Protein Fibril Forming Disease

- Fibril Forming Diseases
  - Alzheimer’s Disease (AD)
  - CAG repeats (Huntington’s Disease)
  - Prion diseases
  - Therapeutics: Senile Systemic Amyloidosis (SSA)/Familial Amyloid Polyneuropathies (FAP)
1° sequence determines structure

Christian Anfinsen b. 1916 d. 1995
(Nobel Prize in Chemistry, 1972)

• 1950’s, hypothesized that information determining 3° structure of a protein resides in chemical features of its 1° sequence

• Experimental proof through reversible denaturation of several proteins, including ribonuclease

Fibril Forming Diseases

• Diverse group of medical disorders
• Conformational instability of proteins (i.e., misfolding)
• Protein aggregates — tissue deposition as amyloid aggregates
• Commonly form β-cross fibrils
β-cross

- Fibers: helical arrangement of β-strands perpendicular to the fiber axis
- Protein fibrils rich in β-sheets, even though native state of the proteins may lack β-sheets
- Large number of fibrils adopt β-cross conformation

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<td>inclusions</td>
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<td>proteins</td>
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*Pathogenic mutations are associated with a toxic gain of function.
†Pathogenic mutations are associated with a loss of function.

Taylor et al. Science 296:1991(02)
**Other Proteins in Fibril Forming Diseases**

<table>
<thead>
<tr>
<th>Amyloid Protein</th>
<th>Precursor</th>
<th>Systemic (S) or Localized (L)</th>
<th>Syndrome or Involved Tissues</th>
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<tr>
<td>AL</td>
<td>Immunoglobulin light chain</td>
<td>S, L</td>
<td>Primary</td>
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<td>AH</td>
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<td>Transthyretin</td>
<td>S</td>
<td>Myeloma-associated</td>
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<tr>
<td>ATTRI</td>
<td></td>
<td></td>
<td>Familial (prototype Portuguese, Japanese, Swedish)</td>
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<td>ATTR2</td>
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<td>L7</td>
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<td>β-amyloidoblin</td>
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<td>Secondary, reactive</td>
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<td>Aβ-Protein AL</td>
<td>Apolipoprotein AL</td>
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<td>C-cell thyroid tumors</td>
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<td>L</td>
<td>Cortical</td>
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</table>

* Preliminary, awaiting for confirmation.

Amyloidosis

- Insoluble, fibrous amyloid proteins
- Mainly extracellular spaces of organs and tissues
- Named by Virchow (1854) on basis of color of staining with iodide and sulfuric acid
- Amyl (Gk. am ylon starch)
- May have no apparent clinical consequence or may lead to severe pathophysiologic changes
- Resistant to proteolysis
- Congo Red binding and birefringence (green)
Other Features of amyloid plaques

• Also contain nonfibrillar components, e.g.,
  — serum amyloid pentraxin (SAP)
  — ubiquitin
  — chaperones (such as Hsp40)
  — glycosaminoglycans (GAG)

Common Features

• Amyloidogenic precursor at appropriate concentration

• Appropriate genetic background

• Abnormalities in proteolysis of fibril precursors and nascent amyloid fibrils

• Alterations in extracellular components (glycosaminoglycans, presence of ApoE)
Potential Treatments

- Reducing precursor production
- Inhibiting synthesis and extracellular deposition
- Promoting lysis or mobilization of existing deposits

QC = Quality Control

Table 1. Features of neurodegenerative disorders characterized by aggregation and deposition of abnormal protein.

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*Pathogenic mutations are associated with a toxic gain of function.
†Pathogenic mutations are associated with a loss of function.
Alzheimer’s Disease (AD)

- Described in 1907 of a 55-year-old woman by Alois Alzheimer
- Risk Factors: Old Age and Positive Family History
- 20-40% population > 85
- 2 million Americans

Neuritic “senile” plaques and neurofibrillar tangles

- Localized amyloidosis (cerebral)
- Two lesions:
  - Neuritic Plaques — Extracellular
    • $\text{A}_\beta$ peptide (also, proteoglycans, ApoE, $\alpha$, antichymotrypsin, and others)
  - Neurofibrillary tangles — Intracellular
    • abnormally phosphorylated $\tau$ protein (cytoskeletal)
Pre-disposing sites of mutations

