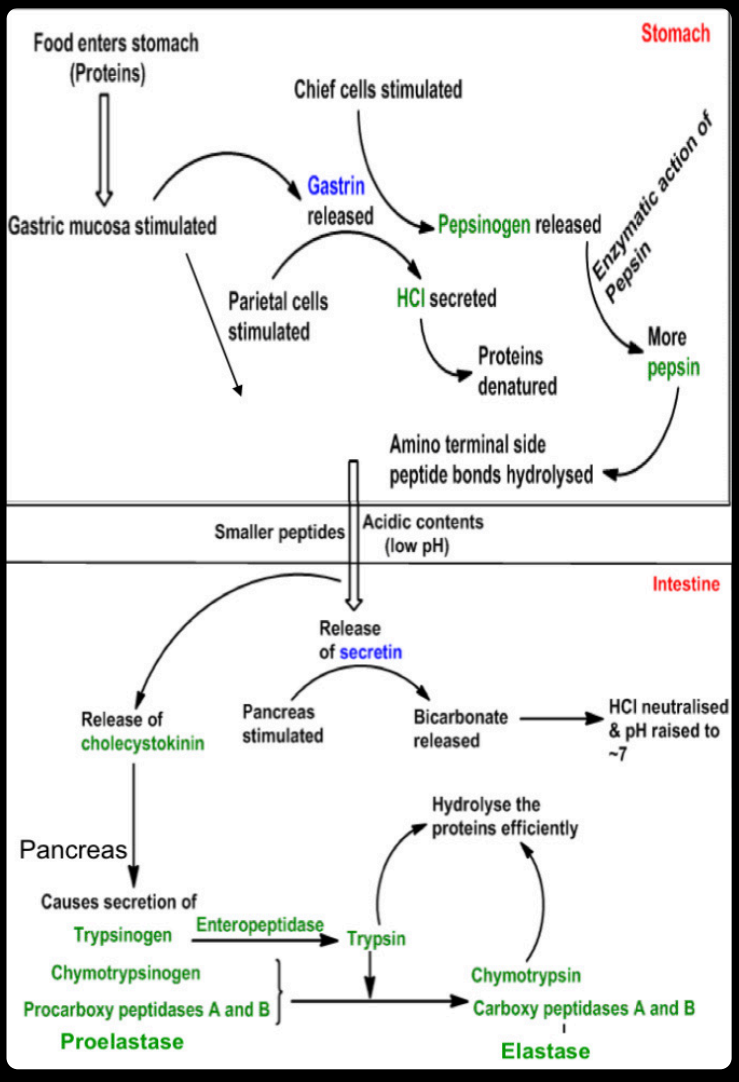
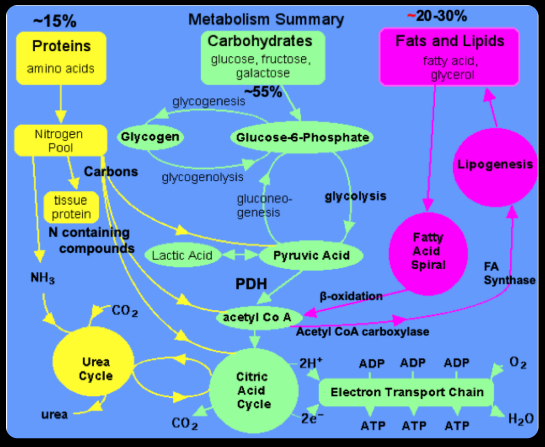
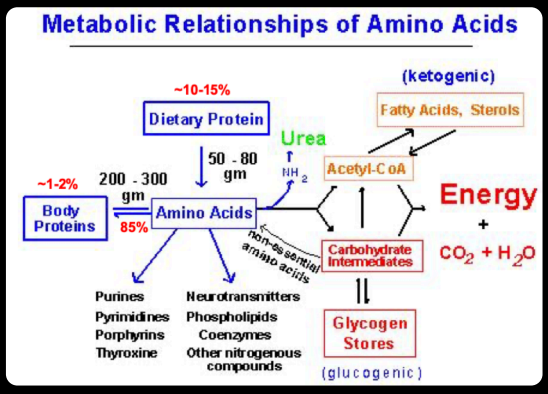
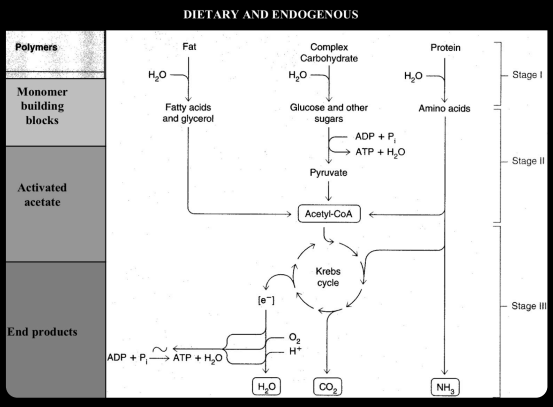
Pathway overview & proteolytic enzymes





