Protein Aggregation in Neurodegenerative Diseases

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Definition

Here we summarize the heterogeneity of structures that are produced from intrinsically disordered protein domains and highlight the routes that lead to the formation of physiological liquid droplets as well as pathogenic aggregates. The most common proteins found in aggregates in neurodegenerative diseases and their structural variability will be addressed.
We will further evaluate the clinical relevance and future applications of the study of the structural heterogeneity of protein aggregates, which may aid the understanding of the phenotypic diversity observed in neurodegenerative disorders.

1. Introduction

The correct function of living organisms depends on the concerted effort of a network of thousands of proteins [1][2][3], which are required to assume a defined structure to exert their function. However, intrinsically disordered regions (IDRs), found in most eukaryotic proteins [4][5][6], appear to show no preference for chaperone binding [7]. Thus, proteins mostly or entirely composed of IDRs, defined as intrinsically disordered proteins (IDPs), may escape the classical folding pathway and maintain a metastable and plastic state. A subset of IDRs termed low complexity domains (LCDs), found in up to 1.2% of the protein-coding human genes [8], is gaining attention due to its presence in aggregated state in most neurodegenerative disorders.

In this review, we will first recapitulate how intrinsic disorder determines the formation of a variety of structures and produces different states of the matter, owning both physiological and pathological features. Next, we will provide evidence of structural variability observed in the proteins most commonly associated with neurodegeneration, specifically the prion protein (PrP), amyloid β-peptides (Aβ), tau, α-synuclein and TAR DNA binding protein 43 (TDP-43). As a caveat, we will focus on the wild-type sequence of these proteins, as most neurodegenerative diseases are sporadic in nature [9]. The clinical relevance and future applications of the study of the structural variability of protein aggregates will be addressed, as it may aid the understanding of the phenotypic diversity observed in human neurodegenerative disorders.

2. Intrinsic Disorder

The presence of IDRs appears to correlate with the complexity of the living organism [10][11], with half of eukaryotic proteins expected to possess at least one IDR in their sequence [4][5][12]. A plethora of biological functions are associated with IDRs [5][6][13], spanning from stress response [14] to high-order assembly [15] to RNA metabolism [16][17][18]. A further level of variability resides in the ability of IDRs to assume non-native states of globular proteins such as the molten-globule or the coiled-coil state [5][6][19][20]. Being metastable and displaying weak and transient interactions [21][22], IDRs may change their structure in time, fluctuating among different low energetic levels.

Intrinsic disorder is encoded by the composition of the sequence rather than the exact string of amino acids [23][24]. Low mean hydrophobicity and high net charge have been reported to increase the disorder and reduce the solubility by reducing compaction and increasing electrostatic repulsion [24][25][26].

Therefore, it is apparent that the lack of structural arrangement may be achieved through different compositions and features, ultimately converging in the ability to coalesce into higher order assemblies.

Overall, low complexity offers another angle to achieve the preservation of the unfolded state, increasing the probability of forming β-structures and allowing for the formation of homomeric and heteromeric assemblies. Moreover, polymerization into supramolecular structures may differ in the state of the matter, implying different routes of aggregation.

3. PrP: The Protein That Started It All

Historically, the concept of the self-propagation and infectivity of a protein was postulated for the scrapie agent, a factor causing neurodegeneration in sheep[27]. The scrapie pathology showed remarkably similar features both to Creutzfeldt–Jakob Disease (CJD) and Kuru. The former is a rare neurodegenerative disease while the latter was an endemic neurodegeneration affecting the Fore tribe in the highlands of Papua New Guinea linked to their cannibalistic rituals[28]. Both Kuru and CJD were shown to be transmissibleto chimpanzees and hamsters[29]. The term “prion” (proteinaceous infectious particle, PrP) was proposed for the scrapie pathology as sole causative agent of the disease. Biochemically, the prion derived from scrapie infected hamsters showed an unusual resistance to non-denaturing detergents and to proteinase K,resulting in 27–30 kDa bands after electrophoresis. the now widely accepted “protein only” hypothesis was proposed for the self-replication of the PrP[30]: its propagation requires the formation of a homotypic complex between the two molecules[31], causing the conversion of the native cellular form (PrP-C) into the pathogenic conformation. Outside of Kuru and CJD, other human pathologies termed transmissible spongiform encephalopathies have been linked to the misfolding of PrP[32], namely the variant CJD (the “mad cow” disease)[33], Gerstmann–Sträussler-Scheinker disease[34], fatal familial insomnia[35] and variably protease-sensitive prionopathy[36].
Overall, the classical studies conducted to delve into the peculiar properties of PrP paved the way for the central role of protein misfolding and transmission in neurodegenerative diseases.

