Lesson (15)

The Ubiquitin Proteosome Protein Degradation Pathway

Protein Degradation

Turnover of protein is NOT constant

Half lives of proteins vary from minutes to infinity

"Normal" proteins – 100-200 hrs

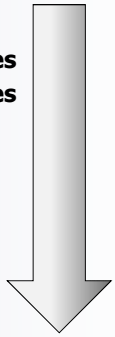
Short-lived proteins
regulatory proteins
enzymes that catalyze committed steps
transcription factots

Long-lived proteins
Special cases (dentin, crystallins)

Protein Degradation

Proteins are not degraded at the same rate

ENZYME	half-life
Ornithine decarboxylase	11 minutes
δ-Aminolevulinate synthetase	70 minutes
Tryptophan oxygenase	2 hours
HMG CoA reductase	3 hours
Glucokinase	1.2 days
Catalase	1.4 days
Tyrosine aminotransferase	1.5 hours
Lactic dehydrogenase	16 days



Protein Degradation

- **May depend on tissue distribution**

  Example: Lactic Acid Dehydrogenase

Tissue	Half-life
Heart	1.6 days
Muscle	31 days
Liver	16 days

- **Protein degradation is a regulated process**

  Example: Acetyl CoA carboxylase

Nutritional state	Half-life
Fed	48 hours
Fasted	18 hours

Two Sites for Protein Degradation

- **Lysosomal vesicles - 10-20% proteins**
  - **Extracellular proteins**
  - **Cell organelles**
  - **Some intracellular proteins**

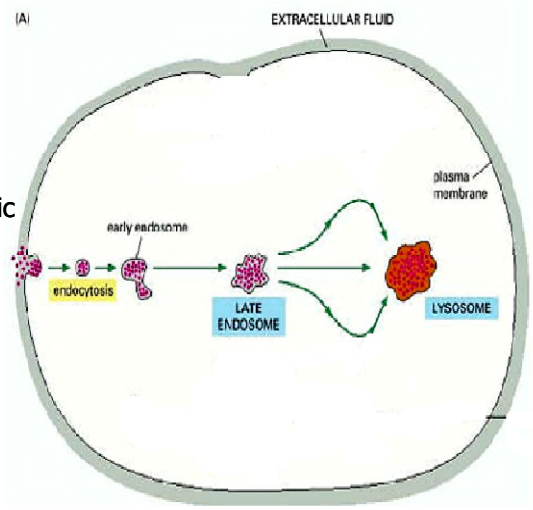
Basal degradation – non-selective
Degradation under starvation – selective for "KFERQ" proteins

- **Ubiquitin/Proteasome Pathway - 80-90% proteins**
  - **Most intracellular proteins**

Large (26S) multiprotein complex (28 subunits)
Degrades ubiquitinated proteins

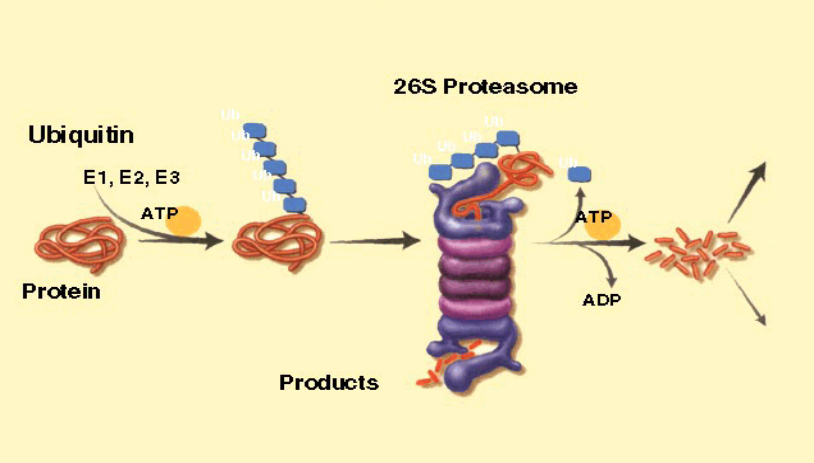
Lysosomal degradation

- Proteins delivered via endocytosis
- Lysosomes
  - The cellular dust-bins
  - Contain many hydrolytic enzymes
    - Proteases
    - Lipases
    - Glycosidases



Cytosolic protein degradation

- The Ubiquitin Proteosome Pathway



www.ihf.de/forschung/ popup/ubiquitin.html

2004 Nobel Prize in Chemistry

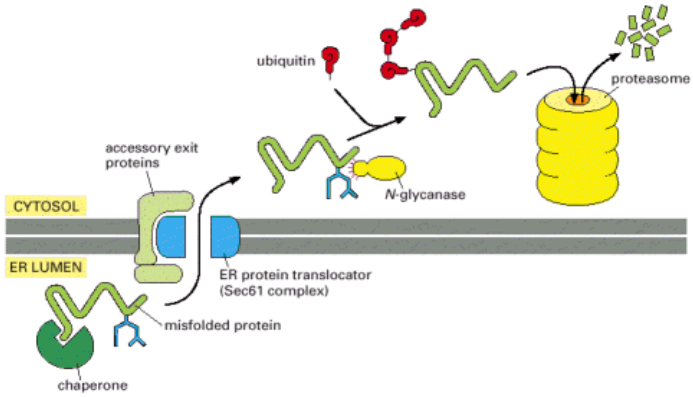
- The discovery of ubiquitin-mediated protein degradation
  - Aaron Ciechanover
  - Avram Hershko
  - Irwin Rose
- Cells give a chemical "kiss of death" to proteins that need to be destroyed.

Degradation of misfolded proteins

- Despite help from chaperones, more than 80% fold incorrectly
- Proteins are dislocated back into the cytosol
  - Oligosaccharides are removed
  - Deglycosylation is catalyzed by N-glycanase*
- One third of the newly made polypeptide chains are selected for degradation

* N-glycanase removes the oligo chains by cleaving the amide bond b/w the carbonyl group and the amino group of the original asparagine to which the oligo was attached. The deglycosylated polypeptide is rapidly ubiquinated by ER bound UB-conjugated enzymes and fed into the proteosome for degradation.