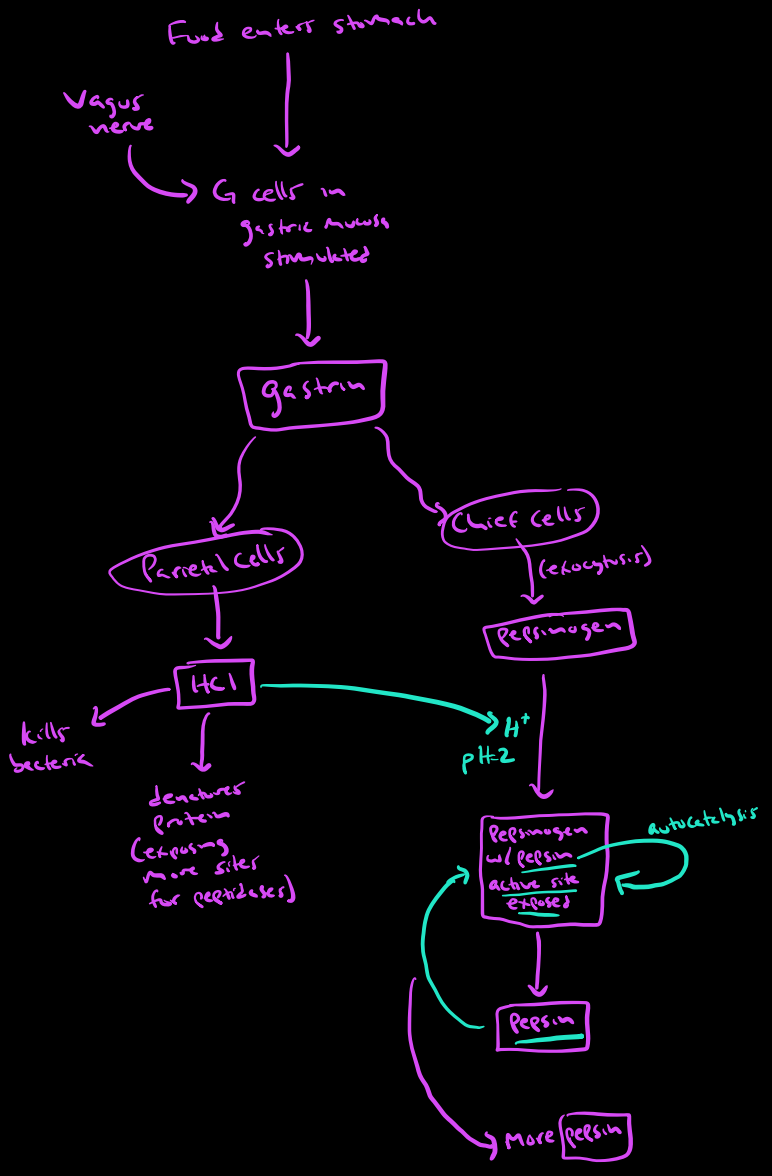


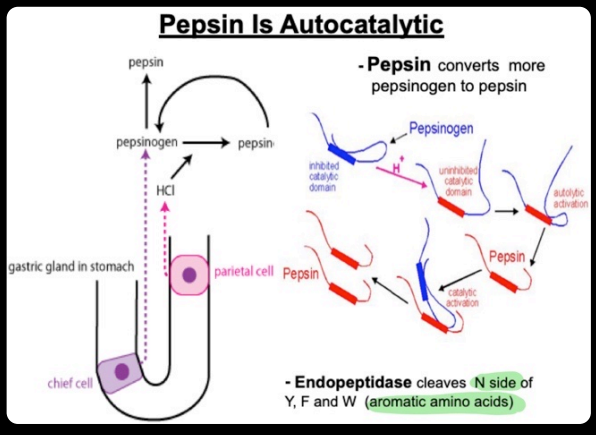


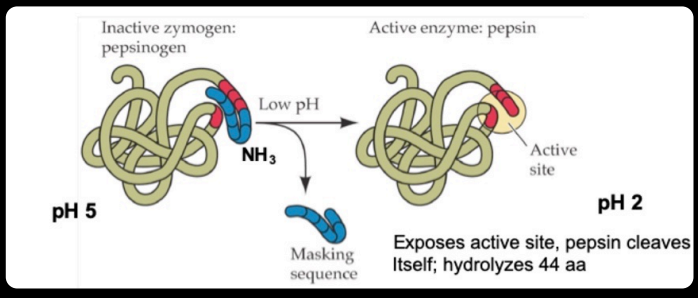
Proteolytic Enzymes in GI tract

	Enzyme	Zymogen (Inactive form)	Activated by:	Site of Synthesis	Site of Action	Optimum pH
Endopeptidases – cleave within peptide @ specific AAs	Pepsin – Phe, Tyr, Trp @ N-term side	Pepsinogen	HCl, Pepsin (autocatalytic)	Stomach epithelium (chief cells)	Stomach	2
	Chymotrypsin – Phe, Tyr, Trp	Chymotrypsinogen	Trypsin	Pancreas	Intestine	7-8
	Trypsin – Lys, Arg @ C-term side	Trypsinogen	Enteropeptidase, Trypsin (autocatalytic)	Pancreas	Intestine	7-8
	Elastase – Gly, Ala, Val, Leu, Ile	Proelastase	Trypsin	Pancreas	Intestine	7-8
Exopeptidases – cleave AA @ carboxy or amino term.	Carboxypeptidase – Carboxy terminal	Procarboxypeptidase	Trypsin, Zn^{2+}	Pancreas	Intestine	8
	Aminopeptidase – amino terminal	(X)	Mn^{2+}	Intestine	Intestine	8-9

Stomach







Pepsin

- endopeptidase that cleaves longer polypeptides w/ large hydrophobic AAs
  - cleaves @ N-term side of AA
  - Tyr
  - Phe
  - Trp

Pancreas & Intestine (lumen & brush border)

- Acute pancreatitis
