

Functional amyloid – from bacteria to humans

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Amyloid – a fibrillar, cross β -sheet quaternary structure – was first discovered in the context of human disease and tissue damage, and was thought to always be detrimental to the host. Recent studies have identified amyloid fibers in bacteria, fungi, insects, invertebrates and humans that are functional. For example, human Pmel17 has important roles in the biosynthesis of the pigment melanin, and the factor XII protein of the hemostatic system is activated by amyloid. Functional amyloidogenesis in these systems requires tight regulation to avoid toxicity. A greater understanding of the diverse physiological applications of this fold has the potential to provide a fresh perspective for the treatment of amyloid diseases.

Amyloid is a unique protein quaternary structure

Proteins usually adopt well-defined, 3D structures as a consequence of their sequence, their environment, and the folding enzymes and chaperone proteins they encounter [1,2]. Although some natively unfolded examples exist (e.g. α -synuclein, tau and A β) [3], proper protein function typically depends on forming the correct structure. Many folded proteins are soluble and globular; however the diversity of functional protein folds has increased considerably in recent years. New structural classes with unusual morphologies and non-canonical folds have been described [3,4], of which functional amyloid represents an intriguing example.

Amyloid is a fibrous quaternary structure formed by the assembly of protein or peptide monomers into intermolecularly hydrogen bonded β -sheets [5] (Figure 1a,b and Box 1). Amyloid was discovered more than a century ago in the context of disease. Alzheimer's disease is perhaps the best-known and most clinically relevant amyloid disease, characterized by neurodegeneration and extracellular amyloidogenesis [5,6]. Subsequently, amyloid formation has been associated with a variety of diseases including type II diabetes, Parkinson and Creutzfeldt–Jacob disease [5]. One hallmark of these diseases is amyloid fibers, which bind the amyloidophilic fluorophores thioflavin and Congo red (see Glossary) [7] and exhibit cross β -sheet X-ray fiber-diffraction patterns (Figure 1c). The factors that initiate amyloidogenesis, and thus disease, are poorly understood in most cases. Small oligomeric species, thought to be intermediates in fiber formation, are linked to tissue damage and disease progression because amyloid, and

especially amyloidogenic intermediates, is toxic to cells [8,9]. Amyloidogenic proteins are diverse in sequence and share few characteristics – they can be large or small, catalytic or structural, abundant or sparse. The lack of sequence or structural homology for amyloidogenic proteins reinforces the notion that amyloid is a primitive structure that can be generated by many polypeptide sequences. Thus, it has been hypothesized that amyloid has existed for as long as proteins, and was probably a prominent fold early in the evolution of life [10].

The functional amyloid hypothesis states that organisms have evolved to take advantage of the fact that many polypeptides can form amyloid, despite the fact that amyloid can be toxic [11–16]. However, the discovery of functional amyloid was surprising because it had been associated solely with human diseases for over a century before its physiological role was discovered. Functional amyloid was identified initially within the past decade in several lower organisms, including bacteria [13,14], fungi [11,17–19] and insects [15] and subsequently in humans [12] (Table 1). In addition, several functional amyloid candidates have been reported [20–22], although there is insufficient evidence to positively classify these as amyloid. Here, we describe the discovery of functional amyloid with a particular focus on the molecular and cellular adaptations necessary to harness amyloid for function in comparatively simple bacteria and then humans.

Lower organisms use amyloid as a structural component

Bacteria, fungi and insects use amyloid fibers for their unique mechanical and biological properties. Amyloid

Glossary

Congo red and Thioflavin S/T: fluorescent dyes used to detect the presence of amyloid in the clinic and in the laboratory.

Cross β -sheet: the amyloid fiber structure is characterized by β -strands that are perpendicular to the fiber axis. This fold is called the cross β -sheet (see Figure 1 and Box 1).

Downhill polymerization: downhill polymerization occurs when all monomer addition events, including dimerization, are energetically favorable. Downhill polymerizations do not have a lag phase.

Nucleated polymerization: a mechanism used to explain the unusual kinetics displayed by some amyloidogenesis reactions. In a nucleated polymerization, there is a lag phase in aggregate appearance that corresponds to the energetically unfavorable, slow formation of the highest-energy oligomeric species on the aggregation pathway, the nucleus. Fiber extension after nucleus formation is rapid.

Prion: a prion is a protein with a conformation, structure or assembly that is infectious and self-propagating.

