Protein degradation and defense against neurodegenerative disease
Prof. Alfred Goldberg

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Protein Degradation and Defense against Neurodegenerative Disease
Part 1 of 2

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Neurodegenerative diseases with inclusion bodies associated with ubiquitin and proteasomes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inclusion</th>
<th>Major constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s</td>
<td>Neurofibrillary Tau</td>
<td></td>
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<tr>
<td>Parkinson’s</td>
<td>Lewy body</td>
<td>α-synuclein, crystallins</td>
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<tr>
<td>Lewy Body Dementia</td>
<td>Lewy body</td>
<td>α-synuclein</td>
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<td>Amyotrophic lateral sclerosis</td>
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<td>G antibodies, neurofilaments</td>
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<td>Polyglutamine extension disorders:</td>
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<tr>
<td>Huntington’s</td>
<td>Nuclear Spinocerebellar</td>
<td>Huntingtin</td>
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<tr>
<td>Spinocerebellar ataxias, 1, 2 &amp; 3</td>
<td>Nuclear Spinocerebellar</td>
<td>Ataxias 1, 2 &amp; 3</td>
</tr>
<tr>
<td>Spinobulbar muscular atrophy (Kennedy’s)</td>
<td>Nuclear Spinocerebellar</td>
<td>Androgen receptors</td>
</tr>
</tbody>
</table>

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Abnormal proteins rapidly degraded in cells

- **Incomplete proteins** – nonsense mutations, incorporation of puromycin, premature termination
- **Missense proteins** – mutations, incorporation of amino acid analogs, biosynthetic errors
- **Free subunits of multimeric complexes** – excess components
- **Post-synthetic damage** – oxygen radicals, intracellular denaturation
- **Genetic engineering** – gene fusions, frame-shifts, incorrect localizations
- **Protein misfolding**
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2012 - an anniversary year

• Goldberg, AL. Degradation of abnormal proteins in E. coli.
  Proc Natl Acad Sci. 1972; 69: 422-426
• Prouty, WF and Goldberg, AL. Fate of abnormal proteins
  in E. coli. accumulation in intracellular granules before catabolism.
  Nature 1972; 240: 147-150

2012 - an anniversary year (2)

35 years old: The ATP-dependent pathway

• Etlinger, J and Goldberg, AL., A soluble ATP-dependent proteolytic
  system responsible for the degradation of abnormal proteins
  in reticulocytes. Proc Natl Acad Sci 1977; 74: 54-58

25 years old: The 26s proteasome

• Hough, R, Pratt, and Rechsteiner, M. J Biol Chem 1987;
  262: 8303-8313
  262: 2451-2457

The ubiquitin-proteasome pathway
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**Ubiquitin conjugation to protein substrates**

- Ub-activating enzyme (E1)
  - Two/cell
- Ub-conjugating enzymes (E2s)
  - 30-40 in mammals
- Ubiquitin ligases (E3s)
  - 650-1000 in mammals, specific for substrates and E2s

**Ubiquitin ligases important in neurological disease**

- UBE3A/E6AP – Angelman’s syndrome
- CHIP - ubiquitinates Huntingtin, SOD1
- Atrogin1, MuRF1, Trim32 - muscle atrophy
- Parkin, Nedd4 – Parkinson’s disease

CHIP, a chaperone-dependent ubiquitin ligase, uses Hsp70 and Hsp90 to recognize misfolded proteins

- Damage, denaturation
- Refolding
- Ubiquitination
- 26S proteasome
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Conditions that induce muscle wasting

Rapid
• Denervation, disuse, neurodegenerative disease
• Cancer cachexia
• Renal failure (Uremia)
• Fasting
• Glucocorticoids (Cushing’s syndrome)
• Diabetes
• Sepsis, AIDS
• Cardiac failure

Slow:
• Aging (sarcopenia)

Ubiquitin ligases induced when muscles atrophy

MURF1, TRIM32
Atrogin 1/MaFBx (SCF complex)

Knockout mice lacking Atrogin-1 (MAFbx) and Atrogin-2 (MuRF1) have reduced rates of denervation atrophy
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The involvement of P97/VCP in the degradation of misfolded proteins by the ubiquitin-proteasome pathway

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P97 is involved in human diseases

• p97/VCP/CDC48 is hexameric ATPase which with its partners, UFD1 and NPL4, is essential for the rapid degradation of misfolded ER protein and damaged cytosolic proteins
• p97 is found in PolyQ inclusions
• Overexpression reduces inclusion formation
• Mutants cause inclusion body myopathy, Paget’s disease, frontotemporal dementia and tauopathy

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Pathways for protein degradation

Proteasomes

Ubiquitin

Lysosomes

Cytosolic, nuclear & misfolded proteins
K48 Ub

Membrane & endocytosed proteins (ESCRT) K63 Ub

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- Studies in yeast have identified more than 27 ATG genes, most have mammalian homologs
- Rapamycin, which inhibits mTOR, activates autophagy and clears inclusions in cellular models of HD, PD and ALS
- Parkin, the E3 mutated in a form of PD, ubiquitinates defective mitochondria and targets them to autophagy by forming K63 chains

Pathways for protein degradation

Proteasomes
- Cytosolic, nuclear & misfolded proteins K48 Ub
- Membrane & endocytosed proteins (ESCRT) K63 Ub

Ubiquitin
- ATP

Lysosomes
- ATP

Cell proteins & organelles
- Autophagy (mitophagy)
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The Nedd4 ubiquitin-ligase ubiquitinates α-Synuclein

Why Nedd4:
1. Potential substrate recognition site (PY motif) on α-synuclein
2. Involved in ubiquitination of membrane-bound proteins
3. Abundant in the brain and up-regulated in brain injury and in Parkinson's disease

α-Synuclein is ubiquitinated by Nedd4

Ubiquitination by Nedd4 requires the C-terminal end of α-Synuclein

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In human neuroblastoma Nedd4 over-expression accelerates α-synuclein degradation and Nedd4 RNAi increases α-synuclein content. Conversely, Nedd4 RNAi increases α-synuclein content.

Pathways for protein degradation

Cytosolic, nuclear & misfolded proteins K48 Ub

Membrane & endocytosed proteins (ESCRT) K63 Ub

Different types of ubiquitin chains exist in vivo

K48-linked chains
Proteasomal degradation

K63-linked chains
Lysosomal degradation
Cell signalling

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Nedd4 is strongly expressed in pigmented neurons with Lewy bodies

Normal brain

Diffusely expressed Nedd4

PD brain

Ring-shape pattern around Lewy bodies

George Tofaris, Thomas Willis Brain Bank, Oxford