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Assessing the causes and consequences of co-polymerization in amyloid formation

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How, and why, different proteins form amyloid fibrils is most often studied in vitro using a single purified protein sequence. However, many amyloid diseases involve co-aggregation of different protein species, including proteins with/without post-translational modifications (e.g., different strains of PrP), proteins of different length (e.g., β_2-microglobulin and ΔN6, Aβ40, and Aβ42), sequence variants (e.g., Aβ and $A\beta_{ARC}$), and proteins from different organisms (e.g., bovine PrP and human PrP). The consequences of co-aggregation of different proteins upon the structure, stability, species transmission and toxicity of the resulting amyloid aggregates is discussed here, including the role of co-aggregation in expanding the repertoire of oligomeric and fibrillar structures and how this can affect their biological and biophysical properties.

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Introduction

It has been well documented that amyloid fibrils can be highly polymorphic, even when formed from proteins with the same amino acid sequence under the same incubation conditions.[1] This polymorphism can be manifested through the formation of fibrils with different numbers and orientations of protofilaments, and also within the protofilament substructure, for example in the residues that contribute to the β-strand segments within the fibrils (reviewed in refs. 2–3). In a comprehensive study of an 11-residue transthyretin (TTR) peptide the structure and orientation of protofilaments from three different fibril polymorphs was described in atomic detail using cryo-electron microscopy (EM), scanning transmission EM and magic angle spinning (MAS) NMR, revealing different numbers of protofilaments within each polymorph.[4] The different packing arrangements available for an amyloid fibril core has also been explored using X-ray crystallography of 4–7-residue peptides from a variety of amyloidogenic proteins, the results revealing eight classes of polymorphs arranged as different steric zippers.[5] The structures of the steric zippers differ in whether the β-strands are parallel or anti-parallel, whether the β-sheets pack face-to-face or back-to-back, and whether the sheets orientate themselves up or down relative to each other[6] (see **Fig. 1A**).

Oligomeric intermediates of amyloid formation can also show polymorphism. HypF-N is the 91-residue N-terminal domain of HypF, a carbamoyl transferase in *Escherichia coli*.[7] In a recent study, oligomers of HypF-N formed under different solution conditions were shown to be similar, morphologically and tinctorially, but differed in their ability to cause cell dysfunction.[8] Further investigation using site-specific labeling with pyrene maleimide and binding of the dye 8-anilinonaphthalene-1-sulfonic acid (ANS) demonstrated that the oligomers that possess less structural order and expose more hydrophobic surface area are more toxic than oligomers with a more ordered core. The increased toxicity of one oligomer morphology over the other is thought to arise because the structural flexibility and hydrophobic exposure of the toxic oligomers allows them to cross the hydrophobic