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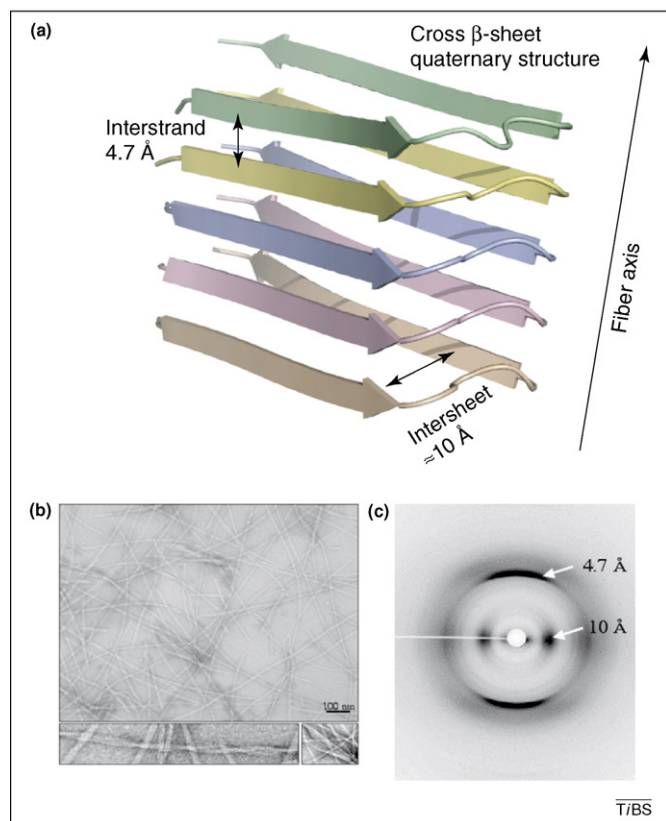


Figure 1. Amyloid is a fibrous protein quaternary structure that has a cross β -sheet fold. (a) The A β (1–42) fiber structure obtained from NMR and complementation mutagenesis methodology reveals the characteristic cross β -sheet amyloid structure (PDB code: 2BEG) [68]. (b) A transmission electron micrograph of negatively stained amyloid fibers formed from full-length islet amyloid polypeptide (IAPP). Higher magnifications (lower panels) reveal twisted-roped and sheet-like arrangements of individual protofilaments. (c) An X-ray fiber-diffraction pattern from partially aligned A β (1–42) amyloid fibers associated with Alzheimer's disease exhibiting the characteristic reflections at 4.7 Å and ≈ 10 Å. The meridional reflection at 4.7 Å results from the interstrand repeats, and the ≈ 10 Å equatorial reflection arises from intersheet packing. Molecular graphics were produced with Pymol (<http://www.pymol.org>). Part (b) reproduced, with permission, from Ref. [76]; part (c) reproduced, with permission, from Ref. [76].

fibers have a yield-strength comparable to steel [23] and are protease and detergent resistant – properties that have been harnessed by a variety of organisms. Bacteria such as *Escherichia coli* and *Salmonella* spp. employ extracellular amyloid, known as curli, to help create a proteinaceous matrix that enables surface adhesion and colony formation [13,24,25]. The primary structural component of the curli fibers, CsgA, is secreted from bacterial cells in a soluble form [13,24]. CsgB nucleates fibrillization of CsgA, and CsgG seems to be the outer membrane pore responsible for enabling the secretion of CsgA and CsgB. Curli fibers are generated in a highly regulated process involving six proteins encoded by two operons. Expression of the components of curli is regulated by several environmental conditions including temperature, salt and nutrient availability [24]. It has been hypothesized that curli fibers are involved in host invasion and pathogenesis through their activation of host extracellular matrix remodeling enzymes [24].

The filamentous, soil-dwelling bacteria *Streptomyces coelicolor* secretes a family of putatively amyloidogenic proteins known as the chaplins. Eight proteins comprise

Box 1. Amyloid is a unique protein quaternary structure

Amyloid is a non-covalent oligomer of extended, intermolecularly hydrogen-bonded β -sheets that laterally self-assemble to yield twisted fibers of ~ 10 nm in diameter. The amyloid fold is sometimes called a cross β -sheet fold because β -strands are orientated perpendicular to the fiber axis. Amyloid has a distinct X-ray diffraction pattern owing to its repeated structure and binds to amyloidophilic fluorophores including Congo red and thioflavin S/T [7].

