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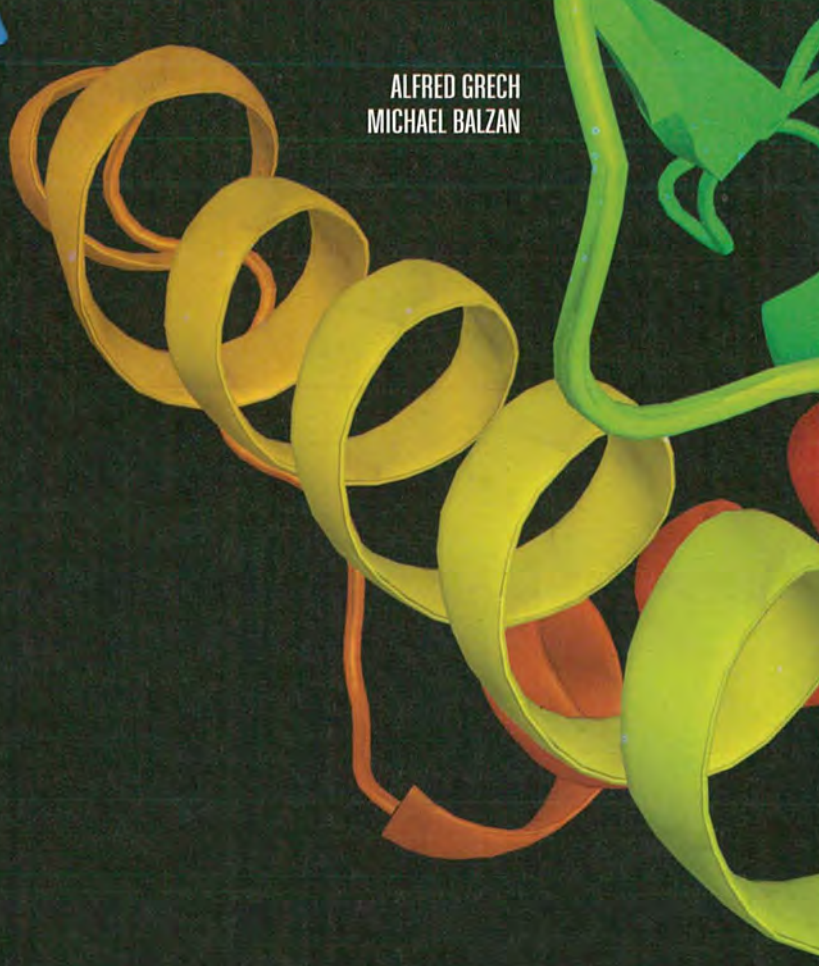


AN UPDATE ON PRIONS AND PRION-LIKE DISORDERS

ABSTRACT

Prions are infectious agents composed of protein material. Prions fold in multiple ways, at least one of which is transmissible to other prions; transmission between species leads to prion diseases, such as the more commonly known "mad cow disease". Similar to prion diseases, other neurodegenerative diseases are also associated with the accumulation of self-templating amyloid forms of specific proteins. For example, β-amyloid and tau misfold in Alzheimer's disease, α-synuclein in Parkinson's disease, huntingtin in Huntington's disease, and TDP-43 and FUS aggregate in neurons in ALS. In this review, we will discuss why these diseases are often dubbed prion-like.

PRIONS AND PRION DISEASES?

Prions are pure proteinaceous infectious particles. They reproduce by recruiting the normal cellular prion protein (PrP^{C}), which is a membrane glycoprotein, and stimulating its conversion to the disease-causing (scrapie) isoform (PrP^{Sc}). PrP^{C} and PrP^{Sc} are identical in composition but differ in their 3D structures; PrP^{C} is rich in α-helixes and has little β-sheet whereas PrP^{Sc} is less rich in α-helixes and has much more β-sheet. In this cross-beta fibrous structure, termed amyloid, the strands of the β-sheets align orthogonal to the fibre axis. Such fibres elongate at both ends, and the fibre ends capture and convert the natively folded proteins to the cross-beta form. Overall, this structural transition from α-helixes to β-sheet in PrP, known as permissive templating, is the fundamental event that underlies prion diseases.[1]