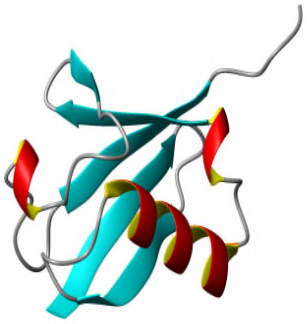
The Export of Misfolded Proteins



The export and degradation of misfolded ER proteins. Misfolded soluble proteins in the ER lumen are translocated back into the cytosol, where they are deglycosylated, ubiquitylated, and degraded in proteasomes. Misfolded membrane proteins follow a similar pathway. Misfolded proteins are exported through the same type of translocator that mediated their import; accessory proteins that are associated with the translocator allow it to operate in the export direction.

Ubiquitin

- 76 amino acids, 8.5 kDa protein
- Heat stable
- Folds into a compact globular structure
- Found throughout the cell
- Found in all eukaryotic cells
- Human and yeast ubiquitin share 96% sequence identity
- Involved in many cellular processes



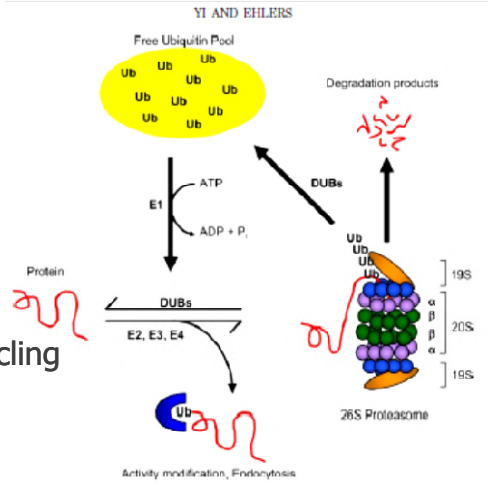
Alpha helices are shown in red & beta sheets are shown in blue.

http://www.sanger.ac.uk/Users/sgj/thesis/html/node93.html

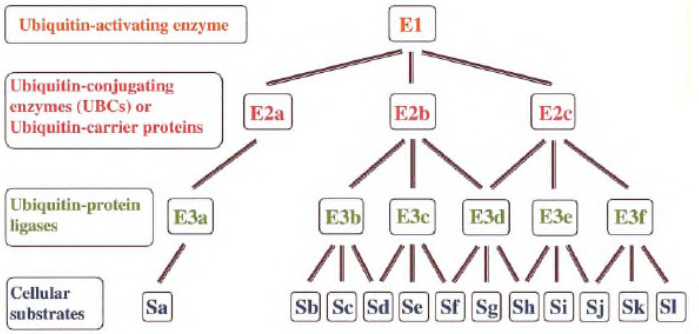
The Ubiquitin/Proteasome System (UPS) protein degradation system.

Five Main Steps:

- Ubiquitin activation
- Ubiquitin conjugation
- Ubiquitin ligation
- Protein degradation
- Deubiquitination & recycling



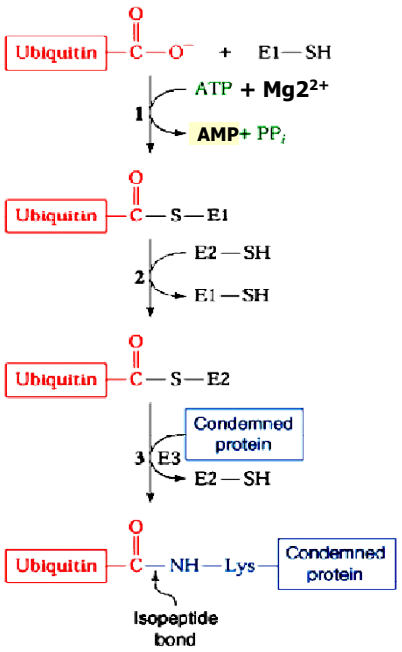
Hierarchical structure



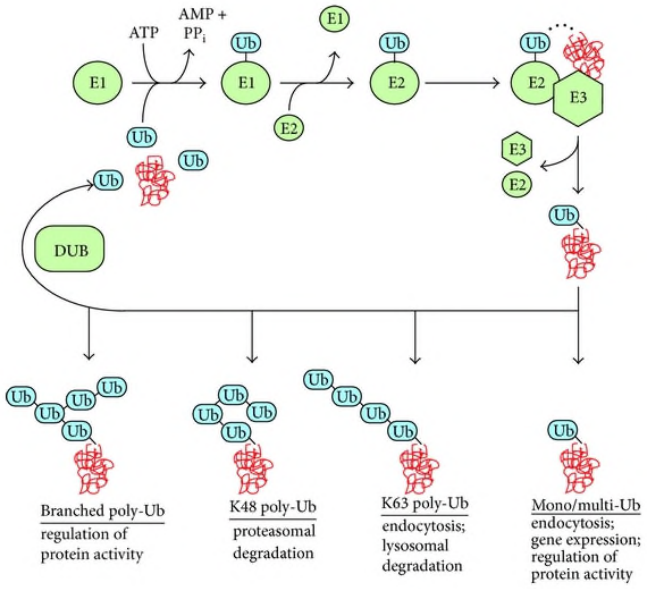
- 3 Enzymes : Ub – Activating enzyme E1
  Ub – Conjugating enzyme E2
  Ub – Ligases E3
- Several E2 transfer Ub from E1 to E3 to which substrate protein is bound
- E3s catalyze covalent attachment to the substrate and recognize the substrate

Ubiquitination of proteins process

- First, Ubiquitin is activated by forming a link to "enzyme 1" **(E1: 1 gene, 2 forms: E1a & E1b)**
- Then, ubiquitin is transferred to one of several types of "enzyme 2" **(E2: ~50 genes)**
- Then, "enzyme 3" **(E3)** catalizes the transfer of ubiquitin from E2 to a Lys ε-amino group of the "condemned" protein **(E3: 2 classes HECT & RING, ~500 genes)**
- Lastly, molecules of Ubiquitin are conjugated to the protein to be degraded by **E3s & E4s**

