals nascent PrPSc in cell-surface, raft-associated amyloid strings and webs. *J Cell Biol* **204**, 423–441.

41 Zhu M, Souillac PO, Ionescu-Zanetti C, Carter SA and Fink AL (2002) Surface-catalyzed amyloid fibril formation. *J Biol Chem* **277**, 50914–50922.

42 McLaurin J, Franklin T, Zhang X, Deng J and Fraser PE (1999) Interactions of Alzheimer amyloid-beta peptides with glycosaminoglycans effects on fibril nucleation and growth. *Eur J Biochem* **266**, 1101–1110.

43 Castillo GM, Ngo C, Cummings J, Wight TN and Snow AD (1997) Perlecan binds to the beta-amyloid proteins (A beta) of Alzheimer's disease, accelerates A beta fibril formation, and maintains A beta fibril stability. *J Neurochem* **69**, 2452–2465.

44 Rodriguez RA, Chen LY, Plascencia-Villa G and Perry G (2017) Elongation affinity, activation barrier, and stability of Abeta42 oligomers/fibrils in physiological saline. *Biochem Biophys Res Commun* **487**, 444–449.

45 Snow AD, Sekiguchi R, Nochlin D, Fraser P, Kimata K, Mizutani A, Arai M, Schreier WA and Morgan DG (1994) An important role of heparan sulfate proteoglycan (Perlecan) in a model system for the deposition and persistence of fibrillar A beta-amyloid in rat brain. *Neuron* **12**, 219–234.

46 Bruinsma IB, te Riet L, Gevers T, ten Dam GB, van Kuppevelt TH, David G, Kusters B, de Waal RM and Verbeek MM (2010) Sulfation of heparan sulfate associated with amyloid-beta plaques in patients with Alzheimer's disease. *Acta Neuropathol* **119**, 211–220.

47 Snow AD, Mar H, Nochlin D, Sekiguchi RT, Kimata K, Koike Y and Wight TN (1990) Early accumulation of heparan sulfate in neurons and in the beta-amyloid protein-containing lesions of Alzheimer's disease and Down's syndrome. *Am J Pathol* **137**, 1253–1270.

48 Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S and Van der Flier WM (2016) Alzheimer's disease. *Lancet* **388**, 505–517.

49 Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB and Teplow DB (2003) Amyloid beta -protein (Abeta) assembly: Abeta 40 and Abeta 42 oligomerize through distinct pathways. *Proc Natl Acad Sci USA* **100**, 330–335.

50 Kirkitadze MD and Kowalska A (2005) Molecular mechanisms initiating amyloid beta-fibril formation in Alzheimer's disease. *Acta Biochim Pol* **52**, 417–423.

51 Jendresen CB, Cui H, Zhang X, Vlodavsky I, Nilsson LN and Li JP (2015) Overexpression of heparanase lowers the amyloid burden in amyloid-beta precursor protein transgenic mice. *T J Biol Chem* **290**, 5053–5064.

52 Nguyen K and Rabenstein DL (2016) Interaction of the heparin-binding consensus sequence of beta-amyloid peptides with heparin and heparin-derived oligosaccharides. *J Phys Chem B* **120**, 2187–2197.

53 Zhang GL, Zhang X, Wang XM and Li JP (2014) Towards understanding the roles of heparan sulfate proteoglycans in Alzheimer's disease. *Biomed Res Int* **2014**, 516028.

54 Lindahl B, Westling C, Gimenez-Gallego G, Lindahl U and Salmivirta M (1999) Common binding sites for beta-amyloid fibrils and fibroblast growth factor-2 in heparan sulfate from human cerebral cortex. *J Biol Chem* **274**, 30631–30635.

55 Madine J, Clayton JC, Yates EA and Middleton DA (2009) Exploiting a (13)C-labelled heparin analogue for in situ solid-state NMR investigations of peptide-glycan interactions within amyloid fibrils. *Org Biomol Chem* **7**, 2414–2420.

56 Woods AG, Cribbs DH, Whittemore ER and Cotman CW (1995) Heparan sulfate and chondroitin sulfate glycosaminoglycan attenuate beta-amyloid(25-35) induced neurodegeneration in cultured hippocampal neurons. *Brain Res* **697**, 53–62.

57 Bravo R, Arimon M, Valle-Delgado JJ, Garcia R, Durany N, Castel S, Cruz M, Ventura S and Fernandez-Busquets X (2008) Sulfated polysaccharides promote the assembly of amyloid beta(1-42) peptide into stable fibrils of reduced cytotoxicity. *J Biol Chem* **283**, 32471–32483.

58 Bergamaschini L, Rossi E, Storini C, Pizzimenti S, Distaso M, Perego C, De Luigi A, Vergani C and De Simoni MG (2004) Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. *J Neurosci* **24**, 4181–4186.