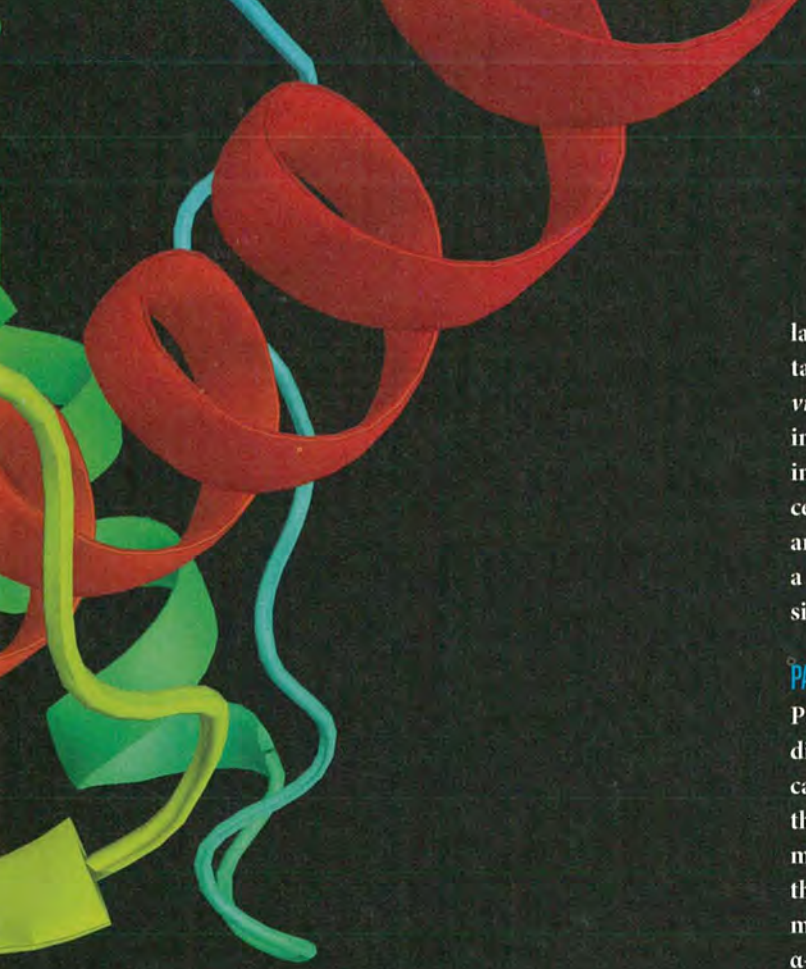
Prion diseases are a collection of fatal neurodegenerative disorders that afflict mammals, including Creutzfeldt-Jakob disease (CJD), Fatal Familial Insomnia (FFI), Gertsmann-Straussler-Scheinker syndrome (GSS), Kuru and Variably Protease-Sensitive Prionopathy (VPSPr), in humans. Transmission between individuals, and sometimes even between species, is possible due to the stability of the cross-beta prion form, which is resistant to denaturation, detergents, and proteases.[2] For example, in Europe, industrial cannibalism has been responsible for "mad cow disease". In general, it is the presence of the PrP amyloid plaques that is diagnostic of these transmissible spongiform encephalopathies.

PRION-LIKE DISORDERS

Interestingly, this ability to access self-templating amyloid forms is not unique to prion proteins. Several neurodegenerative diseases are also associated with the accumulation of self-templating amyloid forms of specific proteins, e.g. β-amyloid (Aβ) and tau misfold in Alzheimer's disease (AD), α-synuclein misfolds in Parkinson's disease (PD), and huntingtin misfolds in Huntington's disease (HD). Several research studies support the possibility that these disorders and their respective amyloid forms are more transmissible and prion-like than previously suspected.[3] Such findings have therapeutic implications and blur the distinctions between amyloid and prion, and between transmissibility and infectivity.

ALZHEIMER'S DISEASE

AD is the most common neurodegenerative disorder, affecting approximately 35 million people worldwide. In AD, the defining pathological lesions are the neuritic plaques



composed of Aβ peptides. Aβ is a cleavage peptide of the amyloid precursor protein (APP). It varies in size from 39 to 43 amino acids, but the main species observed in the plaques are Aβ40 and Aβ42. Aβ40 is more common, whereas Aβ42 is more fibrillogenic and is therefore more associated with disease states.[4] Similar to a prion protein, pure Aβ40 forms fibres with different molecular structures, which are toxic to neurons in culture. Likewise, Aβ amyloid forms accumulate in the extracellular space, and therefore transmissibility becomes a concern. Indeed, when brain extracts from patients with AD were introduced into the brains of transgenic mice generating human APP, the material induced plaque formation and deposition of Aβ. In contrast, introducing brain extracts from age-matched patients without AD showed less Aβ accumulation.[5] Moreover, coherent with the property of permissive templating, plaques did not appear in mice that did not produce human Aβ. It is thus probable that transmission from extract to host brain occurs through pure protein templating.

In AD, Aβ is not the only protein that can access an amyloid form. Tau is a protein that binds to and stabilises microtubules, thus enabling intracellular transport. Six tau isoforms are expressed in the human brain. During AD, tau dissociates from microtubules and forms amyloid accumulations throughout the cell.[6] Specifically, filamentous inclusions, made up of all 6 isoforms, form in a stereotypical manner, underlying the "Braak stages" of tau pathology. Tau pathology begins in discrete regions but ultimately involves larger areas of the brain. Such an intercellular transfer of tau aggregates has been described in both *in vitro*[7] and *in vivo*[8] experiments. In general, tau is considered to be an intracellular protein. However, tau aggregates are detected in the extracellular space, and tau peptide is observed in the cerebrospinal fluid of patients. Frost *et al.*[7] hypothesised and showed that extracellular tau aggregates can transmit a misfolded state from the outside to the inside of a cell, similar to prions.