The molecular structure of amyloid fibers has been extensively studied for >40 years. Recent efforts have yielded both NMR [68–72] and microcrystal-based [73] high-resolution structures of peptide-derived amyloid fibers, revealing an all-parallel β -sheet quaternary structure wherein residue n of strand x interacts with residue n of strands $x - 1$ and $x + 1$. Typically, two or more of these extended parallel β -sheet quaternary structures stack upon one another, affording a supramolecular structure referred to as an amyloid fibril. Many, if not most, polypeptides can form amyloid under modestly denaturing conditions [74], indicating that amyloid is a widely accessible, low-energy protein quaternary structure. Amino acid composition can bias a given polypeptide towards amyloid formation, although specific sequence determinants for amyloid formation remain poorly understood [75].

the chaplin family, ChpA–H. Chaplins isolated from the *S. coelicolor* cell wall can form thioflavin T (ThioT)-positive, β -sheet-rich fibers upon vortexing, indicating that the chaplin proteins form amyloid [14]. *S. coelicolor* hyphae are submerged and secrete ChpE and ChpH, which assemble into an insoluble mat of fibers at the air–water interface. By creating an amphipathic film at this interface, chaplins lower surface tension and enable hyphae to grow into the air, which is an important step in spore formation.

Like *S. coelicolor*, most fungi employ amphipathic proteins known as class I hydrophobins to enable formation of aerial structures such as spores or fruiting bodies. Similar to the chaplins, the hydrophobins also assemble into β -sheet-rich amphipathic fibers at hydrophobic–hydrophilic interfaces, including the air–water interface [11,18,26]. Hydrophobins can coat fungal structures with a hydrophobic shell enabling their adherence to other hydrophobic surfaces, including the surface of a host organism, thereby facilitating pathogenesis [27]. Recently, the nuclear magnetic resonance (NMR) structure of a monomeric EAS class I hydrophobin from *Neurospora crassa* was solved and revealed a two-stranded β -barrel with large disordered regions [28]. These monomers could polymerize, resulting in fibers composed of β -strands approximately perpendicular to the fiber axis (i.e. in a cross β -sheet quaternary structure). The fiber structure might not be identical to that of a typical amyloid; however, aggregated EAS hydrophobin exhibits many characteristics of amyloid, including Congo-red binding, fibrillar morphology and a β -sheet-rich structure. Class I hydrophobins are thus an example of amyloid-like fibers that function in aerial growth, surface attachment and pathogenesis.

Egg-laying creatures must protect their eggs from a myriad of hazards including proteases, microorganisms and physical stress. The eggshell serves as the primary defense against such threats and, in insects and fish, is made up primarily of chorion proteins. These proteins share a conserved central domain, which has been shown to form Congo-red-positive fibers *in vitro* with X-ray

Table 1. Functional amyloid exists in a wide variety of organisms^a

Species	Protein	Function	Experimental evidence	Refs
Bacteria				
<i>Escherichia coli</i> , <i>Salmonella spp.</i>	Curli	Biofilm formation, host invasion	EM, Congo red, ThioT and CD	[13,24,25,27]
<i>Streptomyces coelicolor</i>	Chaplins	Modulation of water surface tension (i.e. development of aerial structures)	CD, ThioT and EM	[14,27]
Fungi				
<i>Podospora anserina</i>	HET-s	Regulation of heterokaryon formation	EM, CD, FTIR, Congo red and NMR	[37,40–42]
<i>Saccharomyces cerevisiae</i>	URE2p	Regulation of nitrogen catabolism	Electron diffraction, X-ray, Congo red, EM and ThioT	[16,35,37]
<i>S. cerevisiae</i>	Sup35p	Regulation of stop-codon read-through	EM, Congo red, CD and X-ray	[16,19,33,37]
Most fungi	Hydrophobins	Fungal coat formation, modulation of adhesion and surface tension	AFM, CD, FTIR, ThioT, NMR, Congo red and X-ray	[11,18,26–28]
Animalia				
Insects and fish	Chorion proteins	Structural and protective functions in the eggshell	Congo red, X-ray, EM, FTIR and CD	[15,29,30]
<i>Nephila clavipes</i>	Spidroins	Structural (i.e. spider silk)	EM and CD	[20]
<i>Homo sapiens</i>	Pmel17	Scaffolding and sequestration of toxic intermediates during melanin synthesis	X-ray, Congo red, EM and ThioT	[12]

^aAbbreviations: AFM, atomic force microscopy; CD, circular dichroism; EM, electron micrograph; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; ThioT, thioflavin T.

diffraction patterns that are indicative of amyloid [15,29,30]. Furthermore, examination of intact insect eggshell chorion reveals lamellar arrays of fibers, the X-ray diffraction patterns of which are consistent with amyloid [15]. Thus, it seems that amyloid protects developing larvae from harm by virtue of its mechanical and chemical stability. From bacteria to insects, a wide range of organisms have evolved to exploit the physical properties of amyloid.