59 Armstrong RA (2014) A critical analysis of the 'amyloid cascade hypothesis'. *Folia Neuropathol* **52**, 211–225.

60 Guo T, Noble W and Hanger DP (2017) Roles of tau protein in health and disease. *Acta Neuropathol* **133**, 665–704.

61 Iqbal K, Wang X, Blanchard J, Liu F, Gong CX and Grundke-Iqbal I (2010) Alzheimer's disease neurofibrillary degeneration: pivotal and multifactorial. *Biochem Soc Trans* **38**, 962–966.

62 Konno T, Oiki S, Hasegawa K and Naiki H (2004) Anionic contribution for fibrous maturation of protofibrillar assemblies of the human tau repeat domain in a fluoroalcohol solution. *Biochemistry* **43**, 13613–13620.

63 Ramachandran G and Udgaonkar JB (2011) Understanding the kinetic roles of the inducer heparin and of rod-like protofibrils during amyloid fibril formation by Tau protein. *J Biol Chem* **286**, 38948–38959.

64 Zhu HL, Fernandez C, Fan JB, Shewmaker F, Chen J, Minton AP and Liang Y (2010) Quantitative characterization of heparin binding to Tau protein: implication for inducer-mediated Tau filament formation. *J Biol Chem* **285**, 3592–3599.

65 Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin ML, Yardin C and Terro F (2013) Tau protein kinases: involvement in Alzheimer's disease. *Ageing Res Rev* **12**, 289–309.

66 Paudel HK and Li W (1999) Heparin-induced conformational change in microtubule-associated protein Tau as detected by chemical cross-linking and phosphopeptide mapping. *J Biol Chem* **274**, 8029–8038.

67 Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP *et al.* (2013) Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci USA* **110**, E3138–E3147.

68 Goedert M and Spillantini MG (2017) Propagation of tau aggregates. *Mol Brain* **10**, 18.

69 Frost B and Diamond MI (2010) Prion-like mechanisms in neurodegenerative diseases. *Nat Rev Neurosci* **11**, 155–159.

70 Zhao J, Huvent I, Lippens G, Eliezer D, Zhang A, Li Q, Tessier P, Linhardt RJ, Zhang F and Wang C (2017) Glycan determinants of heparin-tau interaction. *Biophys J* **112**, 921–932.

71 Thacker BE, Xu D, Lawrence R and Esko JD (2014) Heparan sulfate 3-O-sulfation: a rare modification in search of a function. *Matrix Biol* **35**, 60–72.

72 Przedborski S (2017) The two-century journey of Parkinson disease research. *Nat Rev Neurosci* **18**, 251–259.

73 Nussbaum RL (2017) The identification of alpha-synuclein as the first Parkinson disease gene. *J Parkinsons Dis* **7**, S45–S51.

74 Bourdenx M, Dehay B and Bezard E (2014) Down-regulating alpha-synuclein for treating synucleopathies. *Mov Disord* **29**, 1463–1465.

75 Beyer K (2006) Alpha-synuclein structure, posttranslational modification and alternative splicing as aggregation enhancers. *Acta Neuropathol* **112**, 237–251.

76 Uversky VN (2007) Neuropathology, biochemistry, and biophysics of alpha-synuclein aggregation. *J Neurochem* **103**, 17–37.

77 Cohlberg JA, Li J, Uversky VN and Fink AL (2002) Heparin and other glycosaminoglycans stimulate the formation of amyloid fibrils from alpha-synuclein in vitro. *Biochemistry* **41**, 1502–1511.

78 van Horssen J, de Vos RA, Steur EN, David G, Wesseling P, de Waal RM and Verbeek MM (2004) Absence of heparan sulfate proteoglycans in Lewy bodies and Lewy neurites in Parkinson's disease brains. *J Alzheimers Dis* **6**, 469–474.

79 Liu IH, Uversky VN, Munishkina LA, Fink AL, Halfter W and Cole GJ (2005) Agrin binds alpha-synuclein and modulates alpha-synuclein fibrillation. *Glycobiology* **15**, 1320–1331.

80 Torres-Bugeau CM, Avila CL, Raisman-Vozari R, Papy-Garcia D, Itri R, Barbosa LR, Cortez LM, Sim VL and Chehin RN (2012) Characterization of heparin-induced glyceraldehyde-3-phosphate dehydrogenase early amyloid-like oligomers and their implication in alpha-synuclein aggregation. *J Biol Chem* **287**, 2398–2409.

81 Annus A, Csati A and Vecsei L (2016) Prion diseases: new considerations. *Clin Neurol Neurosurg* **150**, 125–132.

82 Larramendy-Gozalo C, Barret A, Daudigeos E, Mathieu E, Antonangeli L, Riffet C, Petit E, Papy-Garcia D, Barritault D, Brown P *et al.* (2007) Comparison of CR36, a new heparan mimetic, and pentosan polysulfate in the treatment of prion diseases. *J Gen Virol* **88**, 1062–1067.