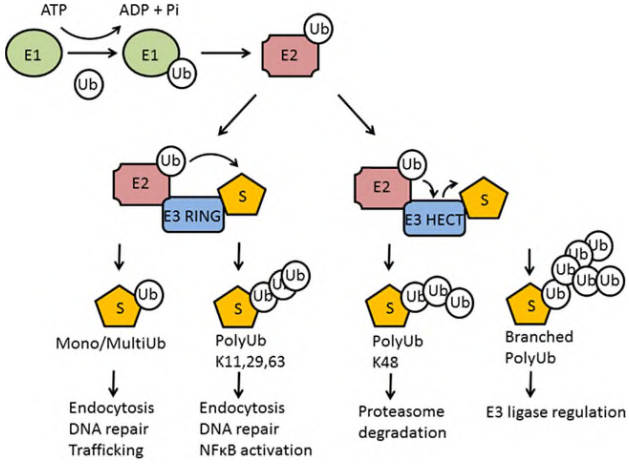


Ubiquitin Pathway: different outcomes



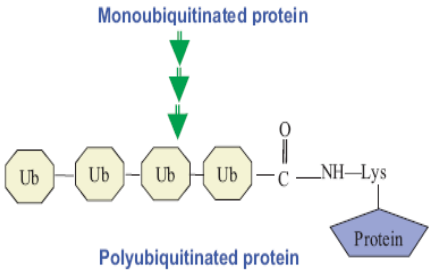
Categories of E3 Ligases

- **HECT domain**: **H**omologous to **E**6-AP **C** **T**erminus
- **RING domain**: **R**eally **I**nteresting **N**ew **G**ene

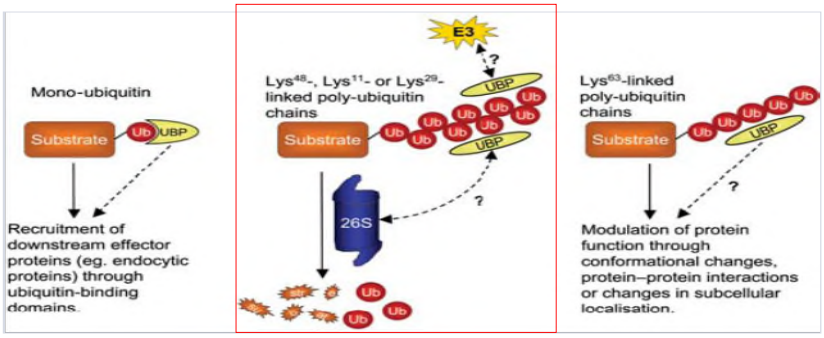


Polyubiquitination by E4

- Poly Ub chain synthesized by adding Ub moieties to Lys of the previous Ub
- Another enzyme **E4** may be catalyzing this step



Types of Ubiquitination

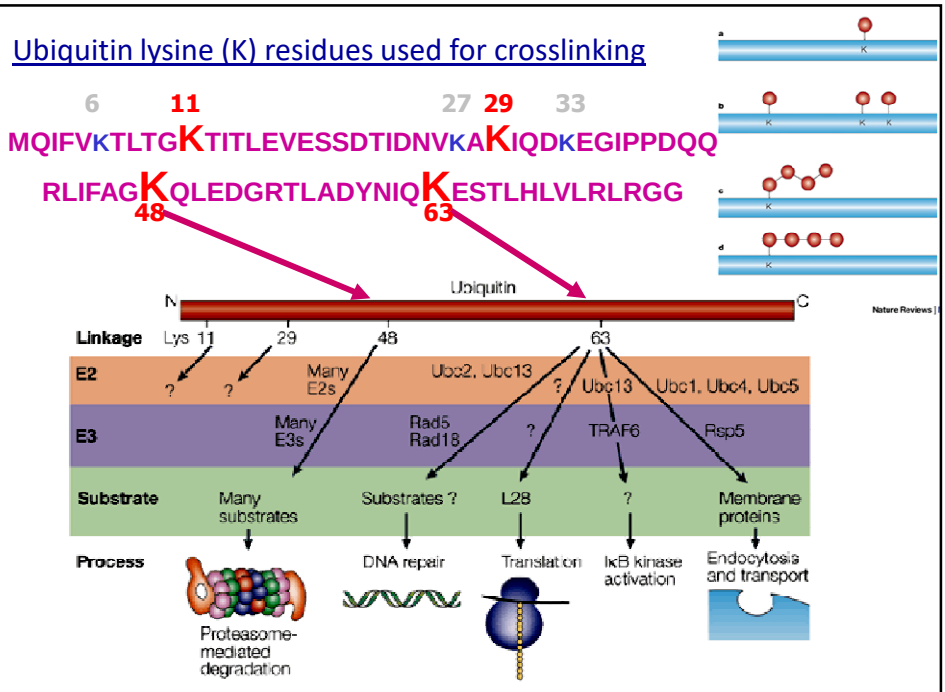


- **Mono-ubiquitination**
  - Transcription, histone function, endocytosis and membrane trafficking
- **Lys48, Lys11 or Lys29 linked poly ubiquitination**
  - Target proteins to the proteasome
- **Lys63 linked poly ubiquitination**
  - Signaling, DNA repair, stress response, endocytosis and signal transduction

Functions of Ubiquitination

- **Mono-ubiquitination**
  - Receptor internalization
  - Endocytosis – lysosome
  - Transcription regulation
- **Poly-ubiquitination**
  - Targets proteins from Cytoplasm, Nuclear & ER for degradation by the PROTEASOME
  - DNA repair

Ubiquitin lysine (K) residues used for crosslinking

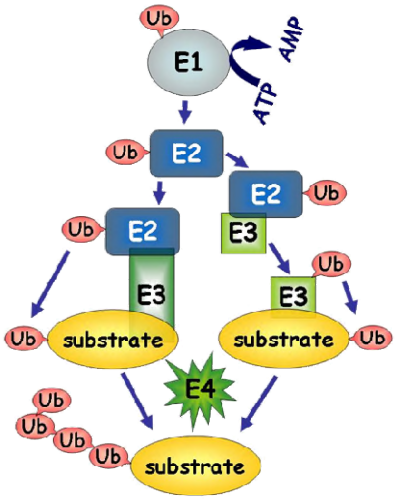


Pay attention

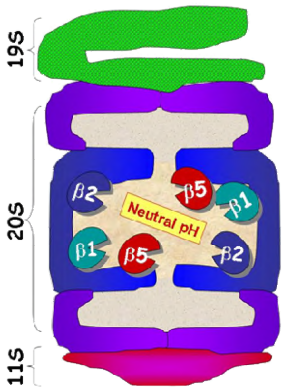
- Ubiquitination is an important and widespread post-translational modification of proteins, which resembles phosphorylation.
- Very importantly, ubiquitination is not only a degradation signal, but also directs proteins to a variety of fates which include roles in ribosomal function, in DNA repair, in protein translocation, and in modulation of structure or activity of the target proteins.
- In order to be efficiently degraded, the substrate must be bound to a polyubiquitin degradation signal that comprises at least 4 ubiquitin moieties, These signals are usually determined by short regions in the primary sequence of the targeted protein.
- The nature of the N-terminal amino acid of a protein (N-end rule) may determine its rate of polyubiquitination and subsequent degradation.