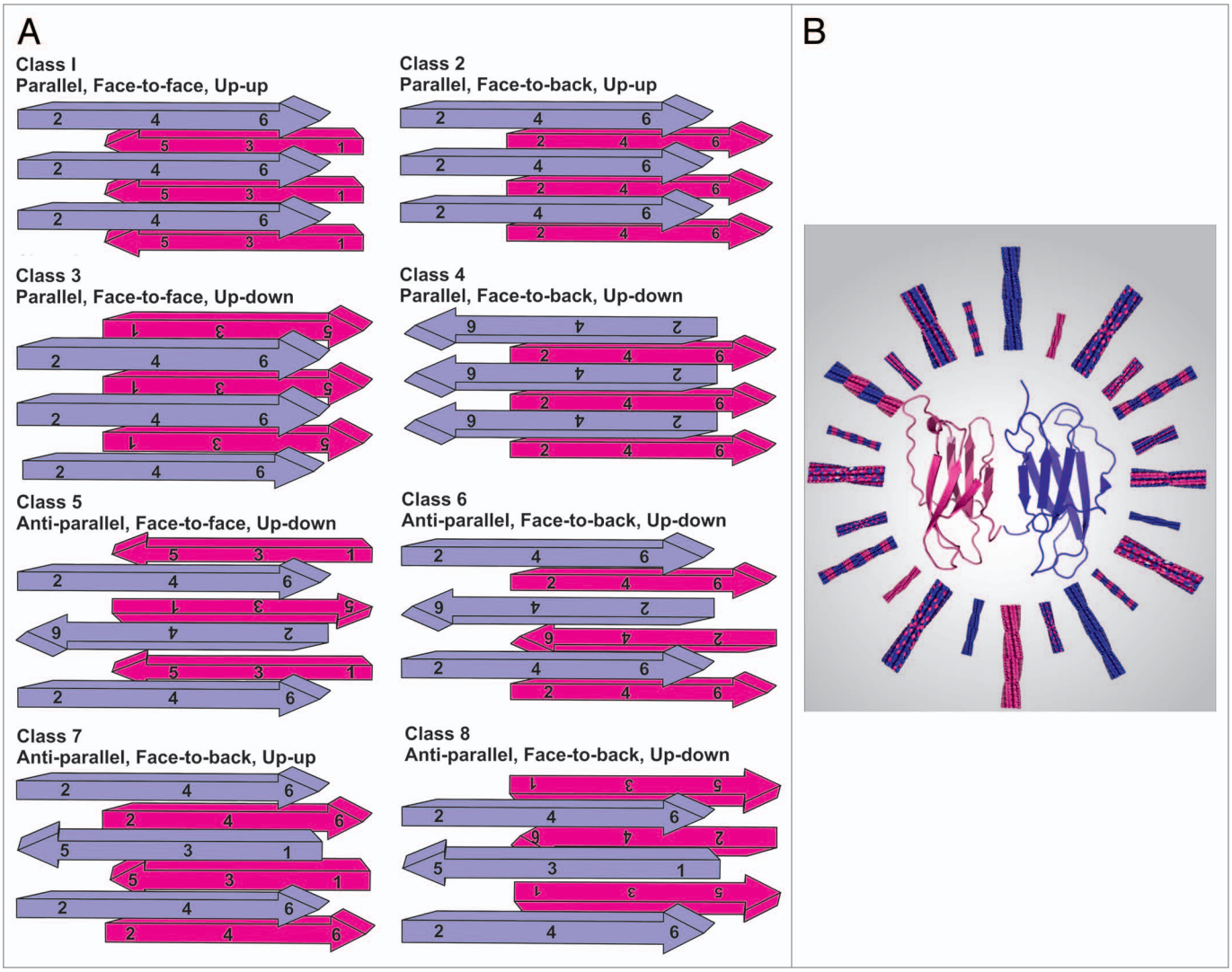

Figure 1. The potential repertoire of fibril polymorphs. (**A**) The eight classes of steric zipper available to a single polypeptide chain (re-drawn from Figure 4 of Sawaya et al.).[5] (**B**) A schematic of different possible fibril polymorphs that can arise from co-aggregation of more than one protein (depicted are ΔN6 [PDB code 2XKU] in pink and β_2m [PDB code 2XKS] in purple).[20]

bilayer of the cell membrane, whereas the non-toxic oligomers can bind to, but not cross the membrane. In other examples the more structured oligomers were shown to be the most toxic.[9] These examples, and many others,[10-12] show the ability of a single polypeptide sequence to adopt an array of oligomeric structures with differing physical, chemical, biophysical and biological functions, even for species of similar molecular weight. This poses significant challenges for the identification and structural characterization of each individual species in the amyloid cascade and in amyloid disease.

The above studies demonstrate that there is a large range of possible polymorphic structures available to an amyloidogenic protein or peptide both as oligomeric intermediates in fibril formation and in the final fibrillar form. The potential assortment of polymorphs can be greatly expanded when mixtures of proteins can co-aggregate, either de novo or via cross-seeding. The resulting increased range of fibril structures expands the repertoire of aggregation propensities and amyloid stabilities. This can be useful, for example by providing enhanced opportunity for tailoring the physical properties of amyloid fibrils for use as nanomaterials. However, it may also be detrimental, for example, by increasing the possibility for toxicity and disease.

Here we discuss the repertoire of amyloid polymorphs formed from different protein and peptide sequences, (the interactions between amyloidogenic proteins and other important factors in amyloid fibril formation, such as chaperones, small molecules, metal ions, lipids, and glycolipids, are not included).[13] We identify three different scenarios that can affect the likelihood of two proteins cross-seeding and/or co-polymerizing with each other. First, we discuss the requirements of sequence in determining co-aggregation propensity and its outcomes. Second, we describe scenarios in which one protein increases the amyloidogenic propensity of another. Finally, we review the extent to which

both proteins need to have the ability to adopt a similar fibril structure in order to co-assemble. A select number of studies are discussed in detail in each section, and a more comprehensive list of protein-protein interactions that expand the repertoire of fibril polymorphs is included in **Table 1**.

Condition 1: Co-Aggregation of Proteins/Peptides with Similar Primary Sequence

The intermolecular interactions that stabilize amyloid fibrils involve the peptide backbone and thus the ability to form fibrils is considered to be a generic property of all polypeptide chains.[14] However side-chain interactions play a crucial role in the formation of amyloid fibrils and in determining the ability of one protein to seed polymerization of another. The importance of amino acid sequence similarity in cross-seeding (adding fibrillar seeds of one polypeptide sequence to monomers of a second polypeptide sequence) was shown in a study of hen lysozyme (Row 1, **Table 1**).[15] Fibrillar seeds created from sequences that are 99.2% (I55T mutational variant of hen lysozyme) or 95% identical (turkey lysozyme) to that of the hen lysozyme monomer produced identical seeding behavior to homologous seeding. A seed with a sequence that is 60% identical to the hen lysozyme monomer (human lysozyme) showed faster fibril elongation than the rate of fibril formation of the unseeded monomer, but with a lag phase that is increased compared with self-seeding. Sequences with 36% identity or no identity to the hen lysozyme monomer had no effect on fibril formation.[15] Thus the efficiency of seeding depends on the similarity between both the seed's and monomer's amino acid sequence.[15]