Aβ (β-amyloid protein)

- Amyloid deposits — cerebrovascular walls and cores of neuritic plaques in AD and Down’s Syndrome patients
- APP = Aβ Protein Precursor
- Aβ 39-43 — Aβ42 pathogenic
- Normal biochemical function of Aβ unknown
- APP neuroprotective and neurotrophic (growth and survival of neurons)
- Cleavage by
  - β-secretase (BACE)
  - γ-secretase (PS1 and PS2)
Genetic Factors

- Amyloid Precursor Protein (APP)
  - chromosome 21 — Down’s Syndrome
  - Early-onset familial AD (FAD) have point mutations in APP — autosomal dominant

- Presenilins — affect $\gamma$-secretase
  - PS-1 on chromosome 14
  - PS-2 on chromosome 1

- Apo E
  - 3 alleles: $\varepsilon2$, $\varepsilon3$, and $\varepsilon4$
  - $\varepsilon4$ shows strong association with AD, but neither necessary nor sufficient.

Predisposing Mutations

CAG Expansion Disease

- At least 9 inherited neurodegenerative diseases are caused by expansion of glutamine repeats.
  - Huntington’s disease, Kennedy’s disease (spinal bulbar muscular atrophy), dentatorubro-pallidoluysian ataxia, 6 forms of spinocerebellar ataxias
- Dominant, autosomal, late-onset
- Progressive degradation of nervous system, usually fatal
- Caused by an unstable (CAG)$_n$ trinucleotide repeat — CAG encodes glutamine
Huntington’s disease

- Most common (incidence of 4 in $10^5$ in European populations)
- Genetic, autosomal dominant degenerative brain disorder
- Chorea (jerky, involuntary movements) and behavioral disturbance
- Degeneration of striatal medium-sized spiny neurons

Huntington’s inclusions (deposited in nuclei) contain huntingtin but also ubiquitin
Huntingtin

- HD gene on chromosome 4p, codes for huntingtin
  - Encoded on 67 exons spread over 180 kb of DNA
  - Polypeptide >3140 residues
  - CAG repeat is part of first exon and followed by CCG, CCA, and CCT repeats that encode Pro
- Huntingtin required for embryonic neurogenesis, but molecular function is unknown
- Huntingtin shows no homology to any known protein

Properties of glutamine repeats

- Proteins with repeats < 38 glutamine residues are harmless
  - Huntingtin normally has 35 repeats

- Repeats > 41 glutamine residues form toxic aggregates in the nucleus of neurons.
  - Likewise, proteins with repeats < 38 glutamine residues are soluble in vitro, but with > 40 glutamine residues precipitate as insoluble fibers.
Hydrogen Bonds

Disulfide Bonds

GNNQQNY: A detailed view of a β-cross spine

RNAase A — 10 x Q insertion forms fibrils

Domain swapping and β-cross fibrils


Domain Swapping

Closed Monomers  Open Monomers  3D Domain Swapped Dimer

Inter-domain Interface Hinge Loop "Swapped" domain Functional Unit (FU)
RNAase A — 10 x Q insertion forms fibrils

Complementation shows domain swapping occurs in solution

<table>
<thead>
<tr>
<th>Construct</th>
<th>Fibril formation</th>
<th>Congo red birefringence</th>
<th>Relative activity (%)</th>
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<tbody>
<tr>
<td>Wild-type RNase</td>
<td>No fibrils</td>
<td>No birefringence</td>
<td>100 (100)</td>
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<td>Q_{12}H12A</td>
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<td>Q_{12}H119A</td>
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<td>Q_{12}H12A + Q_{12}H119A</td>
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Sambashivan et al. Nature 437:266 (05)
Glutamine repeats

- In yeast, proteins which are rich in glutamine- and asparagine-rich domains form β-sheet rich aggregates and mediate inheritance of prion-like traits
  - e.g., Sup35 [PSI+] phenotype

- ~1% of proteins in mammals contain glutamine- or asparagine-rich domains

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Prion Diseases

• Infectious proteins
  – neurodegenerative diseases, the transmissible spongiform encephalopathies