DEGRADATION

Ubiquitin/Proteasome Pathway



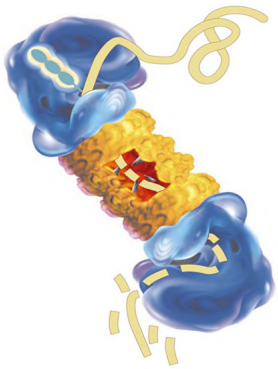
Ubiquitination



Degradation by the 26S PROTEASOME

The Proteasome

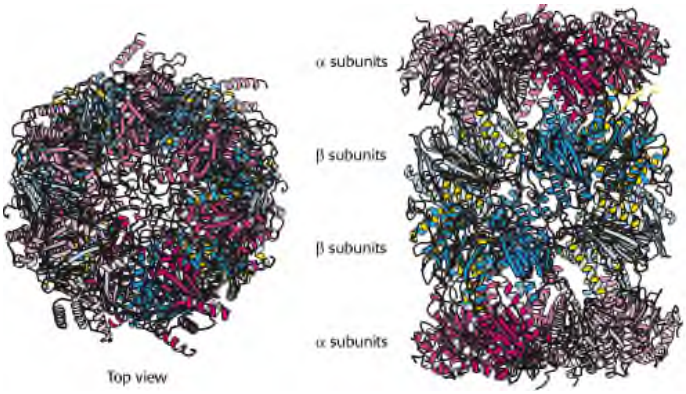
- Professional protein degrading organelles
- An abundant ATP-dependent protease
- Constitutes nearly 1% of cellular protein
- Present in many copies the cytosol and the nucleus
- Consists of a central hollow cylinder (20S)
- Ends of the cylinder are associated with the 19S cap



http://walz.med.harvard.edu/Proteasome_Complexes/

The proteosome consists of a central hollow cylinder, the 20s core proteosome formed from multiple protein subunits that assemble as a cylinder stack of four heptameric rings. Some of these subunits are distinct proteases whose active sites face the cylinder's inner chamber. Each end of the cylinder is normally associated with a large protein complex, the 19S cap containing ~20 distinct polypeptides.

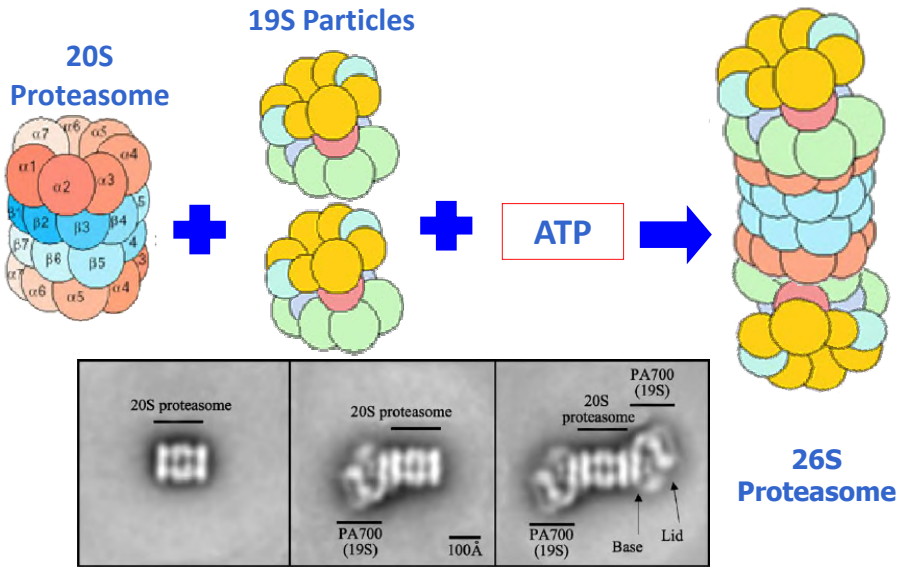
The Structure of 20S Proteasome



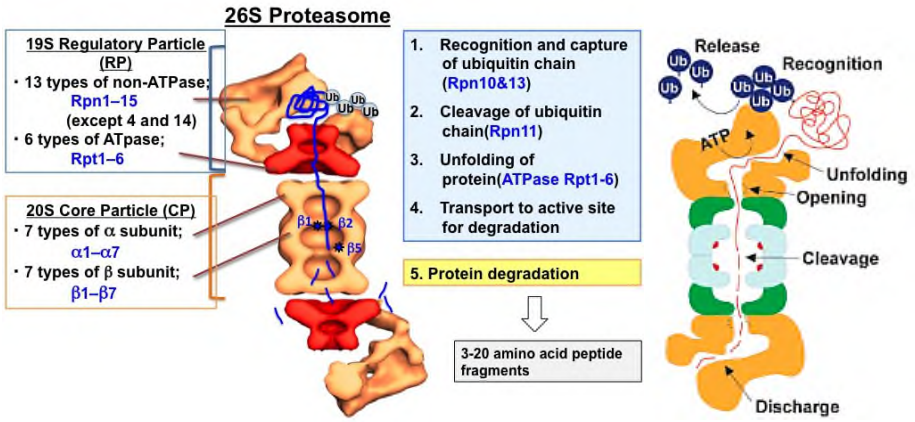
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=stryer.figgrp.3206

The 20S proteasome comprises 28 homologous subunits (a, red; b, blue), arranged in four rings of 7 subunits each. Some of the b subunits (highlighted in yellow) include protease active sites at the amino termini. The top view shows the approximate seven-fold symmetry of the structure