83 Ouidja MO, Petit E, Kerros ME, Ikeda Y, Morin C, Carpentier G, Barritault D, Brugere-Picoux J, Deslys JP, Adjou K *et al*. (2007) Structure-activity studies of heparan mimetic polyanions for anti-prion therapies. *Biochem Biophys Res Commun* **363**, 95–100.

84 Fontaine SN and Brown DR (2009) Mechanisms of prion protein aggregation. *Protein Pept Lett* **16**, 14–26.

85 Muras AG, Hajj GN, Ribeiro KB, Nomizo R, Nonogaki S, Chammas R and Martins VR (2009) Prion protein ablation increases cellular aggregation and embolization contributing to mechanisms of metastasis. *Int J Cancer* **125**, 1523–1531.

86 Privat N, Levavasseur E, Yildirim S, Hannaoui S, Brandel JP, Laplanche JL, Beringue V, Seilhean D and Haik S (2017) Region-specific protein misfolding cyclic amplification reproduces brain tropism of prion strains. *J Biol Chem* **292**, 16688–16696.

87 Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, Sharma AM, Miller TM and Diamond MI (2016) Tau prion strains dictate patterns of cell pathology, progression rate, and regional vulnerability in vivo. *Neuron* **92**, 796–812.

88 Yabe T, Hata T, He J and Maeda N (2005) Developmental and regional expression of heparan sulfate sulfotransferase genes in the mouse brain. *Glycobiology* **15**, 982–993.

89 Quittot N, Sebastiao M and Bourgault S (2017) Modulation of amyloid assembly by glycosaminoglycans: from mechanism to biological significance. *Biochem Cell Biol* **95**, 329–337.

90 Kalaria RN (2017) The pathology and pathophysiology of vascular dementia. *Neuropharmacology*. pii: S0028-3908(17)30627-5.

91 Polanco JC, Li C, Bodea LG, Martinez-Marmol R, Meunier FA and Gotz J (2018) Amyloid-beta and tau complexity – towards improved biomarkers and targeted therapies. *Nat Rev Neurol* **14**, 22–39.

92 Braak H and Del Trecidi K (2015) Neuroanatomy and pathology of sporadic Alzheimer's disease. *Adv Anat Embryol Cell Biol* **215**, 1–162.

93 Eller M and Williams DR (2011) α-Synuclein in Parkinson disease and other neurodegenerative disorders. *Clin Chem Lab Med* **49**, 403–408.

94 Poggiolini I, Saverioni D and Parchi P (2013) Prion protein misfolding, strains, and neurotoxicity: an update from studies on Mammalian prions. *Int J Cell Biol* **2013**, 910314.

95 Koyuncu S, Fatima A, Gutierrez-Garcia R and Vilchez D (2017) Proteostasis of huntingtin in health and disease. *Int J Mol Sci* **18**, pii: E1568.

96 Sangwan S and Eisenberg DS (2016) Perspective on SOD1 mediated toxicity in Amyotrophic Lateral Sclerosis. *Postepy Biochem* **62**, 362–369.

97 Trott A and Houenou LJ (2012) Mini-review: spinocerebellar ataxias: an update of SCA genes. *Recent Pat DNA Gene Seq* **6**, 115–121.

98 Tsuji S (2012) Dentatorubral-pallidoluysian atrophy. *Handb Clin Neurol* **103**, 587–594.

99 Palsdottir A, Snorradottir AO and Thorsteinsson L (2006) Hereditary cystatin C amyloid angiopathy: genetic, clinical, and pathological aspects. *Brain Pathol* **16**, 55–59.

100 Ghiso J, Revesz T, Holton J, Rostagno A, Lashley T, Houlden H, Gibb G, Anderton B, Bek T, Bojsen-Moller M *et al*. (2001) Chromosome 13 dementia syndromes as models of neurodegeneration. *Amyloid* **8**, 277–284.

101 Kuratsu J, Matsukado Y and Miura M (1983) Prolactinoma of pituitary with associated amyloid-like substances. Case report. *J Neurosurg* **59**, 1067–1070.

102 Rusmini P, Crippa V, Cristofani R, Rinaldi C, Cicardi ME, Galbiati M, Carra S, Malik B, Greensmith L and Poletti A (2016) The role of the protein quality control system in SBMA. *J Mol Neurosci* **58**, 348–364.

103 Arciello A, Piccoli R and Monti DM (2016) Apolipoprotein A-I: the dual face of a protein. *FEBS Lett* **590**, 4171–4179.

104 Ueda M and Ando Y (2014) Recent advances in transthyretin amyloidosis therapy. *Transl Neurodegener* **3**, 19.

105 Gasperini RJ and Small DH (2012) Neurodegeneration in familial amyloidotic polyneuropathy. *Clin Exp Pharmacol Physiol* **39**, 680–683.