  - Premature activation of pancreatic zymogens → inflammation & self digestion
    - Trypsin → digests pancreas & activates other zymogens
    - Elastase → breaks down elastic fibers in blood vessels → hemorrhage
    - Phospholipase A_2 → ↑ arachidonic acid → ↑ fat necrosis
    - Kallikrein - Ser protease → inflammation & thrombosis
  - Potentially caused by deficient trypsin inhibitor
  - Tx: stop feeding, IV fluids, pain management

- Celiac Disease
  - Gluten-sensitive enteropathy, Celiac sprue
  - Malabsorption from inflammatory injury to mucosa of small intestine after ingestion of wheat (gluten), or related rye & barley proteins
    - protein = gliadin - ↑ Glu & Pro → resistr hydrolysis
    - → stimulates immune response → immune response damages microvilli → ↓ absorption → diarrhea, malnutrition, etc.
    - immune response sustained b/c once microvilli damaged → ↑ permeability to non-hydrolyzed peptides → encounter Transglutaminase → deamidation of Gln → Glu → ↑ (-) charge → → APCs w/ HLA-DQ2 or -DQ8 have ↑ affinity for (-) charge peptides → ↑ immune response
    - → antibodies
    - → T-lymphocytes release metalloproteinases → ↑ destruction to microvilli