4. The Curious Case of Aβ

The Aβ peptides are small amino acidic chains derived from the proteolytic process of the larger amyloid precursor protein (APP), whose aggregation into plaques represents the major hallmark of Alzheimer’s disease along with tau-derived neurofibrillary tangles [37]. Human APP can be processed through two different routes, defined as non-amyloidogenic and amyloidogenic pathways [38]. In the non-amyloidogenic processing, the enzyme α-secretase cleaves the extracellular domain, producing a short soluble N-terminal fragment and leaving a membrane-bound 83 kDa fragment [38][39][40].

Although molecular dynamics simulation showed that Aβ peptides are IDPs[41], transient secondary structures have been reported for various fragments. Raman and infrared spectroscopy studies showed that peptides containing residues 1–28 fold in a polyproline-II α-helical structure that transition into β-sheet upon binding to phospholipid bilayers [42]. Likewise, Aβ fragments of various sizes were shown by NMR to display very different conformational states (see ref. Overall, NMR structural studies [43] showed that Aβ peptides may populate multiple conformational levels ranging from α-helices to β-sheets, with rapid transitions among different structural features.

Beyond dimerization, the path of Aβ peptides toward aggregation involves a widely heterogeneous population of oligomers, spanning from low molecular weight assemblies such as dimers and tetramers to midrange aggregates, protofibrils and fibrils [44]. Characterization of oligomers through different experimental approaches showed high structural variability, from discoidal shapes devoid of β structures to antiparallel β-turn-β motif to collapsed coil [38][45].

The extreme variability of Aβ structures at the low scale of monomers and oligomers mirrors the diverse landscape of high order aggregates and plaques found in human brains. Fibrillar and dense cored plaques often associate with dystrophic neurites and reactive astrocytes and microglia, forming a unity termed neuritic plaque [46]. Furthermore, Aβ plaques are found in healthy subjects as a normal consequence of aging with a frequency comparable to those found in AD [47]. Recently, prion protein oligomers have been associated with rapid onset forms of AD [48].

The simultaneous presence of Aβ deposits in various neurodegenerative diseases, along with the presence of Aβ plaques in healthy individuals and the lack of neurodegeneration associated with Aβ alone, may suggest a “chaperoning” role for Aβ fragments in assisting neurodegeneration, exacerbating the pathologic phenotype regardless of the main proteinaceous aggregating species. Overall, neurodegenerative diseases are multi-faceted pathologies in which IDPs interact with one another and with the environment in a narrow equilibrium between functionality and uncontrolled aggregation.

5. Protein Quality Control

As already mentioned [7], to prevent the accumulation of potentially pathogenic aggregates, neural cells make use of a series of chaperones capable of recognizing misfolded proteins by means of the exposed hydrophobic portions, thus guiding their correct folding. Proteostasis or homeostasis of proteins, through the protein quality control system (PQC), requires the prompt degradation and eventual recycling of aggregates and misfolded proteins. PQC includes several proteolytic systems, including ubiquitin-proteasome system (UPS), chaperone-mediated autophagy (CMA), and macroautophagy [49].

The UPS is the system responsible for the degradation of most of the misfolded proteins. These are conjugated with ubiquitin, then deubiquitinated, linearized and introduced into the proteasome, which degrades them into smaller peptides [50]. It is important to underline that the proteasome is particularly vulnerable to protein aggregates; in fact, the passage channel of this structure has a very small diameter (just over 10–12 angstroms) and this does not allow the digestion of aggregates that are difficult to linearize. Proteotoxicity resulting from decreased UPS activity could represent potential damage to neurons [51][52][53].