PARKINSON'S DISEASE

PD is the most common neurodegenerative movement disorder, afflicting people over 65 years of age. It is caused by selective damage to dopaminergic neurons in the substantia nigra.[9] While PD is sporadic, a few gene mutations have been associated with familial forms of the disease; these include gene multiplications and point mutations in the *SNCA* gene, which encodes α-synuclein. α-synuclein is a small (140 a/a) presynaptic protein that binds lipids through its amino-terminal repeat region. It is believed to play a role in the assembly of the protein complexes required for chemical neurotransmission. In PD, the signature lesions are cytoplasmic aggregates in dopaminergic neurons known as Lewy bodies (LBs) and Lewy neurites (LNs), consisting of amyloid forms of α-synuclein. Curiously, α-synuclein pathology also hints at a prion-like mechanism of propagation through the brains of patients with PD; the propagation of LB and LN pathology has been described in both *in vitro*[10] and *in vivo*[11] experiments.

Steiner *et al.*[10] showed that the propagation of the amyloid forms of α-synuclein requires two steps. Step 1 is the exit of α-synuclein from the dying neuron; upon cell death, degenerating neurons release α-synuclein fibres. α-synuclein aggregates are then incorporated into vesicles and secreted from neurons via exocytosis. Step 2 is the entry of α-synuclein into the recipient cell. It is however still unclear how α-synuclein escapes a membrane-bound vesicle to form cytoplasmic aggregates. In cell culture, it was discovered that inhibiting lysosomal function results in the deposition of α-synuclein.[12] Interestingly, mutations in PARK9, a lysosomal transmembrane ATPase, are connected with familial Parkinsonism. In addition, overexpression of PARK9 was found to counter α-synuclein toxicity.[13] Therefore, a decline in lysosomal function with age might contribute to α-synuclein pathogenesis by facilitating transmission and reducing clearance of α-synuclein in the lysosome.[14] For this reason, restoring lysosomal function could be one of several strategies for curing PD.

HUNTINGTON'S DISEASE

HD is an autosomal dominant neurodegenerative disorder. It is characterised by a loss of striatal neurons in the basal ganglia; however, pathology is observed in other areas of the brain as well. HD is caused by a trinucleotide (CAG) repeat expansion in the huntingtin gene.[15] Specifically, it is the expansion of a CAG repeat in exon 1 of the huntingtin gene above a threshold of 35 to 40 CAG repeats that causes the disease. In turn, this mutation encodes an expanded polyglutamine (polyQ) tract, which makes the protein prone to aggregate and to form intraneuronal inclusion bodies.[16] It has been found that the longer the polyQ tract, the faster the aggregation, and the earlier the onset. In the *Drosophila melanogaster* brain, Pearce *et al.*[17] observed prion-like transmission of neuronal huntingtin aggregates to phagocytic glia.

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is a neurodegenerative disease that devastates the upper and lower motor neurons. ALS starts in a discrete location but progresses to involve neighbouring regions of the motor system. Recently, mutations in two RNA binding proteins, TDP-43 and FUS, were identified in patients with familial ALS. It was found that in ALS both TDP-43 and FUS form aggregates in neurons. Furthermore, evidence indicates that both proteins contain prion-related modular domains. Indeed, both have domains highly enriched in glutamine (Q) and asparagine (N) residues that meet the criteria for prion-related domains. Also, both form aggregates, and both are cross-seeded into polyQ inclusions, mediated by the Q/N rich region, similar to other prion-related domain containing proteins.[18]

CONCLUSION

Even if most neurodegenerative diseases don't spread from individual to individual like true prion diseases do, the possibility that they may spread from cell to cell in an analogous way has important implications for potential therapies, especially in view of stem-cell and neuronal-graft based therapies. For example, because PrP acts as a receptor for toxic Aβ oligomers, grafted neurons might be useful for the treatment of AD; grafted neurons would resist Aβ oligomer invasion.[19] Likewise, depleting tau or α-synuclein from grafted neurons might prove advantageous in tauopathies and PD, respectively. Indeed, depletion of PrP or tau, even after the onset of the disease, was shown to be an efficient treatment of prion and prion-like diseases in mouse models.[2]

Translational research on prions is fast-paced. On the diagnostic side is a blood-test to diagnose Variant Creutzfeldt-Jakob Disease (vCJD).[20] Indeed, the test has the potential to detect presymptomatic patients. Given the fact that there is evidence of silent carriers, such a diagnostic test will surely limit the risk of vCJD transmission through blood transfusion. On the therapeutic side, multiple pathways are being followed, involving small molecule drugs, antibodies, gene silencing, vaccines and stem cell therapy.

In view of all this, and as the current studies and existing knowledge of prion pathogenesis are augmented, there is optimism that a much better understanding of the pathogenic pathways will lead to more rational theranostics.

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