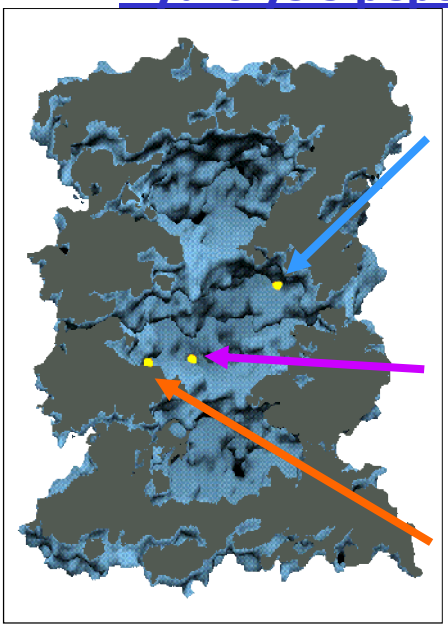
Proteasome components



The 26S proteasome



Hydrolysis peptide bonds after:



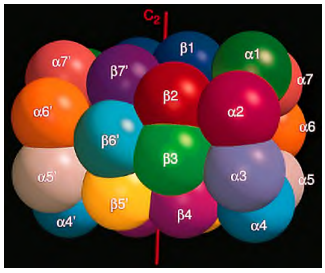
hydrophobic a.a. = CHYMOTRYPSIN-LIKE - β5

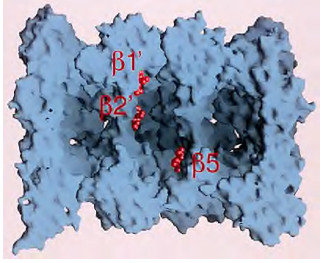
acidic a.a. = (-) CASPASE-LIKE -β1

basic a.a. = (+) TRYPSIN-LIKE -β2

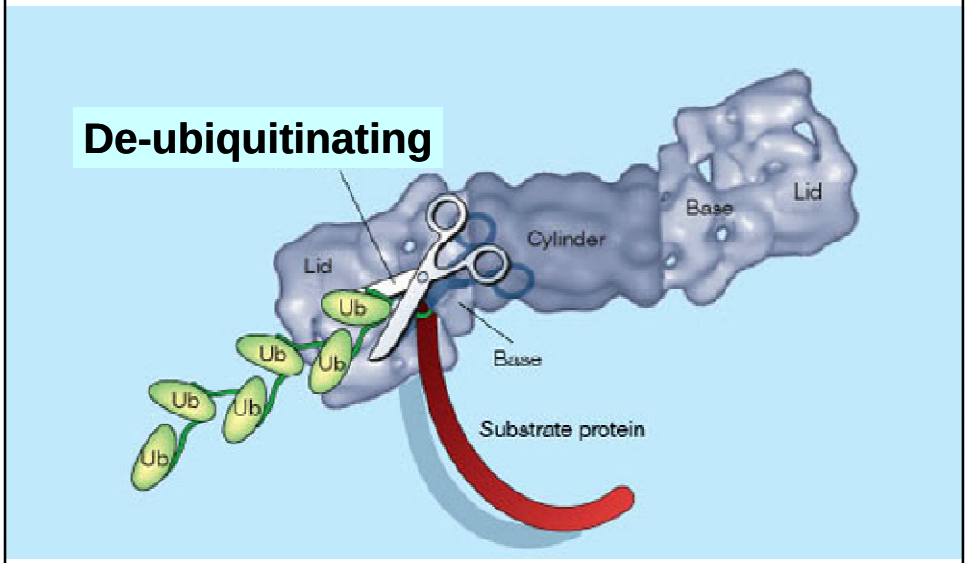
Ubiquitinated proteins are degraded by the proteasome

- Ubiquitinated proteins are degraded in the cytoplasm and nucleus by the proteasome.
- Proteasomal protein degradation ***consumes*** ATP.
- The proteasome degrades the proteins to ~8 amino-acid peptides.
- Access of proteins into the proteasome is tightly regulated.
- The peptides resulting from the proteasome activity diffuse out of the proteasome freely.





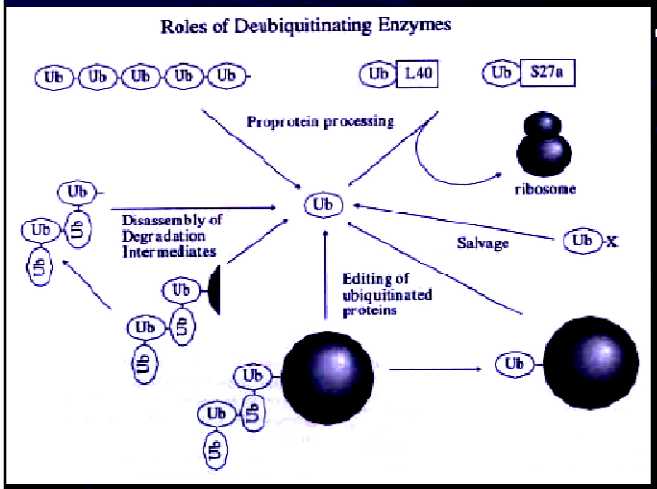
DEUBIQUITINATION



Deubiquitination

- Thiol proteases
- Ubiquitin processing (UBP) enzymes
  - Removes Ub from polyubiquinated proteins
- Ubiquitin carboxy terminal hydrolases (UBH)
  - Regenerates monomeric Ub