The degradation system by CMA is able to act on misfolded cytosolic protein without interfering with normal molecules. Target proteins of CMA include aggregates showing a specific degradation signal, the KFERQ sequence and substrates generated by post-translational modifications. These substrates may be entrusted to the CMA-mediated degradation system in lysosomes by interaction of the chaperone (mainly Hsp70 family) with the lysosome membrane molecule LAMP2A[54].

When aggregates show resistance to both the CMA and the UPS, autophagy comes into play. In the proteostasis of post mitotic neurons the role of autophagy is of fundamental importance. Efficacy of autophagy clearance has been shown to play an important role for neuronal homeostasis and maintenance. Moreover, several studies have shown that a number of signaling molecules responsible for regulating neuronal activity are localized in membrane lipid rafts [55], for example, neuroglobin, which is found in the lipid raft and is involved in neuronal survival mechanisms [56].

As a consequence, alterations in lipid rafts’ components have been hypothesized to contribute to the loss of neural function and potentially to the cell death/cell survival or autophagy balance associated with neurodegeneration. In particular, lipid rafts at mitochondria associated membrane (MAM) level are structures involved in a number of key metabolic functions, shown to be altered in neurodegenerations such as AD, PD and ALS [57][58]. In addition, disruption of mitochondrial dynamics by the knocking down of strategic molecules associated to MAM’s lipid rafts including MFN2, GD3 or ERLIN1 significantly prevented autophagosome biogenesis and maturation [59]. In light of this evidence, a dysregulation during autophagosome maturation might drive the accumulation of protein aggregates and increase neurodegeneration

It is possible to direct misfolded proteins prone to aggregation to the autophagic mechanism for lysosomal degradation thanks to the involvement of molecules that function as adapters, such as p62 and NBR1 [60][61]. After the accumulation of non-degradable autophagic cargoes, the chaperone molecules residing in the ER and participating in this signaling chain are arginylated and, via the N-terminal arginine residue, bind to the ZZ domain of p62 in the cytosol. Once bound, p62 undergoes a conformational modification that induces its polymerization and the interaction with LC3-II, a molecule anchored on the membrane of autophagosomes [62]. The autophagosome thus begins its load and, once completed, fuses

with the lysosome to form the autolysosome, for the degradation by lysosomal hydrolases of both, load and p62.

The failure of the PQC system to remove misfolded proteins in the nervous system is the biochemical process behind most neurodegenerative diseases. During aging, deterioration in the PQC systems causes the failure of protein degradation, which may result in the accumulation of misfolded proteins. The successive modifications of structure and aggregation represent in many cases hallmarks of neurodegenerative diseases.

In light of this, it is clear that molecules involved in the clearance of misfolded proteins could represent new pharmacological targets, for example, by controlling the activation of CMA chaperones and adapters, as well as using autophagy inducers, which could be included in future therapeutic strategies for the improvement of neurodegenerative diseases.

6. Clinical Outlook and Concluding Remarks

Protein aggregation is an emerging concept in biology. Accumulating knowledge is suggesting that a convergent evolution has positively selected IDRs. Living organisms exploited the intrinsic property of IDRs to form amyloids to their advantage, incorporating it as a key signaling mechanism. Functional amyloids have indeed been documented throughout evolution, from yeasts to mammals[63]. Intriguingly, amyloid species of proteins associated with neurodegeneration may, in principle, serve a physiological role as well. For instance, proteinase K-resistant PrP was found in response to chronic morphine withdrawal in rats[64], whereas reversible, hyperphosphorylated tau tangles were found in brains of hibernating mammals[65]. The dysregulation of a physiological function associated with the aggregated state may thus represent the mechanism behind the abundance of IDR sequences found in proteins associated with neurodegenerative diseases.