Similar primary sequences between amyloidogenic proteins may promote the likelihood of a cross-seeding event occurring, but this does not always result in a reciprocal ability for both partners to cross-seed each other's assembly. Islet amyloid polypeptide (IAPP), the amyloidogenic peptide involved in type II diabetes mellitus, is derived from the 89 amino acid precursor protein pro-islet amyloid polypeptide. Aβ, the neurotoxic agent in Alzheimer disease (AD), is derived from the proteolytic processing of amyloid precursor protein which, depending on the splicing isoform, is composed of 365 to 770 amino acids. These precursor proteins are unrelated in sequence and have no obvious functional relationship. Despite this, residues 15–37 of Aβ (residues involved in the amyloid core of Aβ fibrils[16]) share 39% sequence identity and 65% sequence similarity with residues 10–33 of IAPP. The interaction between IAPP and Aβ40 was studied to determine whether cross-seeding could occur.[17] These in vitro studies showed that Aβ40 fibrils, IAPP fibrils, and IAPP amorphous aggregates are all equally effective at seeding elongation with IAPP monomer. Conversely, IAPP aggregates will not cross-seed polymerization of Aβ40, and IAPP fibrils are much less efficient at seeding Aβ40 monomer compared with Aβ40 fibrils, exhibiting only 2% of the seeding efficiency of Aβ fibrils on a weight basis.[17] Thus, there is a surprising lack of equivalence in cross-seeding of Aβ40 and IAPP (Row 2, **Table 1**).

The importance of primary sequence in determining the ability of proteins to co-aggregate is more complex than both partners simply having amyloid-prone primary sequences. In one example, the ability of a variety of different amyloid fibrils to act as a seed for elongation with Aβ40 was compared with the efficiency of homologous seeding (Aβ40 seeds).[17] The majority of fibril types, including fibrils formed from the yeast protein Ure2p and the human protein β_2-microglobulin (β_2m), were as inefficient at cross-seeding Aβ40 as non-amyloid protein aggregates such as collagen or denatured ovalbumin (Row 3 of **Table 1**). Thus simply being amyloidogenic does not necessarily result in an ability to seed elongation with other non-homologous amyloidogenic proteins; sequence similarity is required as well.

Sequence similarity is not the only important factor in determining the propensity for co-aggregation in amyloid formation as the precise positioning of compatible residues within the structure also plays a significant role. For example, co-incubation of murine and human Aβ40 and Aβ42 peptides revealed that interspecies fibrils will form for both Aβ40 and Aβ42 alloforms (Row 4, **Table 1**)[18] despite the three residue difference in the mouse and human amino acid sequences. This is because the residue differences between human and murine Aβ (Arg5Gly, Tyr10Phe, and His13Arg) are located toward the N-terminal region of the peptides, a region not thought to take part in the β-structure of Aβ fibrils.[16] Interestingly the mixed fibrils containing murine and human Aβ42 were more stable in solubilizing buffers than the homopolymeric human Aβ42 fibrils.[18] Murine Aβ peptide contains a larger amount of β-turn structure and is more strongly stabilized by hydrogen bonds than human Aβ.[19] The increased number of hydrogen bonds may stabilize the mixed fibrils, resulting in a greater resistance to buffer solubilization compared with homopolymeric human fibrils. This study demonstrates how mixing proteins with different primary sequences can result in the emergence of new fibril polymorphs with different biophysical properties.

The ability of human Aβ and its murine homolog to co-polymerize is consistent with the other studies outlined above, as reflected by the percentage of overall sequence similarity between the two peptides (~93% identity for Aβ40 and Aβ42). Other studies show that the presence of a non-homologous amyloidogenic protein can inhibit fibril formation because both the number and position of differences in the primary sequences are incompatible with fibril elongation and/or nucleation, e.g., human and murine β_2m (70% sequence similarity).[20] In an elegant study in which heterotetramers of transythyretin (TTR) were formed by mixing its human and murine subunits (Row 5, **Table 1**) Kelly and coworkers demonstrated that incorporation of murine TTR subunits protects the amyloidogenic human TTR from aggregating by stabilizing the native state.[21,22] Similar results were observed for the mutant Thr119Met of human TTR which protects wild-type TTR tetramers from dissociation both in vitro and in vivo (Row 6, **Table 1**).[23] In both cases amyloid formation is inhibited via kinetic stabilization of the resulting heterotetramers relative to their homotetrameric human counterparts. Thus, using variants of amyloidogenic sequences