Smaller peptides
AAs
Acidic contents

Stomach

Pyloric sphincter

Duodenum

Mucosal epithelium

I cells

S cells

CCK

Secretin

Intestinal epithelium

Trypsin inhibitor in Pancreas keeps Zymogens stored as inactive until proper time to release

Enteropeptidase

trypsinogen

Trypsin inhibitor

Pancreas

Cleave basic AAs

trypsin

autocatalytic

HCO_3^- → HCl neutralized → ↑ pH = 7

Intestinal enzymes @ neutral pH function

Cleavage

Chymotrypsin — C-term: Aromatic, hydrophobic

Carboxypeptidases A & B — C-term

Elastase — C-term: aliphatic AAs

Chymotrypsinogen

Procarboxypeptidases A & B

Proelastase

Oligopeptides (3-8 AAs)

3-8+ residue oligopeptides

Dipeptides & Tripeptides

Amino Acids

Penultimate Pro or Ala

Brush Border

Amino Oligopeptidase

AA Transport Proteins

Amino Peptidases

Peptide Transport Protein

Dipeptidyl Aminopeptidase

Cytosol

AAs

Dipeptides & Tripeptides

Cytoplasmic Peptidases
- Aminase
- Dipeptidase
- Tripeptidase

AAs

Enterocytes

Intestinal lumen

brush border

AA

Na^+

Na^+

ATP

K^+

Facilitated Transporter

Serosal side

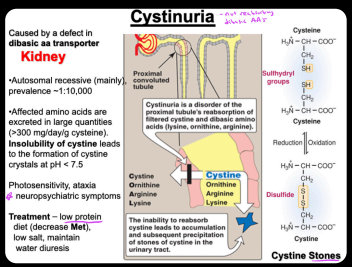
AA

Portal vein → liver → lymph

- 2° active transport b/c AA transporter itself does not require ATP, but does require Na^+ gradient, maintained by Na^+/K^+ ATPase

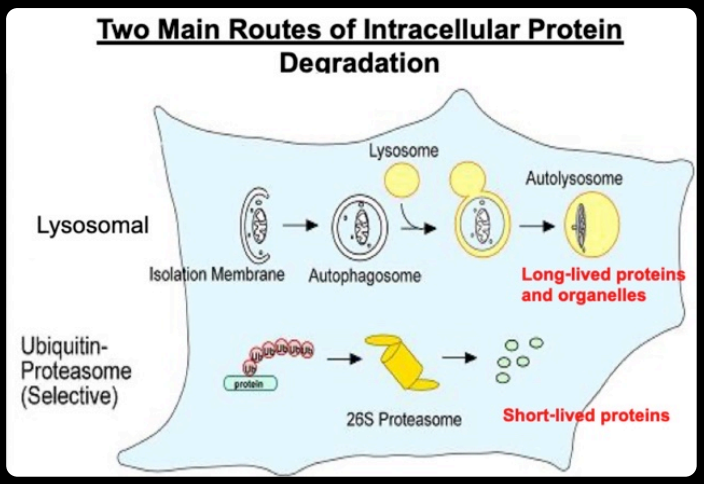
Transporters also present in kidney PT → 95% reabsorbed

Hartnup disorder
- impaired transport of neutral AAs (aromatic) in apical brush border of intestine & PT of kidneys
- ↳ ↓ uptake Trp → ↓ synthesis of Niacin (B_3), Serotonin, Melatonin
- Autosomal recessive
- Pellagra-like Dermatosis = Dermatitis, Diarrhea, Dementia
- Tx: high-protein diet & Niacin supplement



Endogenous Protein Degradation (Protein $t_{1/2}$, Lysosomal, Ubiquitination)