The understanding of the link between structural plasticity of proteins and neurodegeneration has led researchers to investigate this phenomenon as both a diagnostic and therapeutic tool. Particularly, the exploitation of the prion-like seeded conversion mechanism, produced outstanding results in the early and differential diagnosis of neurodegenerative diseases. Protein misfolding cyclic amplification and real-time quaking-induced conversion assays have been applied to an increasing number of pathologies, starting with prion-related pathologies[66] and expanding to synucleinopathies [67],tauopathies and AD[68] and TDP-43-related pathologies[69], yielding a very high diagnostic accuracy from ex vivo human samples.

As we are just beginning to understand the phenomenon of phase separation and protein aggregation, the direction of the molecular pathogenetic study, an expression of basic science, seems right. This approach makes predictable a future in which these devastating diseases may be early and accurately diagnosed, so that personalized and disease-modifying therapies could slow down the insurgence of pathologies.

References

1. Soto, C. Protein misfolding and disease; protein refolding and therapy. FEBS Lett. 2001, 498, 204–207.
2. Soto, C. Unfolding protein misfolding in neurodegenerative diseases. Nat. Rev. Neurosci. 2003, 4, 49–60.
3. Foit, L.; Morgan, G.J.; Kern, M.J.; Steimer, L.R.; von Hacht, A.A.; Titchmarsh, J.; Warriner, S.L.; Radford, S.E.; Bradwell, J.C.A. Optimizing protein stability in vivo. Mol. Cell. 2009, 36, 861–871.
4. Ward, J.J.; McGuffin, L.J.; Buxton, B.F.; Jones, D.T. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J. Mol. Biol. 2004, 337, 635–645.
5. Dunker, A.K.; Silman, I.; Uversky, V.N.; Sussman, J.L. Function and structure of inherently disordered proteins. Curr. Opin. Struct. Biol. 2008, 18, 756–764.
6. Uversky, V.N. Intrinsic Disorder, Protein-Protein Interactions, and Disease. Adv. Protein. Chem. Struct. Biol. 2018, 110, 85–121.
7. Hegyi, H.; Tompa, P. Intrinsically Disordered Proteins Display No Preference for Chaperone Binding In Vivo. PLoS Comput. Biol. 2008, 4, e1000017.