Deubiquitination enzymes

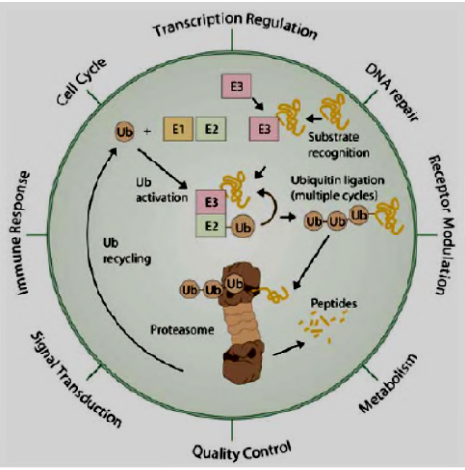


- Roles:
  - Editing
  - Rescue
  - Disassembly
  - Recycling
  - Processing

The role of proteolysis in Neural Plasticity and disease

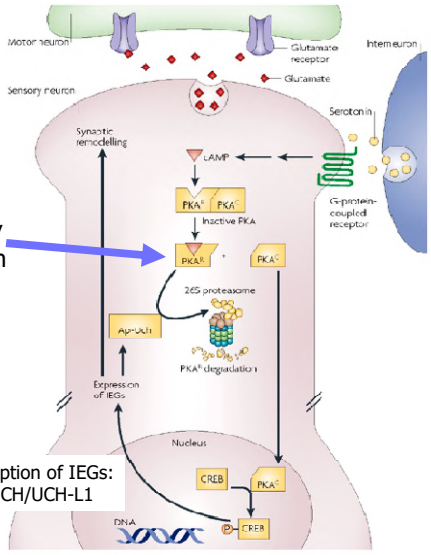
Biological functions of the Ubiquitin/Proteosome pathway

- Ubiquitin proteasome pathway is ubiquitous & targets many processes and substrates.
- Several complex processes are mediated via degradation or processing of specific proteins.
- Aberrations in these systems associates with pathogenic conditions either directly or indirectly.



The role of the Ubiquitin/Proteosome pathway in long-term facilitation in Aplysia

Degradation of PKA-regulatory subunit, permits PKA activation and synaptic strengthening