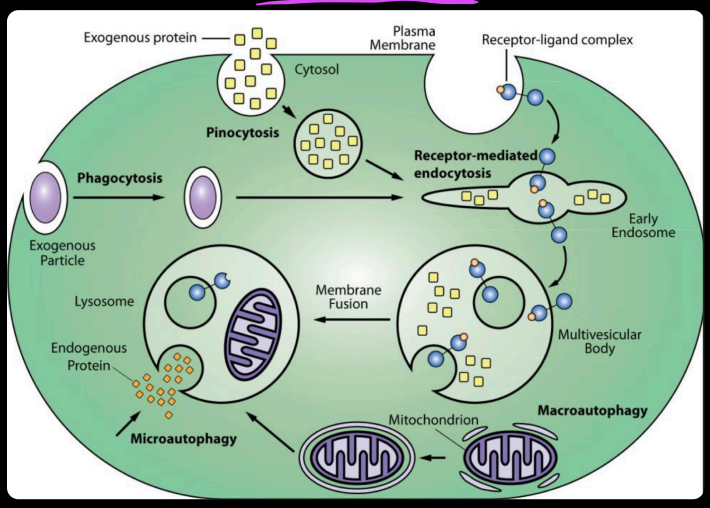
- 1-2% of body proteins turnover daily, 85% recycled
- Degradation = first order kinetics = proportional to [reactant] → rate = k[A]



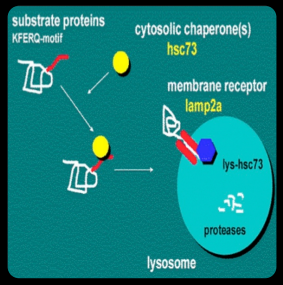
Protein Degradation Signals

- Misfolded, abnormal
- PEST sequences
  - 12-60 residue domains,
  - ↑↑ Proline (P), Glutamate (E), Serine (S), Threonine (T) — targets for phosphorylation
  - $t_{1/2}$ < 2 hours
- N-terminal rule
  - amino terminal recognized by degradative machinery
  - Large, Charged AA's – $t_{1/2}$ ≤ 3 min
    - Phe, Leu, Asp, Lys, Arg
  - Smaller AAs – $t_{1/2}$ > 20 hours
    - Met, Ser, Ala, Thr, Val, Gly
- Protein Domains
  - HECT
    - Homologous to E6-AP Carboxyl Terminus
    - E6 protein of HPV 16 & 18
    - E6/E6AP tag p53 w/ ubiquitin for degradation
  - RING
    - Really Interesting New Gene
    - Zinc finger domain
  - F-box
    - found in E3 Ubiquitin Ligase adapter protein
  - UBA domain
    - Ubiquitin-associated domain
  - SOCS
    - Suppressors of Cytokine Signalling
    - always located @ C-terminus
    - helps target proteins for ubiquitination

Lysosomal Degradation

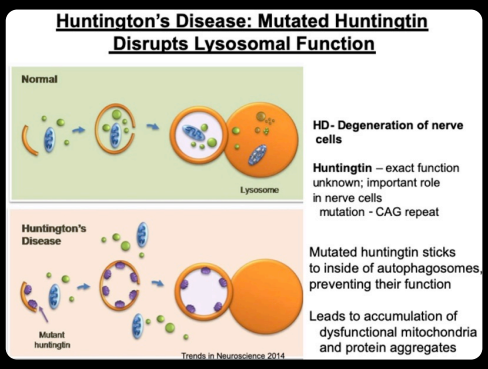


- Microautophagy – direct sequestration of cytosolic components via invaginations in lysosomal membrane
- Macroautophagy – entire organelles (e.g., mitochondria)
  - Phagophore assembles/expands by acquiring membranes from intracellular organelles/carrier proteins
    - ↳ phagophore expansion forms autophagosome
      = entire organelle sequestered within another double membrane
      - ↳ autophagosome fuses w/ lysosome → autolysosome
        - ↳ degradation
- Chaperone-mediated autophagy
  - during periods of nutritional or oxidative stress, body first selectively degrades nonvital proteins containing KFERQ motif
    - Lys, Phe, Glu, Arg, Gln
  - In cytosol, Hsc73 binds KFERQ
    - ↳ Hsc73/protein complex binds to lysosome receptor Lamp2a (lysosome-associated membrane protein)