Table 1. Interactions between amyloidogenic proteins

	Protein 1	Protein 2	Type of cross-interaction	Effect on interaction	References
1	Hen lysozyme	I55T Hen lysozyme (99.2% sequence identity to hen lysozyme) Turkey lysozyme (95% identity) Human lysozyme (60% identity) Human α-lactalbumin (36% identity) Bovine insulin (no identity)	Cross-seeding	Sequences must be > 60% identical to cross-seed efficiently	15
2	Aβ40	IAPP	Cross-seeding	Aβ fibrils will cross-seed IAPP monomer, but IAPP fibrils are inefficient at cross-seeding Aβ40 monomer	17
3	Aβ40	IAPP K^{IV} light chain LEN-(1–30) Polyglutamine Gln_{30} Polyglutamine Gln_{50} Ure2p Λ^{VI} light chain JT05 β_2-microglobulin Collagen Ovalbumin-RA	Cross-seeding	All the proteins listed showed less than 6% of the seeding efficiency of homologous seeding (Aβ40 seeds with Aβ40 monomer)	17
4	Human Aβ40 and Aβ42	Murine Aβ40 and Aβ42	Co-incubation	Interspecies fibril formation occurs and mixed fibrils are more stable than homopolymeric human Aβ fibrils	18
5	Human TTR	Murine TTR	Co-incubation	Murine TTR subunits stabilize the mixed tetramer and inhibition of fibril formation occurs	22
6	Human wild-type TTR	Human T119M TTR	Co-incubation	T119M TTR subunits stabilize the mixed tetramer and inhibition of fibril formation occurs	23
7	Human β_2m	Human ΔN6	Co-incubation	ΔN6 monomer causes β_2m fibril formation and formation of heteropolymeric fibrils with distinct morphology result	20,30
8	Aβ40	$Aβ40_{ARC}$	Co-incubation	Stabilization of protofibrils occurs	32
9	Tau	α-synuclein	Co-incubation	Both proteins induce fibrillation of each other into homopolymers	33,57
10	Aβ42	α-synuclein	Co-incubation	Aβ42 and α-synuclein form hybrid nanopore oligomers	36
11	Human vCJD prions and human classical CJD prions	Mouse PrP	Cross-seeding	Human vCJD prions, but not classical CJD prions, induce formation of prions in mice with faithful strain replication	26,38
12	Hamster PrP R- and S-strain	Mouse PrP	Cross-seeding	The fibrillar R-strain of hamster PrP acts as a catalyst and a template for mouse PrP fibril formation, whereas the fibrillar hamster PrP S-strain could only act as a catalyst	39
13	Rnq1	Ure2p and Sup35	Cross-seeding	The ability to cross-seed Ure2p and Sup35 by the prion form of Rnq1 is strain dependent	41
14	Sup35	Ure2p	Cross-seeding	Sup35 prion inhibits fibril formation of Ure2p	41
15	Aβ40	Aβ42	Co-incubation	Aβ42 monomer stimulates fibril formation of monomeric Aβ40, but Aβ40 monomer inhibits fibril formation of monomeric Aβ42	42
16	Aβ40	Aβ42	Cross-seeding	Aβ40 seeds both monomeric Aβ40 and monomeric Aβ42 equally well, but fibrillar Aβ42 is inefficient at seeding A40 monomer compared with fibrillar Aβ40	42
17	Aβ40 and Aβ42	PrP^C	Co-incubation	PrP^C inhibits Aβ fibril formation and traps it in an oligomeric state. Amyloid-β oligomers bind with nanomolar affinity to PrP^C and the interaction is required for toxicity	43,44
18	Insulin	TTR	Co-incubation	The kinetics of fibril formation of both protein partners must be evenly matched for cross-seeding to occur, as with insulin and TTR	45
19	Aβ40	Cystatin C	Co-incubation	Cystatin C inhibits Aβ40 formation	49,50

Table 1. Interactions between amyloidogenic proteins (continued)