Fungal amyloid facilitates information transfer

Prion proteins were initially discovered in the context of the transmissible spongiform encephalopathies (TSEs), a group of neurodegenerative diseases that, in humans, includes Creutzfeldt–Jacob disease and kuru [31]. These diseases can arise spontaneously or can be acquired through contact with infected tissue. Curiously, the infectious disease-causing agent seems to be an amyloid or aggregated form of the normally soluble prion protein (PrP) [31]. PrP amyloid causes soluble PrP to become amyloid, thus propagating itself. Prions have come to be defined as proteins that can exist in at least two distinct forms, with one form being capable of recruiting additional members to that form. Amyloid shares many characteristics with prions, for example, soluble amyloidogenic proteins usually form insoluble amyloid fibers more rapidly if exposed to a small amount of seed amyloid [32]. Thus, amyloid is a self-replicating protein quaternary structure.

Several amyloid-based prions that are potentially functional have been identified, including those formed by the proteins Sup35p, URE2p and Rnq1p in the yeast *Saccharomyces cerevisiae* [16,19,33–39], and HET-s in the fungus *Podospora anserina* [17,40–42]. Each of these proteins reversibly forms cytoplasmic amyloid, leading to a particular phenotype that is acquired upon mating and passed from mother to daughter cells upon division. The prion aggregation state and resulting phenotype is heritable in an epigenetic, non-Mendelian manner. Sup35p is a translation termination factor that ensures the cessation of protein synthesis at nonsense (stop) codons. Aggregation of Sup35p

prevents translation termination and results in stop-codon read-through and protein C-terminal extension, which generates phenotypic diversity, presumably, by creating an altered proteome [43]. In its soluble form, URE2p regulates nitrogen catabolism by repressing the Gln3 transcription factor that controls expression of the Dal5 ureidosuccinate and allantoin transporter [34]. Dal5 is normally expressed only when good nitrogen sources are unavailable; however, when URE2p is aggregated, Gln3 becomes constitutively active and Dal5 is upregulated. Aggregation of both URE2p and Sup35p leads to a loss-of-function phenotype, presumably, because the proteins cannot bind their targets in the aggregated state. The HET-s prion regulates heterokaryon formation and functions as a meiotic drive element in *P. anserina* [17,44]. When fungal hyphae from adjacent colonies meet, they can fuse and generate multinucleated cells called heterokaryon that are important to mating and vegetative growth. The *het-s* locus has two alleles, *het-s* and *het-S*. The HET-s protein exists in either soluble or amyloid forms, whereas the HET-S protein only exists in a soluble form. When hyphae that contain aggregated HET-s fuse with those containing HET-S, a heterokaryon-incompatibility reaction takes place, which kills the heterokaryon and creates a barrier between the two colonies to prevent further fusion. However, fusion can take place between genetically identical colonies (HET-S–HET-S or HET-s–HET-s) and between those in which HET-s is soluble and those expressing HET-S.

Much evidence exists to indicate that prions and their associated phenotypes are biologically important. The Sup35p prion domain undergoes positive selection and is conserved among a diverse array of *Saccharomyces* spp. [45]. In addition, yeast populations harboring the aggregated form of Sup35p have selective growth advantages under some conditions [16,33,43]. Furthermore, the temporary growth advantages arising from aggregated Sup35p-mediated nonsense-codon read-through can become genetically fixed, indicating that Sup35p has an important role in phenotypic plasticity [33]. Selective growth advantages under certain conditions have also been observed for mutant

URE2p strains [16], indicating that the URE2p prion might function analogously to Sup35p. The HET-s prion mediates heterokaryon incompatibility, which is thought to limit the spread of viral infections by preventing the fusion of genetically dissimilar colonies. Aggregated HET-s is found in wild strains [46], indicating that the prion functionality is important. However, recent studies have shown that neither URE2p nor Sup35p is aggregated in 70 wild *S. cerevisiae* strains [47] and that URE2p is conserved in only a subset of *Saccharomyces* spp. [48,49]. Prions offer an intriguing example of how amyloid can be used for epigenetic inheritance, although careful study is required to establish their function(s).