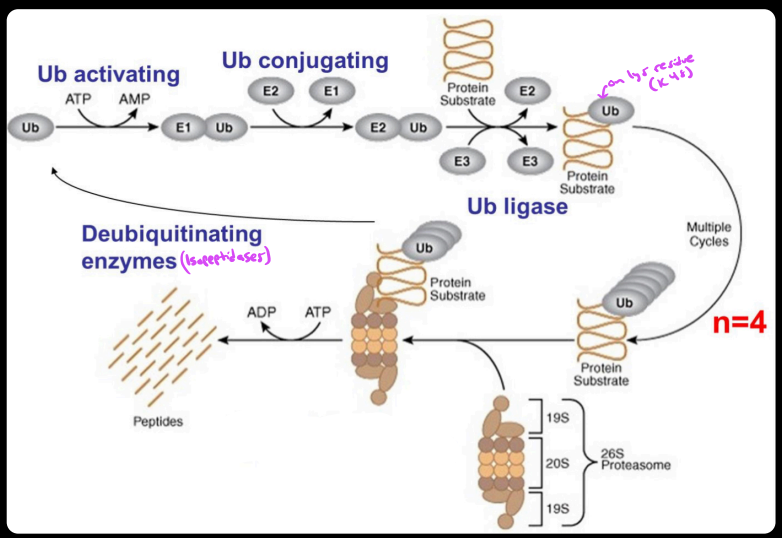
Huntington's Disease

- Autosomal dominant
- Mutated HTT gene, codes for protein Huntingtin
  - ↳ Mutation causes ↑↑ CAG repeats
    - ↳ abnormally long version of Huntingtin protein
- Mutated Huntingtin sticks to inner layer of autophagosomes
  - ↳ prevents autophagosomes from getting "garbage"
    - ↳ autophagosomes arrive @ lysosomes empty
      - ↳ accumulation of junk that should have been degraded



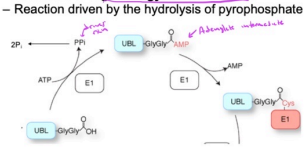
Ubiquitin-Proteasome Pathway

- ~80% of cellular proteins degraded via Ubiquitin (Ub) Pathway
- Involved in degradation of abnormal OR short-lived proteins
- located in cytosol and nucleus
- Ub tags proteins via
  - N-end rule
  - PEST sequences
  - degradation domains
- Proteins degraded via 26S proteasome
  - 2 outer 19S subunits
    - Recognizes polyubiquitinated proteins
    - Deubiquitination via isopeptidases
    - ATPases - unfold protein to allow it to fit into proteolytic core
  - 1 20S subunit - Catalytic
    - Proteolytic core where protein degradation occurs
    - several enzymes
      - Chymotrypsin-like
      - Trypsin-like
      - peptidylglutamyl-peptide hydrolytic-like
        - degrades glutamate
- 26S proteasome usually = ATP-dependent



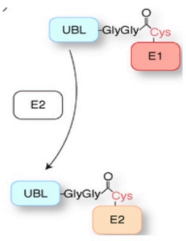
E1: Ubiquitin Activating Enzyme

- Utilizes ATP to activate Ub (mammals 2 enzymes)
  - Initial step involves production of Ub-adenylate intermediate
- Ub is then transferred to **Cys** of Ub Activating Enzyme (E1)
  - Formation of **high energy thioester bond**
  - Reaction driven by the hydrolysis of pyrophosphate



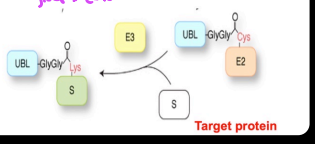
E2: Ubiquitin Conjugating Protein

- Activated Ub is now transferred to Cys of Ub conjugating protein (E2)
  - **Thioester exchange**
- Multiple members