	Protein 1	Protein 2	Type of cross-interaction	Effect on interaction	References
20	Aβ40	TTR	Cross-seeding	Preincubation of Aβ40 with TTR reduced cytotoxicity	51
21	Aβ40	Neuroserpin	Co-incubation	Aβ40 acts as a catalyst of neuroserpin polymerization	58
22	Aβ42	Neuroserpin	Co-incubation	Neuroserpin accelerates Aβ42 aggregation to form non-toxic oligomers	59
23	Murine β_2m	ΔN6	Co-incubation	Inhibition of fibril formation	20
24	Gliadin	Amylase/myoglobin	Co-incubation	Varied fiber morphologies form when the proteins are incubated together	60
25	Aβ6–40 Aβ26–42 Aβ34–42 Aβ26–43	Aβ26–39 Aβ26–40	Cross-seeding	All the proteins in the Protein 1 column accelerate fibril formation of monomeric Aβ26–39 and Aβ26–40	61
26	Aβ42	Pyroglutamate Aβ42	Cross-seeding	Cross-seeding accelerated fibril formation and resulted in increased toxicity	62
27	Aβ40	α-synuclein	Cross-seeding	Fibrillar α-synuclein is more effective than fibrillar Aβ40 at cross-seeding Aβ40, oligomeric α-synuclein is less effective than oligomeric Aβ40 at cross-seeing Aβ40. Aβ40 fibrils and oligomers are a poor seed of monomeric α-synuclein	63
28	Aβ42	α-synuclein	Cross-seeding	Fibrillar and oligomeric α-synuclein are less effective than oligomeric and fibrillar Aβ42 at seeding monomeric Aβ42. Aβ42 fibrils and oligomers are both less effective than α-synuclein fibrils and oligomers at seeding α-synuclein	63
29	Aβ40	BRICHOS	Cross-seeding	Extension of the Aβ40 lag phase in the presence of BRICHOS	64
30	Aβ40	IAPP mimic ([N-Me]G24, [N-Me]I26)-IAPP	Cross-seeding	An IAPP mimic inhibits Aβ40 fibril formation	65

Interactions between two amyloidogenic proteins of different primary sequences are shown. The types of interactions are either cross-seeding, where one protein is oligomeric or fibrillar and the other protein is monomeric, or co-incubation, where the two proteins are mixed as monomers. The effect of the interaction on the propensity for fibril formation is also described.

allows amyloid formation to be controlled and/or inhibited by introducing a non-homologous primary sequence into a fibril formation reaction.

Condition 2: Co-Aggregation Occurs by One Partner Protein Affecting the Rate of Fibril Formation of Another

Amyloidoses can occur when susceptible proteins are exposed to conditions that promote global unfolding of the native state[24] or enhance the population of amyloidogenic intermediates.[25] Statistically, mixed fibril formation is less likely than the generation of fibrils from a single sequence, as the production of heterofibrils requires a change in conformation of two proteins instead of one. However, this assumes that both partners must undergo a change to an amyloid conformer independently. By contrast with this observation, monomer-monomer interactions can promote protein unfolding, and/or formation of an amyloidogenic fold.[20,26] Thus if one protein partner acts to enhance the amyloid potential of another, the probability of forming heteropolymeric assemblies is increased.

An excellent example of one protein enhancing the amyloid propensity of another has been shown for β_2m and its N-terminally truncated variant ΔN6.[20] β_2m forms amyloid fibrils in vivo resulting in the disease dialysis-related amyloidosis (DRA).[27] However, in the absence of other co-factors or co-solvents human β_2m will not form fibrils in vitro at neutral pH within an experimentally tractable timescale,[28,29] while ΔN6 is highly aggregation prone.[20,30] Surprisingly, when monomeric human β_2m is mixed with monomeric ΔN6 fibril formation from both proteins occurs, resulting in heteropolymeric fibrils, even at neutral pH (Row 7, **Table 1**).[20,30] Interestingly ΔN6 was shown to stimulate fibril formation of human β_2m at sub-stoichiometric levels, with fibril formation occurring at a 1:99 ΔN6:β_2m molar ratio.[20] An atomistic description of how ΔN6 converts β_2m into an amyloidogenic form has been provided using NMR.[20] These studies showed that collision with ΔN6 results in increased conformational dynamics of human β_2m, allowing β_2m to undergo further structural rearrangements, critically via cis-trans isomerization of Pro32, that subsequently allows access to the amyloid state.

By enhancing the amyloid potential of β_2m, both β_2m and ΔN6 are incorporated into heteropolymeric fibrils when incubated at a 1:1 ratio.[30] As discussed in the introduction, aggregation of a single polypeptide sequence can result in a range of fibril structures dependent on the experimental conditions employed, such as pH, temperature, ionic strength, and

agitation.[31] In the case of mixed fibrils, for example those formed from β_2m and ΔN6 where both proteins are incorporated into the fibril structure, the possibilities for polymorphism are vast. Perhaps unsurprisingly then, the heteropolymeric fibrils formed from β_2m and ΔN6 do not resemble those formed from β_2m alone at acidic pH or from ΔN6 alone at neutral pH. Instead biophysical experiments have shown that the heteropolymeric fibrils of β_2m/ΔN6 form a unique polymorph that is thermodynamically less stable than both β_2m and ΔN6 homopolymeric fibrils.[30] These findings show that the heteropolymorphic fibrils do not possess "average" structural or thermodynamic characteristics resulting from mixing of the two precursors. Instead, through the enhancement of the amyloidogenic potential of one protein by another, a new area of the protein (mis)folding landscape is sampled, and a new polymorph is created.