8. March, Z.M.; King, O.D.; Shorter, J. Prion-like domains as epigenetic regulators, scaffolds for subcellular organization, and drivers of neurodegenerative disease. Brain Res. 2016, 1647, 9–18.
9. Chiti, F.; Dobson, C.M. Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. Annu. Rev. Biochem. 2017, 86, 27–68.
10. Babu, M.M. The contribution of intrinsically disordered regions to protein function, cellular complexity, and human disease. Biochem. Soc. Trans. 2016, 44, 1185–1200.
11. Vymetal, J.; Vondrášek, J.; Hlouchová, K. Sequence Versus Composition: What Prescribes IDP Biophysical Properties? Entropy 2019, 21, 654.
12. Peng, Z.; Yan, J.; Fan, X.; Mizianty, M.J.; Xue, B.; Wang, K.; Hu, G.; Uversky, V.N.; Kurgan, L. Exceptionally abundant exceptions: Comprehensive characterization of intrinsic disorder in all domains of life. Cell Mol. Life Sci. 2015, 72, 137–151.
13. Wright, P.E.; Dyson, H.J. Intrinsically Disordered Proteins in Cellular Signaling and Regulation. Nat. Rev. Mol. Cell Biol. 2015, 16, 18–29.
14. Chavali, S.; Gunnarsson, A.; Babu, M.M. Intrinsically Disordered Proteins Adaptively Reorganize Cellular Matter During Stress. Trends Biochem. Sci. 2017, 42, 410–412.
15. Uversky, V.N. The multifaceted roles of intrinsic disorder in protein complexes. FEBS Lett. 2015, 589, 2498–2506.
16. Calabretta, S.; Richard, S. Emerging Roles of Disordered Sequences in RNA-Binding Proteins. Trends Biochem. Sci. 2015, 40, 662–672.
17. Basu, S.; Bahadur, R.P. A structural perspective of RNA recognition by intrinsically disordered proteins. Cell Mol. Life Sci. 2016, 73, 4075–4084.
18. Zagorvic, B.; Bartonek, L.; Polyansky, A.A. RNA-protein interactions in an unstructured context. FEBS Lett. 2018, 592, 2901–2916.
19. Crick, S.L.; Ruff, K.M.; Garai, K.; Frieden, C.; Pappu, R.V. Unmasking the roles of N- and C-terminal flanking sequences from exon 1 of huntingtin as modulators of polyglutamine aggregation. Proc. Natl. Acad. Sci. USA 2013, 110, 20075–20080.
20. Bergeron-Sandoval, L.P.; Safaee, N.; Michnick, S.W. Mechanisms and Consequences of Macromolecular Phase Separation. Cell 2016, 165, 1067–1079.
21. Wallman, A.; Kesten, C. Common Functions of Disordered Proteins across Evolutionary Distant Organisms. Int. J. Mol. Sci. 2020, 21, 2105.
22. Cieplak, M.; Chwastyk, M.; Mioduszewski, L.; de Aquino, B.R.H. Transient knots in intrinsically disordered proteins and neurodegeneration. Prog. Mol. Biol. Transl. Sci. 2020, 174, 79–103.
23. Das, S.; Pal, U.; Sad, S.; Bagga, K.; Roy, A.; Mrigwani, A.; Maiti, N.C. Sequence Complexity of Amyloidogenic Regions in Intrinsically Disordered Human Proteins. PLoS ONE 2014, 9, e89781.
24. Kumari, B.; Kumar, R.; Kumar, M. Low complexity and disordered regions of proteins have different structural and amino acid preferences. Mol. Biosyst. 2015, 11, 585–594.
25. Uversky, V.N.; Gillespie, J.R.; Fink, A.L. Why are “natively unfolded” proteins unstructured under physiologic conditions? Proteins 2000, 41, 415–427.
26. Babinchak, W.M.; Haider, R.; Dumm, B.K.; Sarkar, P.; Surewicz, K.; Choi, J.K.; Surewicz, W.K. The role of liquid–liquid phase separation in aggregation of the TDP-43 low-complexity domain. J. Biol. Chem. 2019, 294, 6306–6317.
27. S. Prusiner; Novel proteinaceous infectious particles cause scrapie. *Science* **1982**, *216*, 136-144, 10.1126/science.6801762.
28. D C Gajdusek; Unconventional viruses and the origin and disappearance of kuru. *Science* **1977**, *197*, 943-960, 10.1126/science.142303.
29. R. H. Kimberlin; Carol A. Walker; Evidence that the Transmission of One Source of Scrapie Agent to Hamsters Involves Separation of Agent Strains from a Mixture. *Journal of General Virology* **1978**, *39*, 487-496, 10.1099/0022-1317-39-3-487.
30. J. S. Griffith; Nature of the Scrapie Agent: Self-replication and Scrapie. *Nature* **1967**, *215*, 1043-1044, 10.1038/2151043a0.
31. Stanley B. Prusiner; Michael Scott; Dallas Foster; Keh-Ming Pan; Darlene Groth; Carol Mirenda; Marilyn Torchia; Shu-Lian Yang; Dan Serban; George A. Carlson; et al.Peter C. HoppeDavid WestawayStephen J. DeArmond Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **1990**, *63*, 673-686, 10.1016/0092-8674(90)90134-z.
32. Schmitz, M.; Dittmar, K.; Llorens, F.; Gelpi, E.; Ferrer, I.; Schulz-Schaeffer, W.J.; Zerr, I.; Hereditary Human Prion Diseases: An Update. *Mol. Neurobiol* **2017**, *54*, 4138–4149, 10.1007/s12035-016-9918-y..
33. S. Notari; J. Yuan; I. Cali; Q. Kong; W.-Q. Zou; Variant Creutzfeldt-Jakob Disease☆. *Reference Module in Neuroscience and Biobehavioral Psychology* **2017**, *153*, 191-205, 10.1016/b978-0-12-809324-5.00855-5.
34. Laura Cracco; Xiangzhu Xiao; Satish K. Nemani; Jody Lavrich; Ignazio Cali; Bernardino Ghetti; Silvio Notari; Witold K. Surewicz; Pierluigi Gambetti; Gerstmann-Sträussler-Scheinker disease revisited: accumulation of covalently-linked multimers of internal prion protein fragments. *Acta Neuropathologica Communications* **2019**, *7*, 1-9, 10.1186/s40478-