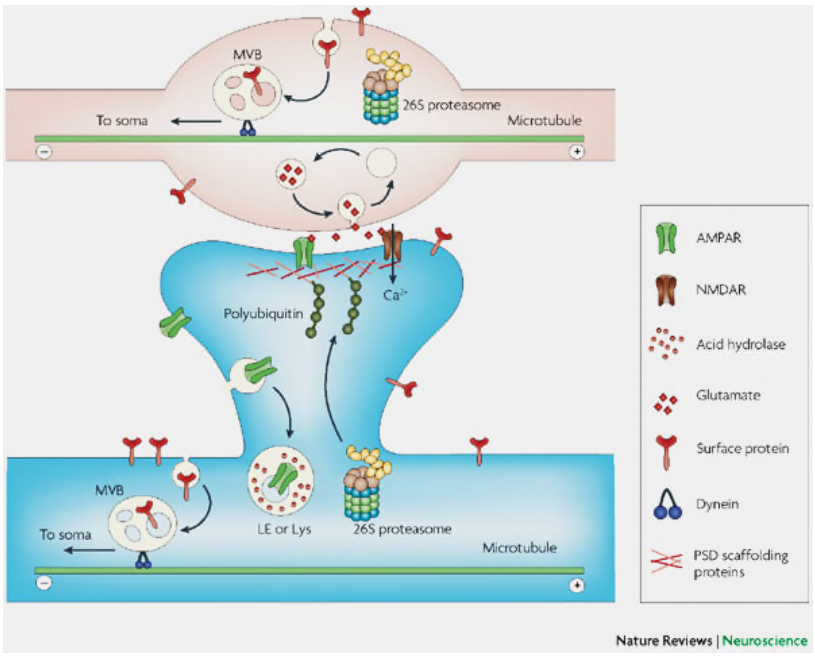


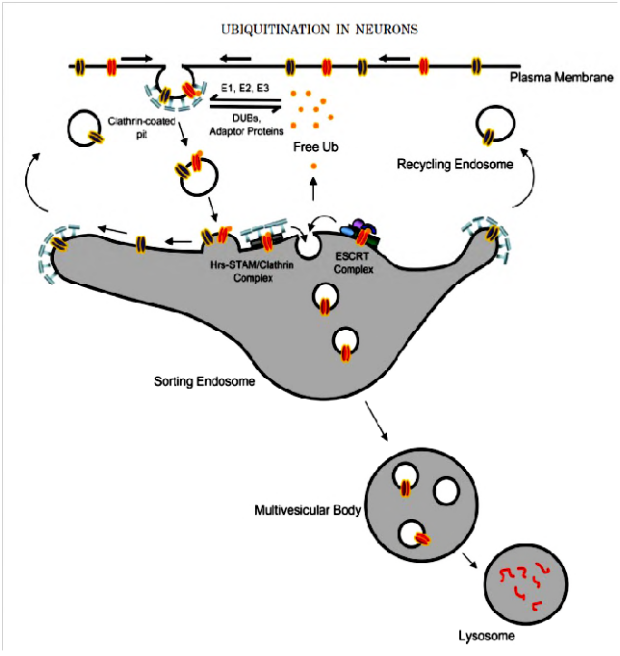
Trascription of IEGs: ApUCH/UCH-L1

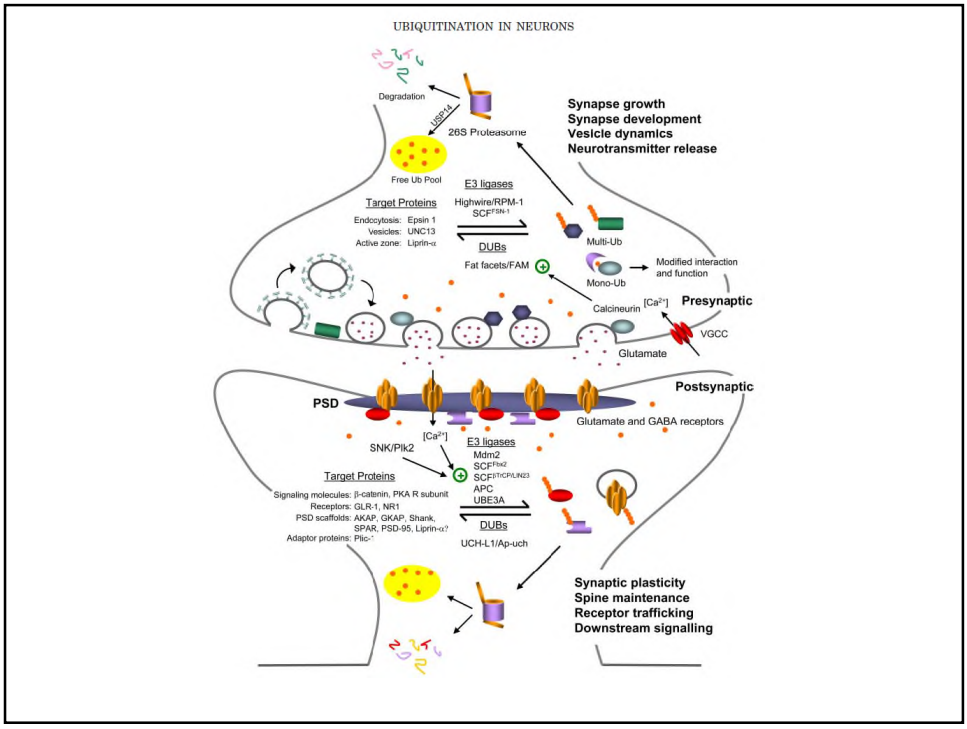
Nature Reviews | Neuroscience

Pathological implication of defective ubiquitin-proteosome pathway

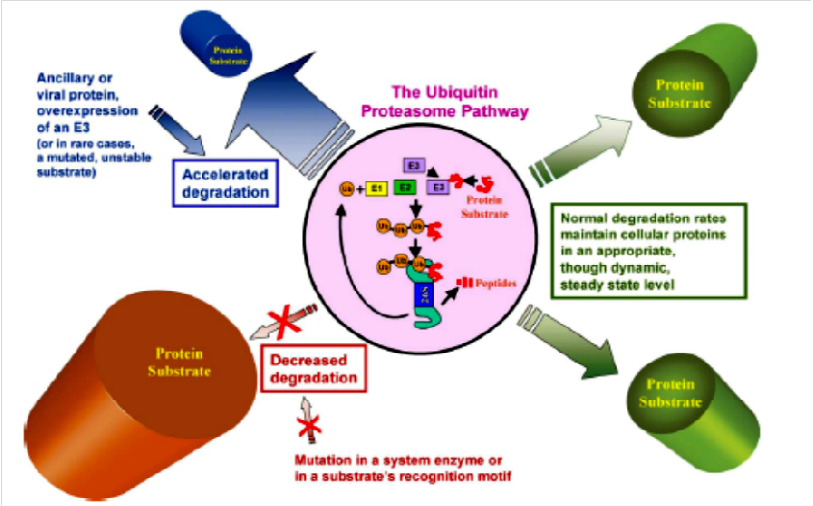
http://www.youtube.com/watch?v=4DMqnfrzpKg


MVB
To soma
26S proteasome
Microtubule
Polyubiquitin
Ca^{2+}
MVB
To soma
LE or Lys
26S proteasome
Microtubule
AMPAR
NMDAR
Acid hydrolase
Glutamate
Surface protein
Dynein
PSD scaffolding proteins
Nature Reviews | Neuroscience


UBIQUITINATION IN NEURONS
Plasma Membrane
E1, E2, E3
DUBs, Adaptor Proteins
Clathrin-coated pit
Free Ub
Recycling Endosome
Hrs-STAM/Clathrin Complex
ESCRT Complex
Sorting Endosome
Multivesicular Body
Lysosome



Consequences of Defects in Ubiquitination



Pathological Conditions Associated with Ubiquitin Proteosome Pathway

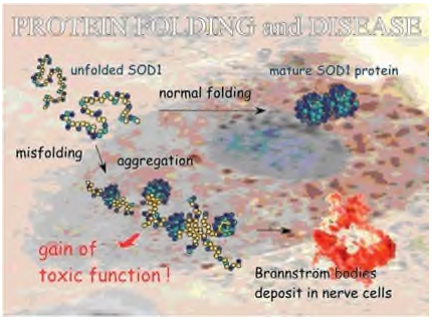
- Malignancies
- Neurodegenerative disorders
- Genetic diseases

  Cystic fibrosis, Angelman's syndrome & Liddle's syndrome
- Immune and inflammatory responses

Neurodegenerative disorders

- Alzheimer's disease
- Parkinson's disease
- Huntington's disease
- Spinocerebellar ataxias
- Spinobulbar muscular dystrophy (Kennedy's syndrome)

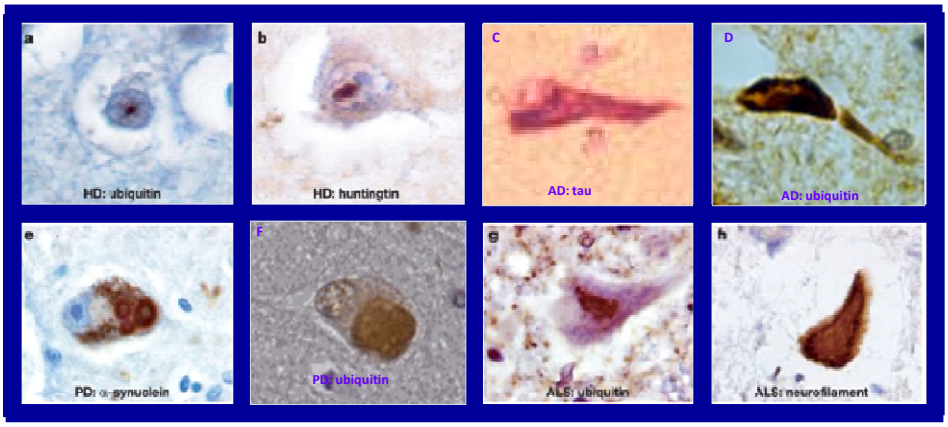
Formation of inclusion bodies



(Ref: http://w3.dbb.su.se/~oliveberg/images/bildstrat1.jpg)

Ubiquitin-Protein Aggregates

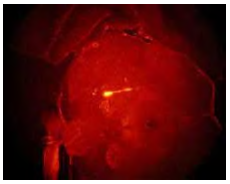
Huntington's **Alzheimer's**

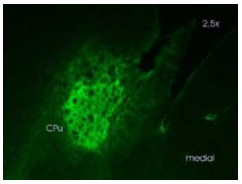


Parkinson's **Lou Gehrig's**

Parkinson's disease and Lewy Bodies

- Accumulation of ubiquitin may be secondary reflecting unsuccessful attempts of ubiquitination.
- Abnormal protein associate with each other forming aggregates.
- Hypothesis: Aggregated proteins inhibit ubiquitin proteosome pathway.





