Amyloid as a natural product

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Amyloid fibrils, such as those found in Alzheimer's and the gelsolin amyloid diseases, result from the misassembly of peptides produced by either normal or aberrant intracellular proteolytic processing. A paper in this issue by Marks and colleagues (Berson et al., 2003) demonstrates that intra-melanosome fibrils are formed through normal biological proteolytic processing of an integral membrane protein. The resulting peptide fragment assembles into fibrils promoting the formation of melanin pigment granules. These results, along with the observation that amyloid fibril formation by bacteria is highly orchestrated, suggest that fibril formation is an evolutionary conserved biological pathway used to generate natural product nanostructures.

Protein folding disorders are now recognized to result in a large number of human diseases including those involving amyloid fibril deposition (Aridor and Balch, 1999; Kelly, 1996). Amyloid is generally considered to be a nonnative quaternary structure that forms in response to a defect in the normal folding or clearance pathways. Amyloid fibrils appear in electron micrographs as ∼100-Å diameter twisted rods composed of a cross–β-sheet structure that selectively bind the dye Congo red and the environment-dependent fluorphore thioflavin T. Two publications now provide tantalizing evidence that fibril formation (amyloidogenesis) may be an evolutionary conserved mechanism for creating biologically active quaternary structures. One paper, by Marks and colleagues (Berson et al., 2003, this issue), examines the mechanism of fibril formation in the melanosome, and a second describes the formation of extracellular fibers on the cell surface of *E. coli* and *Salmonella* that are involved in the colonization of inert surfaces (Chapman et al., 2002).

Melanosomes are subcellular organelles that specialize in the synthesis of pigment granules (melanin) in melanocytes and retinal epithelial cells (Marks and Seabra, 2001; Raposo

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and Marks, 2002). Melanin is a detergent-insoluble matrix whose assembly is a consequence of a carefully orchestrated metabolic pathway. Marks and colleagues (Berson et al., 2003) have now extended our understanding of this pathway. They found that mammalian melanocytes produce a glycoprotein called Pmel17, a type I integral membrane protein, that polymerizes into amyloid-like fibrils, on which melanins are sequestered and concentrated during the multistage process of melanosome biogenesis. Remarkably, they demonstrate that endoproteolysis is required for the intralumenal assembly of Pmel17 into fibrils. The key protease is the proprotein convertase furin, a Ca^{2+}-dependent membrane-associated protein that recycles between the cell surface, endocytic compartments, and the trans-Golgi network. Furin cleaves Pmel17 during the maturation of stage I premelanosomes to stage II–III melansomes to yield a 28-kD β fragment associated with the membrane and an 80-kD α cytosolic fragment (Mα). Only the proteolytically processed Mα fragment of Pmel17 is able to form fibrils that can be recovered in a detergent-insoluble fraction, a characteristic of most amyloid fibrils.

In bacteria, electron microscopy has shown that the Curli protein (17.5 kD) forms a tangled fibrous matrix on the outside of the cell wall. Curli has all the hallmarks of typical amyloid (Chapman et al., 2002). Formic acid treatment is required to depolymerize the CsgA protein comprising Curli. Curli CsgA fibers are SDS resistant and adopt a cross–β-sheet amyloid fibril structure as revealed by far-ultraviolet circular dichoism spectroscopy, a red shifted Congo red spectrum and a thioflavin T binding–induced fluorescence identical to that exhibited by pathological amyloid fibrils. Curli amyloidosis, like melanosome biogenesis, is highly orchestrated by two *E. coli* operons, csgAB and csgDEFG. The CsgB protein, likely in collaboration with CsgF, is thought to nucleate CsgA fiber formation. The CsgG lipoprotein localizes to the inner leaflet of the outer membrane, possibly serving as the Curli assembly platform. CsgD is a FixJ-like transcription factor on the same operon with CsgE, -F, and -G, which may be assembly factors. Purification of CsgA-His$_6$ on a nickel column revealed a metastable fiber-free solution of CsgA. Upon standing, this CsgA-His$_6$ solution formed fibrils indistinguishable from those characterizing membrane-associated Curli. Thus, other genes in the operon may be required to prevent intracellular amyloidosis, consistent with the recent results of Lindquist and colleagues (Ma et

al., 2002), demonstrating that intracelulllar prion (amyloid) formation in yeast may be very toxic.

Extracellular Curli formation by bacteria together with the intralumenal formation of pMel17 Mα fibrous striations by mammalian cells demonstrate that amyloid-like fibrils can be a natural product—a quaternary protein nanostructure formed by biological systems. The fact that mammalian cells utilize a fibril structure to mediate function emphasizes that tuning of protein structure by proteolysis can be a very powerful determinant for expanding the biological diversity of polypeptides. It is apparent that *E. coli* and *Salmonella* go to great lengths to control amyloidogenesis, as evidenced by the dedication of at least two operons to harness the potential of the amyloid quaternary structure to perform a useful biological function, and to prevent misassembly elsewhere in the cell. It is almost certain that numerous mammalian genes are also used to control amyloid-like fibril formation within organelles such as the melanosome to allow to its potential to be recognized. Interestingly, the process of normal mammalian fibril formation in the melanosome pathway demonstrated in this issue (Berson et al., 2003) is similar to the regulated proteolytic processing of precursor proteins leading to the formation of other lysosome-related organelles, including lamellar bodies in lung epithelial cells and Weibel-Palade bodies in endothelial cells. The proposal that biological control of fibril formation is stringently controlled is consistent with the idea that transport from the ER to intracellular and extracellular compartments is likely to require rigid control of the assembly state of the protein (Aridor and Balch, 1999). These examples of biological fibrillogenesis from three different organisms are likely only a prelude to the discovery of other amyloid-like nanostructures that represent intermediates or end products in metabolic folding pathways.

Pathways whereby fibril functions as a natural product are strikingly reminiscent of the pathological process of amyloidogenesis, where a subset of "amyloidogenic" precursor proteins are proteolytically processed into amyloidogenic peptide fragments by one or more proteases (Kelly, 1996). Human amyloid pathologies known to require proteolytic processing of a precursor protein include Alzheimer's disease where the A-β peptide is liberated from a large APP precursor protein by β- and γ-secretases, secondary systemic amyloidosis where the SAA protein is liberated by a minimum of one proteolytic cleavage, and type II diabetes where the aberrant proteolytic processing of pro-islet amyloid polypeptide leads to amyloid inclusions in the endoplasmic reticulum. Most relevant to mammalian melanosome amyloidogenesis is familial amyloidosis of Finnish type (Chen et al., 2001) and familial British dementia (Kim et al., 2002). The D187N and D187Y variants of gelsolin are aberrantly processed by furin. Normally, wild-type gelsolin is stabilized by Ca^{2+} binding in the trans-Golgi resulting in the burial of the furin consensus site. In the mutants, this stabilization does not occur and furin is allowed access to a protein it does not normally cleave. Furin cleavage liberates an amyloidogenic fragment that then assembles into amyloid fibrils.

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