The ability to endow an amyloid-prone form on a less amyloidogenic protein can also result in altered stabilities of on-pathway intermediates. The Arctic mutation (E22G) of Aβ40 results in a highly amyloidogenic protein ($A\beta40_{ARC}$) that forms both protofibrils and fibrils under conditions in which Aβ40 remains monomeric.[32] Interestingly in the absence of Aβ40, $A\beta40_{ARC}$ protofibrils will convert to fibrils, but in the presence of Aβ40 at a 1:1 molar ratio, the two proteins form mixed protofibrils that are incapable of further assembly. The mixed protofibrils are more stable than the protofibrils formed by $A\beta40_{ARC}$ alone and hence these species accumulate (Row 8, **Table 1**). Lashuel et al. postulate that these mixed protofibrils may be responsible for the enhancement of neurotoxicity and accelerated disease progression that is seen in patients carrying this mutation.[32] Co-aggregation of two different peptides, therefore, can alter the kinetics of fibril formation, and thereby alter the morphology and stability of intermediates, resulting in a prolonged lifetime of toxic species.

A highly specific enhancement of one protein's amyloidogenic potential by another is demonstrated by the co-incubation of tau with a familial mutant of α-synuclein (Ala53Thr). This amino acid substitution results in increased α-synuclein and tau inclusions, suggesting that it not only increases the propensity for α-synuclein to form fibrils, but also promotes the formation of tau inclusions.[33] Further in vitro studies have shown that co-incubation of tau and α-synuclein induces polymerization of both proteins and this effect was observed for all six tau isoforms (Row 9, **Table 1**).[33] Immunogold labeling revealed that the individual fibrils are mostly homopolymers, but some fibrils were labeled for both α-synuclein and tau but in spatially separate domains, suggesting that end-to-end annealing of the homopolymers occurs.[33] Furthermore, at low concentrations of α-synuclein, the presence of tau was required for α-synuclein fibrils to form. Similarly, tau requires α-synuclein pre-aggregation to induce the requisite conformational change to allow tau inclusion formation. Interestingly, this effect was specific to α-synuclein; other amyloidogenic proteins such as Aβ40 are unable to initiate tau polymerization.[33]

Enhancing a protein's amyloid potential not only results in increased formation of fibrils, but new species in the amyloidogenic pathway can also form by mixing protein monomers. Aβ has been shown to promote the aggregation and toxicity of α-synuclein in a dose- and time-dependent manner.[34] The two proteins may also interact in vivo as evidenced by an overlap in the pathology of AD and Parkinson Disease in Lewy Body Disease (LBD), where the initial signs are dementia followed by parkinsonism.[35] In samples of human brains from sufferers of LBD Aβ and α-synuclein were shown to co-immunoprecipitate, but no interaction between the two proteins was observed in non-demented controls. In vitro studies have shown that solubilized Aβ42 induces formation of α-synuclein tetramers and higher-order oligomers (Row 10, **Table 1**).[36] Electron microscopy images of the mixture after 6 h show well-defined hybrid ring oligomeric structures 9–15 nm in diameter, which may form functional cation nanopores.[36] Mixing two amyloidogenic proteins, therefore, can change the stability and characteristics of species that form during amyloid assembly, in this case resulting in the formation of a hybrid nanopore that could be involved in disease progression.

Condition 3: Structure and Post-Translational Modifications Affect the Efficiency of Cross-Seeding

One of the most striking examples of post-translational modification altering protein co-aggregation is in prion diseases. In these disorders the efficiency of cross-species infectivity is partly determined by primary sequence, but also relies on compatible structures and post-translational modifications (typically glycosylation).[37] Furthermore, to efficiently cross-seed, both the seed and the monomer must be able to adopt the same amyloidogenic conformation.

The importance of structure in prion propagation was demonstrated with the bovine spongiform encephalopathy (BSE) outbreak in the UK in the mid-1980s which resulted in the infection of more than 2 million UK cattle.[37] This was followed by an outbreak of a novel human prion disease, variant Creutzfeldt-Jakob disease (vCJD) in the mid-1990s. CJD is not a new disease: "classical" CJD prions, formed from spontaneous misfolding of the prion protein (PrP), occur at a rate of approximately one case per 1 million population per year.[37] However, despite sharing identical primary sequences, classical CJD prions and vCJD prions behave very differently in their ability to convert PrP^{C} (the non-infectious form of PrP) to PrP^{Sc} (the prion form) in a new species. This was shown when human classical CJD prions were used to infect wild-type mice, wherein no transmission was observed. However, when human vCJD prions were used instead, transmission was much more successful and the resultant mouse PrP showed faithful strain replication of the vCJD strain.[38] Fascinatingly, this shows that two proteins with identical amino acid sequences can experience different species barriers, confirming that prion propagation is conformation dependent (Row 11, **Table 1**).