• Lack nucleic acid and are composed of fibrils of prion protein (PrP)

Prions

• Humans
  – Sporadic Creutzfeldt-Jakob disease
  – Familial Creutzfeldt-Jakob disease
  – Gerstmann-Straussler-Scheinker syndrome
  – Fatal familial insomnia
  – Kuru (ritualistic cannibalism)
Transmissible

- Corneal transplantation, treatment with cadaveric HGH, variety of neurosurgical procedures
- Scrapie (sheep), bovine spongiform encephalopathy (BSE, mad cow disease), chronic wasting disease (elk and deer)

Conversion of PrP\text{C} to PrP\text{Sc}

- PrP\text{C} (PrP\text{sen}) is normal form of protein (function unknown)
- PrP\text{Sc} (PrP\text{res}) is pathogenic form

NUCLEATION-POLYMERIZATION

\[
\begin{align*}
\text{PrP}^\text{C} & \rightarrow \text{PrP}^\text{Sc} \\
\text{Nucleus} & \\
\end{align*}
\]
Structure of portion of PrP<sub>C</sub>

- Antiparallel β-strands are thought to be nucleation centers for the conversion of PrP<sub>C</sub> to PrP<sub>Sc</sub>

Domain Swapping in PrP<sub>C</sub>(90-231)

Zahn et al. PNAS 97:145 (00); Knaus et al. NSB 8:770 (01)
Infectivity

- PrP\textsuperscript{Sc} converts conformation of PrP\textsuperscript{C} to PrP\textsuperscript{Sc} form
- Species barrier observed in transmission between human and mouse, and between hamster and mouse.
  - Lack of species barrier between cattle (mad cow disease) and human?
Therapeutic strategies: SSA and FAP

• Senile Systemic Amyloidosis (SSA)
  – Amyloid fibrils infiltrate heart (cardiomyopathy)
  – Transthyretin amyloid deposition
• Familial Amyloid Polyneuropathies (FAP)
  – Early onset (30 vs. 80 yr)
  – Patients present with peripheral neuropathy and/or organ dysfunction as a result of massive transthyretin amyloid deposition

Transthyretin

• 14 kDa (prealbumin)
• Transporter for retinal-binding protein (carries 20% of vitamin A) and the hormone thyroxine
• Forms tetramers
Potential Therapies

- Stabilization of native form: Thyroxine or drugs that bind to transthyretin and stabilize tetramer

Flufenamic acid (Flu)

Stabilization of Native State of TR


Fibrils

<table>
<thead>
<tr>
<th>Plot symbol</th>
<th>TTR sequence</th>
<th>pH 8.0</th>
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<tr>
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<td>Leu-55-Pro</td>
<td>5.4</td>
<td>4.1 μM</td>
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<td>Val-26-Met</td>
<td>4.7</td>
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<tr>
<td></td>
<td>wild type</td>
<td>4.4</td>
<td>2.7 μM</td>
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Stabilization of the folded state

Unfolded $\rightleftharpoons$ Folded

$$K = \frac{\text{Folded}}{\text{Unfolded}}$$

$$K = \exp\left(-\frac{\Delta G}{RT}\right)$$

If $\Delta G$ is -5 kcal/mol, then $\frac{\text{Folded}}{\text{Unfolded}} \sim 4 \times 10^3$

If $\Delta G$ is -7 kcal/mol, then $\frac{\text{Folded}}{\text{Unfolded}} \sim 10^5$

Stabilization of the folded tetramer or Destabilization of the transition

<table>
<thead>
<tr>
<th>Small Molecule</th>
<th>$\Delta G_1$ (kcal/mol)</th>
<th>$\Delta G_2$ (kcal/mol)</th>
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<td>6</td>
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<tr>
<td>7</td>
<td>1.53</td>
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<td>8</td>
<td>1.57</td>
<td>1.54</td>
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<td>9</td>
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<td>2.97</td>
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<tr>
<td>10</td>
<td>1.69</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Hammarström et al., Science 299:713 (03)
FAP

• >60 mutations with varying phenotypes
  – V30M exacerbates disease
  – L55P early onset and rapidly progressing

  – T119M protective (in trans)