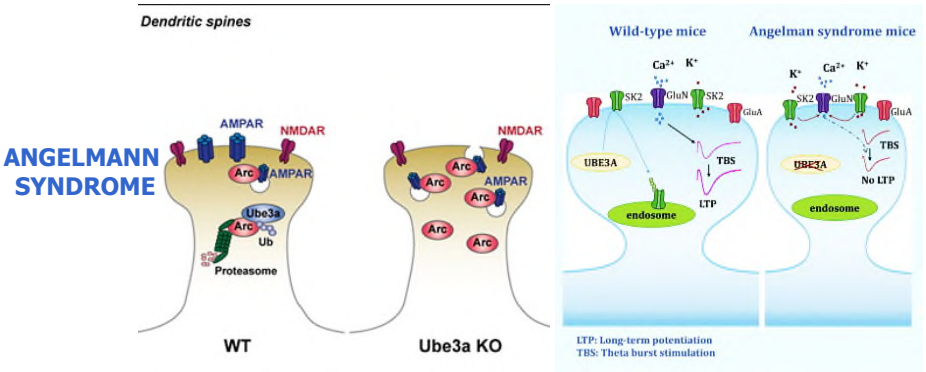
(Ref: http://www.neurodegeneration.uni-goettingen.de/index.html?/en/p311.html)

Angleman syndrome

- Ubiquitin system is considered to be involved in brain development.
- Defective synthesis of gene coding for E3 ligase E6-AP
- Characteristic symptoms involve mental retardation, seizures, out of context frequent smiling and laughter.
- Brain proteins that could be stabilized by mutation have not been identified.

Cystic fibrosis

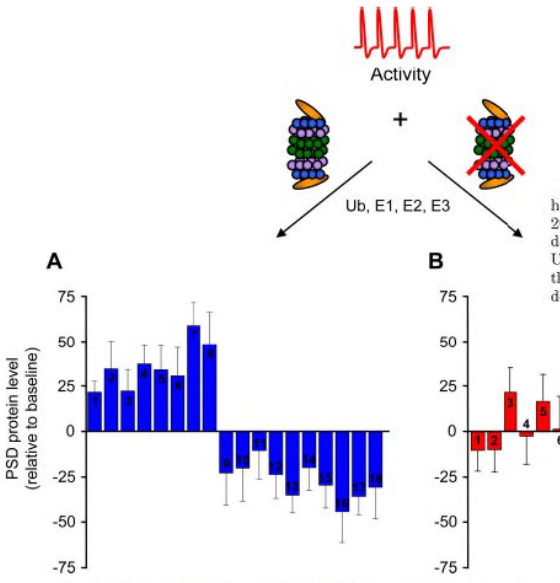
- Gene codes for a protein, CFTR, which is chloride ion channel.
- Small fraction of protein matures to the cell surface.
- Mutation in protein ΔF508, CFTR$^{\Delta F508}$ doesn't reach the cell surface.
- Ubiquitination degrades mutant CFTR$^{\Delta F508}$, resulting in complete lack of cell surface expression.



UBE3A also ubiquitinates and promotes the degradation of Arc (activity-regulated cyto-skeleton-associated protein). Arc is only found in the brain and is rapidly upregulated in response to increases in neuronal activity. UBE3A regulates AMPAR (AMPA receptor) endocytosis by controlling the amount of Arc. By doing so, UBE3A helps to restructure pre-existing synapses and this allows the development of functional neural circuits.

In cases where there is excessive Arc, endocytosis of GluA1-containing AMPARs from glutamatergic synaptic sites is unusually high. This leads to a reduction in excitatory synaptic transmission and also an increase in the number of silent (AMPAR-lacking) synapses. The defective synapses and neural circuits could be the possible cause to the various Angelman Syndrome phenotypes, such as intellectual and developmental delay, and impaired social skills.

Mabb et al.also mentioned that the UBE3A protein interacts with the tumor suppressor protein p27. In the cortex, p27 protein promotes neuronal differentiation and migration in cortical projection neurons. With the absence or low amount of the UBE3A protein, there is an increase in the p27 protein level, which might cause the premature migration and differentiation of cortical neuronal progenitors, and also change the laminar architecture of the cortex.



2. Parkin. Parkin is a RING E3 ubiquitin ligase. It has a C-terminal PDZ-binding domain (Fallon et al., 2002), a specially modified C-terminal in-between RING domain flanked by two RING motifs, and an N-terminal UBL domain that binds Rpn10 of the proteasome and is thought to aid the shuttling of ubiquitinated proteins for degradation (Sakata et al., 2003). Genetic studies have

FIG. 5. PSD proteins are coregulated by the UPS in an activity-dependent manner. Changes in PSD protein levels are observed during activity elevation by bicuculline treatment in the absence (A) and presence (B) of the proteasomal inhibitor MG-132. Prolonged activity in cultured cortical neurons induces coregulated changes in the proteomic composition of the PSD (A), whereas these coordinated changes are lost during proteasomal inhibition by MG-132 (B). 1, PKCγ; 2, CaMKIIβ; 3, CaMKIIα; 4, myosin Va; 5, Homer; 6, PSD-95; 7, mGluR1α; 8, NR2A; 9, PKA RIIβ; 10, PKA catalytic subunit; 11, PP1; 12, spinophilin/neurabin II; 13, AKAP79/150; 14, GKAP/SAPAP; 15, Shank; 16, SAP102; 17, NR2B; 18, NR1. Graphs adapted by permission from Macmillan Publishers Ltd.: Ehlers (2003a) Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat Neurosci* 6:231–242. Copyright 2003.

Drug Development for Ubiquitin Dysfunction

- **Inhibition of enzymes common to entire pathway would target the process non- specifically.**
- **Narrow window between benefits and toxicity needs to be identified.**
- **Develop specific E3 ligase inhibitors that would affect the pathways of interests.**
- **Better approach would be development of small molecules specific for substrates**

Conclusions

- Ubiquitylation plays a fundamental role of protein degradation at cellular level.
  (Levels of proteins in nucleus, cytoplasm, ER lumen and transmembrane protein are kept in check by ubiquitin proteosome pathway.)
- Ubiquitylation is highly complex, temporally controlled and tightly regulated process.
- Enzymologically Ubiquitination is more complex pathway compared to other post translational modification.
- Mechanism of catalysis by E3 ligase still remains unclear.
- Elucidation of complete catalytic mechanism of ubiquitylation will provide considerable insight on cellular functions.

The End