Amyloid as a regulatory motif in human hemostasis and disease

The discovery of functional amyloid in humans is particularly interesting because of the compelling links between human pathogenic amyloid formation and disease. Recent work has linked amyloid and hemostasis in humans. The human hemostatic system regulates blood clot formation and removal through a series of proteolytic cascades. Upon activation, factor XII initiates a proteolytic cascade that, ultimately, leads to the conversion of fibrinogen to fibrin. Fibrin then polymerizes and forms a major component of blood clots [27]. Interestingly, factor XII seems to be activated by various amyloids *in vitro* [50]. Fibrin itself has a high β -sheet content [51] and stains with Congo red under certain circumstances [52]. Furthermore, fibrin-derived peptides assemble into amyloid fibers [52]. Therefore, blood clots composed of aggregated fibrin probably contain cross β -sheet structures and could be composed primarily of amyloid.

Polymerized fibrin is degraded by the protease plasmin, which is generated from plasminogen by tissue-type plasminogen activator (tPA). Both tPA and plasminogen are recruited to fibrin clots, thereby increasing their effective concentration and leading to plasmin formation. Amyloid fibers derived from different sources, including fibrin peptides, can also function as a scaffold to activate plasmin [52]. Thus, cross β -sheet-based motifs seem to be important in targeting both factor XII and tPA activity. Therefore, amyloid could represent a crucial regulatory element in coagulation and clot-clearance mechanisms.

Interestingly, microbes seem to exploit the amyloid-based components of the hemostatic system to enhance virulence. *E. coli* and *Salmonella* spp. that generate amyloid have an advantage in colonization and tissue invasion [27,53]. In *E. coli*, curli amyloid-fiber formation confers the ability to bind tPA and generate plasmin [27]. Plasmin is a non-specific protease and can degrade extracellular matrix components including laminin and fibronectin in addition to fibrin. Amyloid-mediated plasmin activation has been hypothesized to enhance bacterial cell invasion and virulence via extracellular matrix breakdown [27]. These data indicate that hemostasis, amyloid and microorganismal pathogenesis are closely related and demonstrate the interplay between eukaryotic and prokaryotic amyloid. The exciting hypothesis that amyloid motifs are involved in the formation and disassembly of blood

clots illustrates the potential physiological scope and importance of amyloid in humans.

Amyloid orchestrates the chemistry of melanin biosynthesis in mammals

Amyloid composed of the protein Pmel17 is important in the biosynthesis of melanin in mammals, both facilitating and regulating the chemical reactivity of melanin small molecule quinone precursors [12]. Pmel17 was discovered in connection with the *silver (si)* recessive pigmentation defect in mice [54]. *Si/si* mice display a progressive pigmentation defect due to loss of melanocytes [54]. Melanocytes are cells found in the skin and eyes that are responsible for synthesizing melanin from tyrosine-based indolequinone monomers. Melanin synthesis takes place in highly specialized secretory lysosome organelles called melanosomes.

Recent work has illustrated that Pmel17 is responsible for forming the fibrous structures found in stage II melanosomes [55] (Figure 2). These fibers consist of a fragment of Pmel17, referred to as $M\alpha$, which is derived from the full-length transmembrane Pmel17 protein by proprotein convertase cleavage [54,56]. Isolated melanosomes containing $M\alpha$ fibers stain with Congo red and thioflavin S. Furthermore, recombinant $M\alpha$ forms Congo-red- and ThioT-positive β -sheet-rich fibers that exhibit an X-ray diffraction pattern typical of amyloid [12].

The function of $M\alpha$ amyloid within the melanosome is related to templating the chemical synthesis of melanin (Figure 2a). Interestingly, both recombinant $M\alpha$ fibers and $M\alpha$ derived from biological sources accelerate the polymerization of small molecule melanogenic precursors such as 5,6-indolequinone (DHQ) into melanin *in vitro* [12,54]. $M\alpha$ amyloid binding to DHQ probably decreases the entropic requirements for DHQ polymerization by increasing the effective DHQ concentration and by orienting DHQ monomers along the $M\alpha$ fiber. Amyloid fibers composed of other proteins afford an equal rate enhancement, indicating that the amyloid structure itself is responsible for enhancing the melanin polymerization rate. Thus, the amyloid fold has a fundamental chemical function within the melanosome, effectively enhancing the rate of melanin formation by functioning as a multivalent receptor that templates DHQ polymerization.