019-0734-2.
35. Franc Llorens; Juan-José Zarranz; Andre Fischer; Inga Zerr; Isidro Ferrer; Fatal Familial Insomnia: Clinical Aspects and Molecular Alterations. *Current Neurology and Neuroscience Reports* **2017**, *17*, 444, 10.1007/s11910-017-0743-0.
36. Wen-Quan Zou; Pierluigi Gambetti; Xiangzhu Xiao; Jue Yuan; Jan Langeveld; Laura Pirisinu; Prions in Variably Protease-Sensitive Prionopathy: An Update. *Pathogens* **2013**, *2*, 457-471, 10.3390/pathogens2030457.
37. Zhang, W.; Tarutani, A.; Newell, K.L.; Murzin, A.G.; Matsubara, T.; Falcon, B.; Vidal, R.; Garringer, H.J.; Shi, Y.; Ikeuchi, T.; et al. Novel tau filament fold in corticobasal degeneration. Nature 2020, 580, 283–287.
38. Scheltens, P.; De Strooper, B.; Kivipelto, M.; Holstege, H.; Chételat, G.; Teunissen, C.E.; Cummings, J.; van der Flier, W.M. Alzheimer's disease. Lancet 2021, 397, 1577–1590.
39. Chen, G.F.; Xu, T.H.; Yan, Y.; Zhou, Y.R.; Jiang, Y.; Melcher, K.; Xu, H.E. Amyloid beta: Structure, biology and structure-based therapeutic development. Acta Pharmacol. Sin. 2017, 38, 1205–1235.
40. Selkoe, D.J.; Schenk, D. Alzheimer's disease: Molecular understanding predicts amyloid-based therapeutics. Annu. Rev. Pharmacol. Toxicol. 2003, 43, 545–584.
41. Muvva, C.; Murugan, N.A.; Subramanian, V. Assessment of Amyloid Forming Tendency of Peptide Sequences from Amyloid Beta and Tau Proteins Using Force-Field, Semi-Empirical, and Density Functional Theory Calculations. Int. J. Mol. Sci. 2021, 22, 3244.
42. Eker, F.; Griebenow, K.; Schweitzer-Stenner, R. Abeta(1-28) fragment of the amyloid peptide predominantly adopts a polyproline II conformation in an acidic solution. Biochemistry 2004, 43, 6893–6898.
43. Sgourakis, N.G.; Yan, Y.; McCallum, S.A.; Wang, C.; Garcia, A.E. The Alzheimer's peptides Abeta40 and 42 adopt distinct conformations in water: A combined MD/NMR study. J. Mol. Biol. 2007, 368, 1448–1457.
44. Wildburger, N.C.; Esparza, T.J.; LeDuc, R.D.; Fellers, R.T.; Thomas, P.M.; Cairns, N.J.; Kelleher, N.L.; Bateman, R.J.; Brody, D.L. Diversity of Amyloid-beta Proteoforms in the Alzheimer's Disease Brain. Sci. Rep. 2017, 7, 9520.
45. Hayden, E.Y.; Teplow, D.B. Amyloid β-protein oligomers and Alzheimer's disease. Alzheimers Res. Ther. 2013, 5, 60.
46. Xu, G.; Fromholt, S.E.; Chakrabarty, P.; Zhu, F.; Liu, X.; Pace, M.C.; Koh, J.; Golde, T.E.; Levites, Y.; Lewis, J.; et al. Diversity in Aβ deposit morphology and secondary proteome insolubility across models of Alzheimer-type amyloidosis. Acta Neuropathol. Commun. 2020, 8, 43.
47. Jagust, W. Is amyloid-β harmful to the brain? Insights from human imaging studies. Brain 2016, 139, 23–30.
48. Shafiq, M.; Zafar, S.; Younas, N.; Noor, A.; Puig, B.; Altmeppen, H.C.; Schmitz, M.; Matschke, J.; Ferrer, I.; Glatzel, M.; et al. Prion protein oligomers cause neuronal cytoskeletal damage in rapidly progressive Alzheimer's disease. Mol. Neurodegener. 2021, 16, 11.