Although the vCJD and classical CJD strains of PrP have identical primary sequences, they differ in their glycosylation patterns. Classical CJD strains are

modified predominantly with low molecular mass glycosylation, whereas the vCJD strain has high molecular mass glycosylation.[26] Furthermore, the ratio of the three PrP glycoforms (unglycosylated, monoglycosylated, and diglycosylated) is faithfully maintained on passage in transgenic mice expressing human PrP.[26] Similarly, transmission of human prions and bovine prions to wild-type mice results in murine PrP with glycoform ratios that correspond to the initial inocula.[26] Interestingly, PrP glycosylation occurs before conversion to PrP, thus the different glycoforms may be determined by initial PrP conformation. Different cell types may also glycosylate proteins differently, thus particular PrP glycoform strains may replicate most favorably in cell types with a similar PrP glycoform expressed on the cell surface.[37] Glycosylation similarities (or differences) between PrP and the infectious prion may help to explain the different incubation periods seen in individuals with prion disease.[26] Thus post-translational modifications can be crucial in determining the propensity of a protein to cross-seed, as well as affecting the location and timescale of disease developing.

In a recent study the importance of strain for cross-species infectivity was demonstrated using two morphologies of hamster prion protein, R and S.[39] The two strains were formed using the same stock of recombinant PrP under identical solvent conditions, but with different agitation modes; the S-strain was produced under shaking, the R-strain was produced with rotation. The R- and S-fibrils display substantial differences in their cross-β cores, epitope exposure and morphology.[40] Despite sharing the same amino acid sequence, the difference in morphology between the R- and S-fibrils has a profound effect on their ability to reproduce their strain faithfully in a different species (mouse). The hamster R-fibrils successfully recruit mouse-PrP into fibrils and maintain their morphology, thus acting both as a so-called "catalyst" and a template for fibril formation of mouse PrP. By contrast, the hamster S-fibril form also acts as a catalyst for structural conversion of mouse-PrP, but does not act as a template, i.e., the strain morphology was not propagated (Row 12, **Table 1**).[39] In the latter case, fibril elongation occurred but involved mouse-PrP switching to a new conformational state that resembled the R-fibril form. This demonstrates that primary sequence (condition 1) is not wholly responsible for the ability to act as an enhancer of amyloidogenicity in another protein (condition 2). Instead structure also affects the ability to transmit disease across a species barrier.

Yeast prion proteins have also provided a powerful model for developing understanding of both sequence and strain effects in prion propagation. When the yeast protein Rnq1 is aggregated into its prion form, it can promote prion formation of two other proteins, Sup35 and Ure2p.[41] Interestingly the efficiency with which the prion form of Rnq1 can cross-seed the latter two proteins is dependent on its strain. One Rnq1 prion strain will seed Ure2p inefficiently, but will seed Sup35 efficiently. However an alternative Rnq1 prion strain behaves in the opposite manner, inefficiently seeding Sup35, but efficiently seeding Ure2p. Both Rnq1 prion strains can propagate their morphology by homologous seeding, yet their ability to cross-seed other proteins is entirely dependent on their structure, rather than their (identical) amino acid sequence (Row 13, **Table 1**).[41] Further revelations from yeast models show that not all prions can cross-seed, and some can even inhibit homologous seeding.[41] For example, the prion form of Sup35 will inhibit Ure2p prion seeds from seeding non-prion Ure2p, through "poisoning" of the Ure2p prion seeds (Row 14, **Table 1**).[41]

The importance of structure in amyloid propagation is not limited to prion interactions. Despite Aβ40 and Aβ42 differing by only two residues, the two peptides have different effects on each other's aggregation propensity. In co-polymerization experiments Aβ42 monomers stimulate Aβ40 monomers to aggregate, whereas Aβ40 monomers shows a concentration-dependent inhibitory effect on Aβ42 fibril formation (Row 15, **Table 1**).[42] However, the same effect is not seen in cross-seeding experiments (Row 16, **Table 1**),[42] where one partner is in fibrillar form and one partner is monomeric. In this case Aβ42 fibrils are less efficient than fibrillar Aβ40 at inducing fibril formation of monomeric Aβ40. Why does Aβ42 efficiently stimulate Aβ40 amyloidogenesis only when it is monomeric, and not when in the fibrillar form? This phenomenon is explained by the importance of structure, as well as primary sequence for promoting amyloid formation. Accordingly, it is Aβ42 oligomers, rather than fibrils, that are the optimal template for incorporating Aβ40 monomers, and these are mainly populated during incubation of soluble Aβ40 and Aβ42 (co-polymerization), rather than when Aβ42 is added as fibrils (cross-seeding). Thus, although primary sequence is important in allowing Aβ42 and Aβ40 co-aggregation to occur (condition 1), and Aβ42 is an enhancer of Aβ40 amyloidogenesis (condition 2), the inclusion of the structure of Aβ42 aggregates must also be considered in assessing the likelihood of co-aggregation occurring.