Si/si mice that lack $M\alpha$ fibers can still generate melanin. However, these animals suffer from a progressive loss of melanocytes and cultured *si/si* melanocytes grow more slowly than wild type [54]. These data indicate that, in addition to enhancing the rate of melanin synthesis, $M\alpha$ fibers have an important role in protecting melanocytes from the toxic side effects of generating melanin. Melanin precursors including DHQ are highly cytotoxic [57], and probably diffuse across membranes. Leakage of these compounds out of melanosomes into the cytosol would have egregious effects on cellular function because these compounds are potent oxidizers and can react both as electrophiles and nucleophiles. $M\alpha$ amyloid seems to bind and sequester reactive melanogenic intermediates, preventing their diffusion out of the melanosome.

This hypothesis explains observations made in the *si/si* mouse and in chicken pigment mutants. Chicken coat color

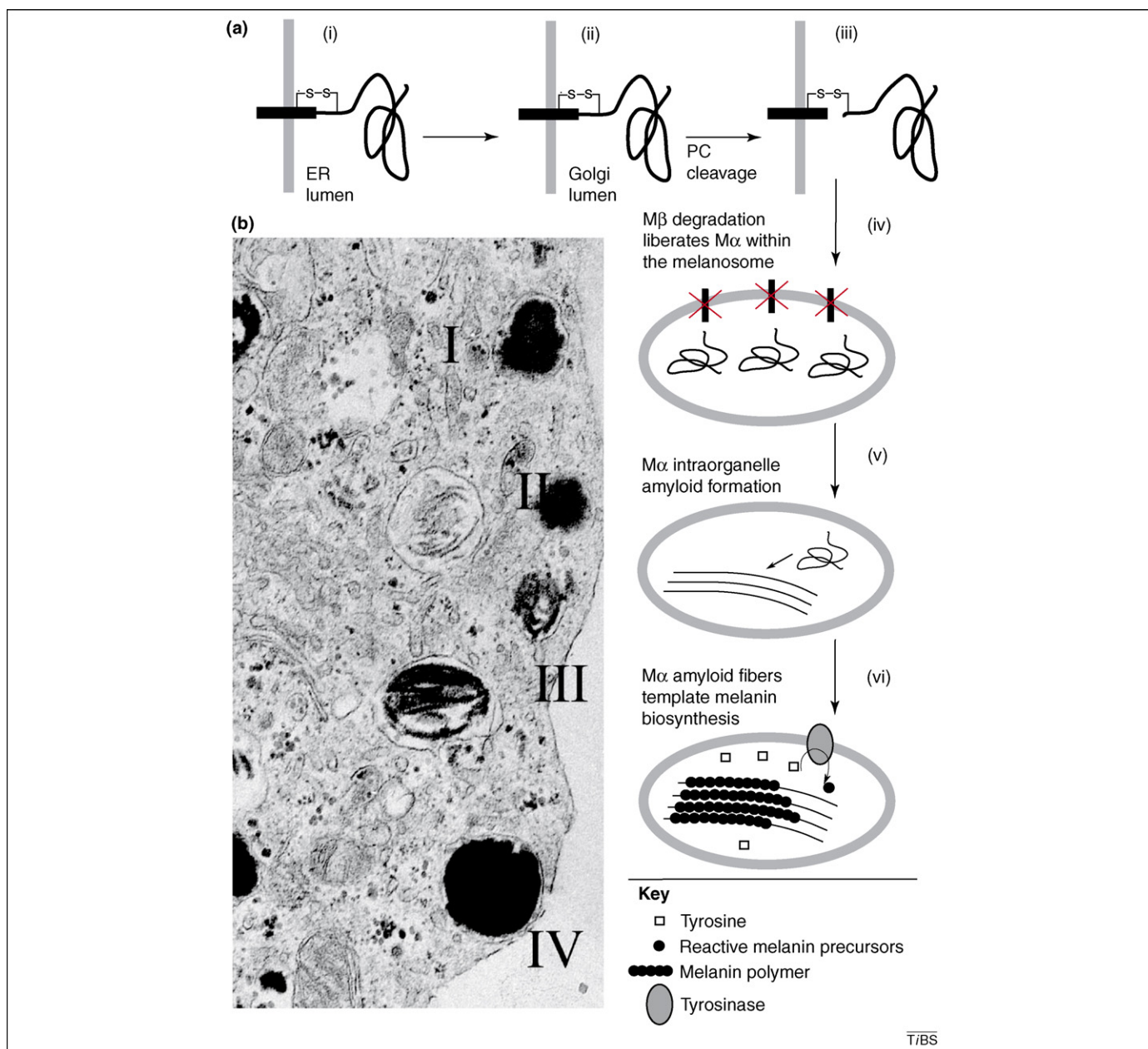


Figure 2. Pmel17 functions in melanin biosynthesis. (a) Pmel17 is synthesized as a transmembrane protein in the ER (i); it is trafficked first to the Golgi (ii) and finally to melanosome organelles. Proprotein convertase (PC) cleavage in a post-Golgi compartment creates a luminal fragment, M α , and a transmembrane fragment, M β . M α remains disulfide-bonded to M β to prohibit aberrant M α amyloidogenesis (iii). Degradation of M β releases M α from the membrane (iv), enabling M α to form amyloid fibers within the melanosome organelle (v). M α amyloid fibers orchestrate the synthesis of melanin from tyrosine-derived reactive indolequinones and protect the melanocyte from melanin-associated toxicity (vi). (b) Electron micrograph depicting the four-stage process of melanosome maturation. Stage I: melanosomes are vesicles of endosomal origin. Stage II: M α amyloid fibers form within the nascent melanosome [equivalent to (v) in part (a)]. Stage III: melanin appears along M α fibers [equivalent to (vi) in part (a)]. Stage IV: melanin occludes the M α fiber structure in melanosomes. Part (b) reproduced, with permission, from Ref. [77].

has been studied intensively, and one of the most common chicken pigment variants results from the *dominant white (I)* allele at the Pmel17 locus [58]. The *I* allele contains a three-amino-acid insertion in the transmembrane region of Pmel17. Thus, *I*^{*} chickens presumably do not have properly formed M α fibers. Like *si/si* mouse melanocytes, melanocytes from *I*^{*} chickens are lost prematurely *in vivo* [59]. Melanocytes isolated from *I*^{*} chickens have depressed superoxide dismutase (SOD) and glutathione (GSH) levels and die more rapidly in culture than wild-type melanocytes [60]. When added exogenously to the media of cultured *I*^{*} melanocytes, SOD and GSH restore a wild-type-like lifespan [60]. Melanogenic intermediates

