Misfolded Proteins

- Result from translational errors
- Increased by oxidative stress and/or heat shock
- Misfolded proteins impair cellular physiology as exposed hydrophobic residues lead to the formation of insoluble oligomers and larger aggregates that alter essential protein-protein interactions thereby leading to cellular toxicity.
- Accumulation of toxic protein aggregates (inclusion bodies) is a hallmark of several diseases i.e. inclusion body myositis, inclusion body dementia
- Misfolded protein increase with cellular age
- It remains unclear whether autophagy is a parallel or compensatory degradation system which the Ub-Proeosome system is impaired
Chaperone Proteins

- Cells continuously evaluate the quality of their proteins
- Chaperone proteins identify abnormal or unstable proteins and often assist them in regaining stability.
- If repair is not possible the aberrant protein is removed via the Ub-proteasome (where amino acids are recycled) or autophagy pathways.
- If the chaperone system is overwhelmed proteins will form aggregations e.g. acute oxidative stress or heat shock can lead to massive protein unfolding

Introduction to Ubiquitin

- Ubiquitination targets substrates to either the:
  - Proteosome
  - Lysosome
  - Autophagosome
Introduction to Ubiquitin

- Ubiquitin (Ub) is a small protein, 79aa highly conserved from yeast to man.
- Determines protein fate by “tagging” them
- First described as playing a role in protein degradation but also participates in:
  - Endocytosis
  - Signal transduction
  - Gene transcription
  - Cell cycle progression
  - DNA repair
  - Apoptosis
Introduction to Ubiquitin

- Conjugation of Ub is a complex reaction requiring E1 (activation), E2 (conjugation) and E3 (ligation) enzymes
- Ubiquitin can be repeatedly attached to itself forming chains with various topologies and functions

Introduction to Ubiquitin

- E1 activating enzyme
  - Catalyses the formation of a thioester bond between the ubiquitin and E1. The ubiquitin is now activated and is transferred to...
- E2 conjugating enzyme
- The E2–ubiquitin complex then binds to E3 ligase
- E3 ligase catalyses the transfer of the activated ubiquitin to a lysine residue on the protein substrate
Introduction to Ubiquitin

• Modification of a protein with Ub chains (polyubiquitination) targets the substrate to the 26S proteosomal pathway
• Attachment of a single Ub marks the substrate for degradation in lysosomes

Ubiquitination is reversible

• Deubiquitinating enzymes (DUBs) can cleave Ub from modified proteins.
Introduction to Ubiquitin

- Recently evidence has found a role for ubiquitination in another fundamental lysosome dependent degradation system autophagy
- **Autophagy** is a catabolic pathway capable of targeting:
  - Individual proteins
  - Macromolecule complexes
  - Complete organelles
- Analogous to cellular “cleaning”
- The process is important for cellular homeostasis and survival
- Through autophagy the cell recycles nutrients during limited energy supply and also acts as a quality control system.
- Dysregulated autophagy has been implicated in pathological conditions e.g. neurodegenerative diseases and cancer

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Ub links proteosomal and lysosomal degradation

*Shaid et al Cell death and Diff 2013*
Types of Autophagy “Self-eating”

- There are 3 mechanisms by which the lysosome acquires cytosolic cargo:

1. **Macroautophagy**: formation of a crescent shaped structure that fuses with the lysosome; 3 known Autophagy-related genes (ATG)

2. **Microautophagy**: lysosomes invaginate and directly sequester cytosolic components; less is known about this process especially in humans

3. **Chaperone-mediated autophagy (CMA)**: involves translocation of unfolded proteins across the lysosomal membrane
   - Lysosomes are single membrane vesicles that contain cellular hydrolases
   - Both Macroautophagy and CMA are maximally upregulated in response to nutrient deprivation

Autophagy Pathways

*Wong and Cuervo Cold Spring Har Persp in Biology 2010*
Macroautophagy

- Autophagy was first thought to be bulk-non-selective “self-eating” e.g. in states of starvation autophagosomes sequester cytosolic material non-specifically
- However there is evidence for selective autophagic degradation
- Autophagy is considered selective when precise cargo is specifically and exclusively targeted into autophagosomes.
- The exact mechanism of cargo recognition remains obscure
- Autophagy receptors bind Ub substances and autophagy-specific light-chain 3 e.g. p62/SQSTM1 and NBR1 simultaneously bind ubiquitin and autophagy-specific ubiquitin-like modifiers
- These receptors provide a molecular link between ubiquitination and autophagy and tether the Ub cargo to the autophagosome.

Non-selective vs selective autophagy

Shaid et al Cell death and Diff 2013
Ub and selective Autophagy: Clinical implications

- The first clinical associations in this area showed cells exposed to abnormal proteins associated with Parkinson's or Huntington's disease displayed upregulated macroautophagy.
- In animal models of Huntington's disease upregulation of the macroautophagy system slows disease progression.
- Subsequently disorders of the macroautophagy systems have been demonstrated in these diseases:
  - Parkinson's disease
  - Huntington's Disease
  - Alzheimer's Disease
  - ALS
  - Prion disease
- Role of inducers of macroautophagy e.g. mTOR inhibitors and others

Failure of macroautophagy in pathogenesis of disease

Wong and Cuervo Cold Spring Har Persp in Biology 2010
Ub and selective Autophagy: Clinical implications

- Autophagy receptors are involved in the elimination of protein aggregates, organelles and pathogens
- P62/SQSTM1 is a common component of Ub-inclusion bodies found in neurodegenerative and liver disease
- P62 is able to bind mono or poly-Ub
- P62 binds LC3 becoming a selective autophagy receptor
- Paget’s disease is associated with mutations in p62 in the Ub-binding domain. The loss of functional Ub-mediated autophagy leads to increased osteoclastogenesis through a TNF-mediated pathway

Ub receptors involved in selective Autophagy:

Shaid et al Cell death and Diff 2013
Ub and Autophagy of Pathogens: Xenophagy

- Autophagosomes have been implicated in the host’s defense against bacterial invasion
- Pathogen-containing phagosomes are targeted for autophagic degradation

Selective autophagy of pathogens

Jiang and Chen Nat Rev Immunol 2012
Ub and Autophagy of Pathogens: Xenophagy

- 3 adaptor proteins recognise Ub-cytosolic bacteria and bring them to autosomes:
  - P62
  - NDP52
  - OPTN
- Depletion of any of these leads to hyperproliferation of S. typhi (non-redundant); have different Ub-binding domains.

Ub recruitment on Shigella vacuolar membrane remnants

Figure 2: Ub recruitment on Shigella vacuolar membrane remnants (from Ub movie)
Infection by DeReD protein-expressing S. flexneri of HeLa cells transiently expressing the Ub–YFP protein. Time is indicated in h:mm:s. The arrow points to membrane remnants.

Dupont et al. Biol Cell 2010
Ub and Autophagy of Bacteria

- There is growing list of bacteria shown to interfere with the host Ub system to achieve successful infection.
- Many bacteria interfere with the Ub system either directly (e.g. by producing E3 mimics, DUBs and small Ub-related modifier SUMO) or indirectly.

Ub and Autophagy : Future Directions

- Increasing number of Ub-receptors being identified as playing a role in selective autophagy
- What determines proteosomal vs lysosomal degradation is an area of current research
- Further understanding into how autophagy substrates are selected and tagged
- Important area of cell biology to understand the role selective autophagy plays in cellular homeostasis.
- Therapies based on manipulating the proteosome and/or autophagy pathways may play a role in the future
Thank you

• Questions?