A final example of a structure-dependent interaction of two amyloidogenic proteins is between PrP and Aβ. Interactions between Aβ42 oligomers and PrP leads to the inhibition of long-term potentiation in hippocampal slices from mice.[43] Interestingly, experiments using NMR have shown that PrP does not interact with disordered monomeric Aβ40, but Aβ40 must first change its conformation, possibly forming a misfolded monomer or dimer.[44] These studies not only highlight the importance of structure in determining co-aggregation, but also show that interactions are not limited to the monomer-monomer or monomer-fibril stages in fibril formation. Instead, recognition between two amyloidogenic proteins can be specific for a key intermediary structure in the amyloid formation pathway.

Conclusions

This review broadly categorizes interactions between two amyloidogenic proteins into "conditions" that prescribe the likelihood of a co-polymerization or cross-seeding event occurring. The first condition is that both partners must have a similar amino acid sequence. Although the importance of the protein backbone in fibril stability means most proteins may have the propensity to form fibrils under defined conditions,[14] side-chain packing

also plays a vital role.[5] Interestingly the ability to cross-seed is not necessarily reciprocal, even between closely related amyloid proteins.[17] The second condition is that at least one of the partners must enhance the amyloidogenic potential of the other, such as the effect of ΔN6 on β_2m[20,30] or α-synuclein on tau.[33] Finally, the third condition is that structure and/or post-translational modifications can affect the efficiency of cross-seeding, for example in prion propagation across different species. The three conditions are not mutually exclusive: primary sequence can influence fibril/oligomer structure, and a similarity in sequences between amyloidogenic proteins with their less amyloidogenic partners is important in defining the ability of proteins to co-polymerize. The network of hydrogen bonds within a fibril is known to be vital for monomer addition, and this would be influenced by both side-chain and fold. The conditions outlined here are also not comprehensive: another property that can regulate the propensity for co-polymerization is similar aggregation kinetics of the co-incubated monomers (Row 18, **Table 1**).[45] Additionally the ratio of the two components may also be vital: under normal physiological conditions the Aβ42:Aβ40 ratio in the brain is ~1:9.[46] However, in familial AD patients a ratio of 3:7 Aβ42:Aβ40 is observed, suggesting that a change in the proportion of the two alloforms may be significant in the development of AD.[47]

Examples of co-polymerization between unrelated sequences in vivo are relatively rare. The BSE crisis followed by the outbreak of vCJD has shown that cross-species prion transmission can occur.[37] An increased risk of AD has been shown for type II diabetes sufferers, suggesting a possible link between Aβ and IAPP aggregation.[48] In fact, Aβ seems to be promiscuous, with in vivo interactions reported between Aβ and cystatin c (Row 19, **Table 1**)[49,50], TTR (Row 20, **Table 1**)[51], and neuroserpins (Rows 21 and 22, **Table 1**).[52] The lack of more examples in vivo suggests that perhaps all three conditions described here need to be met, in a timely and spatially defined manner, for co-polymerization and/or cross-seeding to occur. Additionally the rapid increase in examples reported in the literature of co-polymerization and cross-seeding between different amyloidogenic proteins in vitro confirms the possibility for co-polymerization, giving weight to a possible in vivo relevance to at least some of these interactions.

Interactions between unrelated, or sequence distinct amyloidogenic proteins are fascinating and important because of the possibilities for polymorphism that arise from co-aggregation. In the introduction of this review the eight different classes of steric zippers that comprise the amyloid core were discussed[5] and detailed studies of polymorphism in homopolymeric fibrils have been performed.[1,4,53] Additionally polymorphism can occur at the earliest stages of aggregation, with subsets of oligomers showing structural polymorphism that also affects the ability of the species to be toxic (reviewed in ref. 54). Polymorphism has been induced in proteins with the same sequence through alteration of solution conditions such as pH, agitation, temperature and/or ionic strength.[31,55] Fibril length can also be controlled via fragmentation, allowing the physical dimensions and surface interactions of fibrils to be studied.[56] However, more parameters are also open to manipulation in systems that co-aggregate, such as the ratio of the protein species involved and the identity of the species that are mixed (monomers, oligomers or fibrils). By adjusting these different factors, in addition to altering experimental constraints, the potential repertoire of species that can form, both at the level of oligomers and fibrils is vast (see the schematic in **Fig. 1B**). Furthermore, as all proteins are potentially able to form amyloid,[14] and some amyloidogenic proteins can cross-seed unrelated polypeptide sequences, the risk of co-polymerization could potentially extend to the entire proteome. Unravelling the different structural possibilities for oligomer and fibril architectures and defining their physical and biological characteristics will be challenging. However these multi-component assemblies will need to be defined for developing our understanding of the causative agents of amyloid disease, as well as to harness the future potentials of amyloid fibrils as designer nanomaterials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Note Added in Proof

Recent work has shown that a specific and transient protofibrillar species of Abeta42, with a novel triple helical structure, is the most potent aggregate involved in the interaction between PrP and Abeta.[66] This works supports the importance of structure for influencing amyloid interactions between different proteins.

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