including DHQ are potent oxidizers and their release into the cytosol would be expected to disrupt the oxidative balance of the cell. The oxidative damage and consequent melanocyte death observed in *si/si* mice and *I*^{*} chickens can be explained by a lack of M α amyloid, which seems to function to sequester reactive melanogenic precursor compounds such as DHQ within the melanosome [12].

Regulation of functional amyloidogenesis

Organisms from bacteria to humans have evolved to take advantage of the physical and biological properties of the amyloid fold. However, for several reasons, the potential toxicity of the process of amyloidogenesis poses a challenge

to organisms using amyloid for function. Some amyloid forms via a nucleated polymerization mechanism associated with a lag phase – an obvious disadvantage to organisms that need to respond rapidly and precisely to their environment. Unregulated release of functional amyloid seeds could initiate pathological amyloidogenesis. In fact, injection of functional amyloid fibers, such as curli and Sup35p, into an amyloid A (AA) reactive amyloidogenesis mouse model accelerates amyloid formation and disease progression [61]. Amyloid is highly resistant to degradation, making turnover a potential problem. Oligomeric amyloid assemblies and amyloid fibers are cytotoxic, and this toxicity seems to be inherent to the cross β -sheet structure [8,9]. Therefore, a key question is how the disadvantageous properties of amyloid are overcome.

Comparison of systems for which data exists indicates that different organisms have dealt with the regulation of amyloid formation in different ways. Yeast prions depend upon interactions with a system of chaperones to function properly. The first chaperone known to be involved in yeast prion maintenance is heat shock protein 104 (Hsp104), which is required for prion propagation and solubilizes aggregated proteins [16]. Recently, Hsp104 was shown to fragment Ure2p and Sup35p fibers, forming seeds that could enhance both *in vitro* amyloid formation and infectivity [62]. The yeast Hsp70 family members Ssa and Ssb have opposing roles in regulating the Sup35p prion by enhancing and suppressing prion formation, respectively [16,63]. Sis1, a yeast Hsp40 ortholog, functions in the maintenance of the Rnq1p prion [64]. These chaperone networks regulate yeast prions enabling propagation, degradation and preventing prion-associated toxicity [16,63,65]