49. Ciechanover, A.; Kwon, Y.T. Protein Quality Control by Molecular Chaperones in Neurodegeneration. Front. Neurosci. 2017, 11, 185.
50. Wang, J.; Maldonado, M.A. The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. Cell Mol. Immunol. 2006, 3, 255–261.
51. Andre, R.; Tabrizi, S.J. Misfolded PrP and a novel mechanism of proteasome inhibition. Prion 2012, 6, 32–36.
52. Tai, H.C.; Serrano-Pozo, A.; Hashimoto, T.; Frosch, M.P.; Spires-Jones, T.L.; Hyman, B.T. The synaptic accumulation of hyperphosphorylated tau oligomers in Alzheimer disease is associated with dysfunction of the ubiquitin-proteasome system. Am. J. Pathol. 2012, 181, 1426–1435.
53. Hegde, A.N.; Upadhya, S.C. Role of ubiquitin-proteasome-mediated proteolysis in nervous system disease. Biochim. Biophys. Acta 2011, 1809, 128–140.
54. A.M. Cuervo; J.F. Dice; Regulation of Lamp2a Levels in the Lysosomal Membrane. *Traffic* **2000**, *1*, 570-583, 10.1034/j.1600-0854.2000.010707.x.
55. John A. Allen; Robyn A. Halverson-Tamboli; Mark M. Rasenick; Lipid raft microdomains and neurotransmitter signalling. *Nature Reviews Neuroscience* **2006**, *8*, 128-140, 10.1038/nrn2059.
56. Garofalo, T.; Ferri, A.; Sorice, M.; Azmoon, P.; Grasso, M.; Mattei, V.; Capozzi, A.; Manganelli, V.; Misasi, R. Neuroglobin overexpression plays a pivotal role in neuroprotection through mitochondrial raft-like microdomains in neuroblastoma SK-N-BE2 cells. Mol. Cell. Neurosci. 2018, 88, 167–176.
57. Area-Gomez, E.; de Groof, A.; Bonilla, E.; Montesinos, J.; Tanji, K.; Boldogh, I.; Pon, L.; Schon, E.A. A key role for MAM in mediating mitochondrial dysfunction in Alzheimer disease. Cell Death Dis. 2018, 19, 335.
58. Ciarlo, L.; Manganelli, V.; Matarrese, P.; Garofalo, T.; Tinari, A.; Gambardella, L.; Marconi, M.; Grasso, M.; Misasi, R.; Sorice, M.; et al. Raft-like microdomains play a key role in mitochondrial impairment in lymphoid cells from patients with Huntington's disease. J. Lipid Res. 2012, 53, 2057–2068.
59. Garofalo, T.; Matarrese, P.; Manganelli, V.; Marconi, M.; Tinari, A.; Gambardella, L.; Faggioni, A.; Misasi, R.; Sorice, M.; Malorni, W. Evidence for the involvement of lipid rafts localized at the ER-mitochondria associated membranes in autophagosome formation. Autophagy 2016, 12, 917–935.
60. Hyunjoo Cha-Molstad; Ki Sa Sung; Joonsung Hwang; Kyoung A. Kim; Ji Eun Yu; Young Dong Yoo; Jun Min Jang; Dong Hoon Han; Michael Molstad; Jung Gi Kim; et al.Yoon Jee LeeAdriana ZakrzewskaSu-Hyeon KimSung Tae KimSun Yong KimHee Gu LeeNak Kyun SoungJong Seog AhnAaron CiechanoverBo Yeon KimYong Tae Kwon Amino-terminal arginylation targets endoplasmic reticulum chaperone BiP for autophagy through p62 binding. *Nature* **2015**, *17*, 917-