Melanosome-associated Pmel17 fiber formation is also highly regulated, but in a different way. Proteolytic cleavage of the full-length Pmel17 amyloid precursor protein in a post-Golgi compartment [55] produces the amyloidogenic, luminal M α fragment and the transmembrane M β fragment (Figure 2). Full-length Pmel17 is incapable of forming fibers [56] so cellular compartments upstream of the melanosome, such as the ER and Golgi, are protected from aberrant M α assembly. After proteolytic cleavage, M α and M β remain joined by a disulfide bond [55], which might serve as a second physical barrier against improper assembly of M α . M α is, presumably, then released from the membrane when M β is degraded, thereby initiating amyloid formation. In addition, Pmel17 stability and processing seems to be positively influenced by the melanosomal MART-1 (melanoma antigen recognized by T cells) protein [66], indicating that MART-1 might be a functional regulator of Pmel17 folding and amyloidogenesis. Soluble, oligomeric amyloid assemblies [67] are currently thought to be more toxic than insoluble amyloid fibers [8]. In particular, pore-like amyloid oligomers that compromise membrane integrity have been observed and implicated in toxicity [8]. M α amyloid oligomers, if formed, could rupture the melanosomal membrane, causing leakage of toxic melanogenic precursor compounds, and could possibly compromise other membranes within the cell. Oligomers are probably not populated to any significant extent because M α amyloidogenesis is at least four orders of

Box 2. Functional amyloid – implications for disease

The discovery of functional amyloid in humans has several important implications for amyloid diseases, which have proven to be extremely difficult to understand and treat. Because pathology and physiology are often only subtly different, careful study of functional amyloid could yield new strategies for the treatment of amyloid diseases. Functional amyloid formation and degradation seem to be highly regulated, but the details are just beginning to emerge. Novel therapies for amyloid diseases could be based on enhancing and using existing functional amyloid regulatory systems to prevent pathological amyloidogenesis.

Presently, one of the methods under development for the treatment of amyloid disease involves directly inhibiting the formation of pathological amyloid. Given that pathological and functional amyloid share a common structure, some amyloidogenesis inhibitor drugs intended to prevent disease could disrupt functional amyloid formation. This could lead to deleterious side effects because functional amyloid seems to have a role in vital physiological processes in humans, including hemostasis and melanin synthesis (as discussed here). Thus, amyloidogenesis inhibitor drugs must be designed with sufficient specificity to avoid interfering with these functions.

magnitude faster than pathological A β or α -synuclein amyloid formation [12]. Rapid amyloidogenesis, probably resulting from an energetically favorable downhill polymerization, minimizes the concentration of oligomers and thereby avoids one of the toxic side effects of amyloid formation. In generating a protein that can form amyloid so rapidly, evolution seems to have circumvented one of the fundamental challenges facing functional amyloid – the generation of toxic oligomeric intermediates [12]. Careful regulation of the formation of the functional amyloid fold is necessary because of the potential for toxic side effects – yeast prions and Pmel17 are well-characterized examples illustrating this principle. Further study is required to understand the broader principles of regulation of functional amyloidogenesis and the potential interplay between functional and pathological amyloid (Box 2).

Concluding remarks – amyloid can be a functional protein quaternary structure

The amyloid fold underlies a large, diverse and growing list of functional protein structures (Table 1). Based on the presence of functional amyloid in organisms ranging from bacteria to humans, amyloid seems to be an evolutionarily conserved quaternary structure [12]. The unique physical and morphologic properties of amyloid and the fact that amyloid can be formed in a regulated manner suggest that amyloid could be as widespread in biology as some other common quaternary structures. Thus, amyloid does not always represent a ‘misfolded’ toxic protein structure; rather, it is a low-energy protein quaternary structure that can be formed in the context of either function or disease.

The work highlighted here indicates that further study of functional amyloid is warranted and might shed light on the treatment of amyloid disease. The precise molecular function of currently identified functional amyloids remains unknown in many cases. Improvement of amyloid detection methods, particularly in intracellular contexts, could enable the discovery of many currently unrecognized functional amyloids. Elucidation of the regulatory mechanisms that facilitate the use of amyloid for function might

reveal novel therapies for amyloid disease (Box 2). Increased availability of such data on greater numbers of functional amyloids in a diversity of organisms will yield a more accurate picture of the evolutionary and functional importance of the amyloid fold, and might lead to new therapeutic strategies for amyloid disease.

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