929, 10.1038/ncb3177.
61. Manganelli, V.; Matarrese, P.; Antonioli, M.; Gambardella, L.; Vescovo, T.; Gretzmeier, C.; Longo, A.; Capozzi, A.; Recalchi, S.; Riitano, G.; et al. Raft-like lipid microdomains drive autophagy initiation via AMBRA1-ERLIN1 molecular association within MAMs. Autophagy 2020.
62. Bourdenx, M.; Martín-Segura, A.; Scrivo, A.; Rodriguez-Navarro, J.A.; Kaushik, S.; Tasset, I.; Diaz, A.; Storm, N.J.; Xin, Q.; Juste, Y.R.; et al. Chaperone-mediated autophagy prevents collapse of the neuronal metastable proteome. Cell 2021, 184, 2696–2714.
63. Stephan, J.S.; Fioriti, L.; Lamba, N.; Colnaghi, L.; Karl, K.; Derkatch, I.L.; Kandel, E.R.; The CPEB3 Protein Is a Functional Prion that Interacts with the Actin Cytoskeleton.. *Cell Rep.* **2015**, *11*, 1772-1785, doi:10.1016/j.celrep.2015.04.060.
64. Vincenzo Mattei; Stefano Martellucci; Francesca Santilli; Valeria Manganelli; Tina Garofalo; Niccolò Candelise; Alessandra Caruso; Maurizio Sorice; Sergio Scaccianoce; Roberta Misasi; et al. Morphine Withdrawal Modifies Prion Protein Expression in Rat Hippocampus. *PLOS ONE* **2017**, *12*, e0169571, 10.1371/journal.pone.0169571.
65. Thomas Arendt; Torsten Bullmann; Neuronal plasticity in hibernation and the proposed role of the microtubule-associated protein tau as a “master switch” regulating synaptic gain in neuronal networks. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **2013**, *305*, R478-R489, 10.1152/ajpregu.00117.2013.
66. Ryuichiro Atarashi; Katsuya Satoh; Kazunori Sano; Takayuki Fuse; Naohiro Yamaguchi; Daisuke Ishibashi; Takehiro Matsubara; Takehiro Nakagaki; Hitoki Yamanaka; Susumu Shirabe; et al.Masahito YamadaHidehiro MizusawaTetsuyuki KitamotoGenevieve M J A KlugAmelia McGladeSteven J CollinsNoriyuki Nishida Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nature Medicine* **2011**, *17*, 175-178, 10.1038/nm.2294.
67. Marcello Rossi; Niccolò Candelise; Simone Baiardi; Sabina Capellari; Giulia Giannini; Christina D. Orrù; Elena Antelmi; Angela Mammana; Andrew G. Hughson; Giovanna Calandra-Buonaura; et al.Anna LadoganaGiuseppe PlazziPietro CortelliByron CaugheyPiero Parchi Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. *Acta Neuropathologica* **2020**, *140*, 49-62, 10.1007/s00401-020-02160-8.
68. Allison Kraus; Eri Saijo; Michael A. Metrick; Kathy Newell; Christina J. Sigurdson; Gianluigi Zanusso; Bernardino Ghetti; Byron Caughey; Seeding selectivity and ultrasensitive detection of tau aggregate conformers of Alzheimer disease. *Acta Neuropathologica* **2018**, *137*, 585-598, 10.1007/s00401-018-1947-3.
69. Carlo Scialò; Thanh Hoa Tran; Giulia Salzano; Giovanni Novi; Claudia Caponnetto; Adriano Chiò; Andrea Calvo; Antonio Canosa; Fabio Moda; Paola Caroppo; et al.Vincenzo SilaniNicola TicozziAntonia RattiBarbara BorroniLuisa BenussiRoberta GhidoniGiovanni FurlanisPaolo ManganottiBeatrice SenigagliesiPietro ParisseRomain BrasseletEmanuele BurattiGiuseppe Legname TDP-43 real-time quaking induced conversion reaction optimization and detection of seeding activity in CSF of amyotrophic lateral sclerosis and frontotemporal dementia patients. *Brain Communications* **2020**, *2*, 142, 10.1093/braincomms/fcaa142.

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