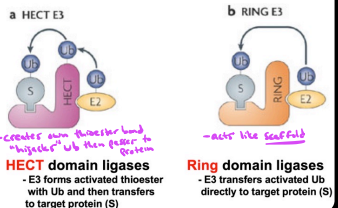


E3: Ubiquitin Protein Ligase

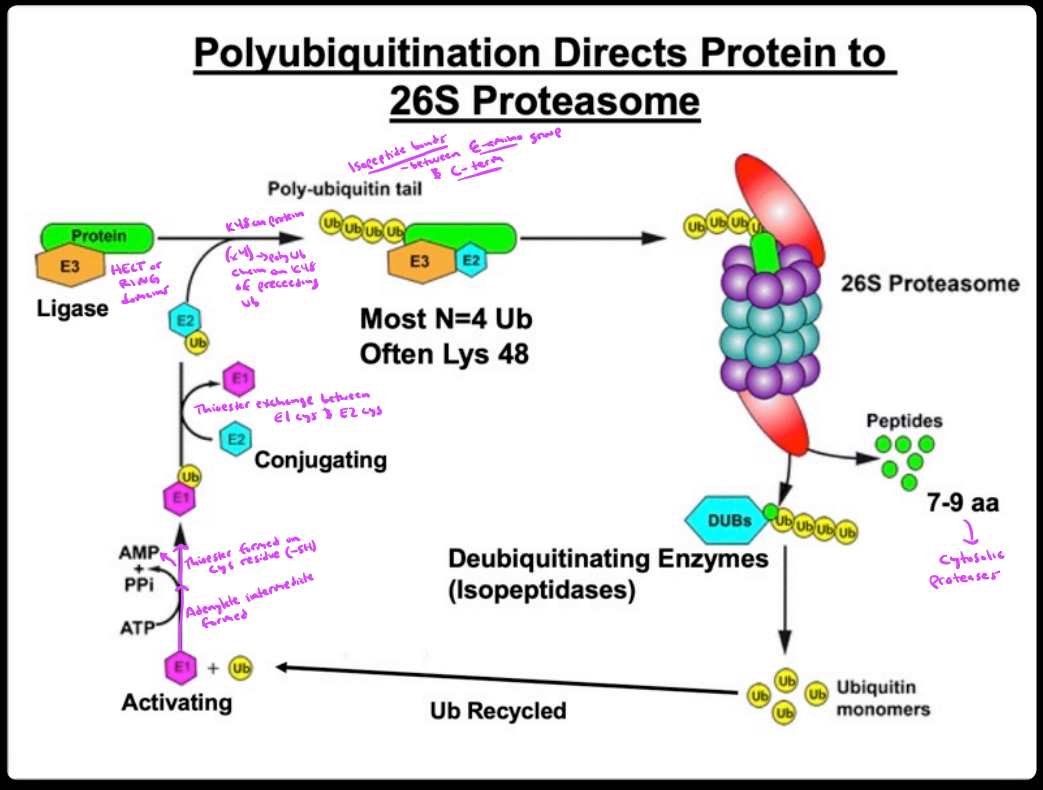
- **Recognizes** target protein and **transfers** activated Ub to target protein (multiple members)
- Transfers Ub to **epsilon amino** group of **Lys** forming **isopeptide** bond



Two Main Families of E3 Ligases



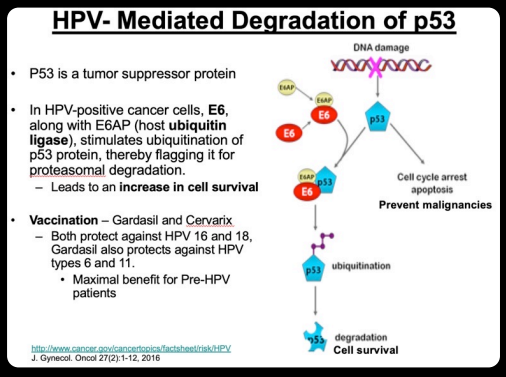
Polyubiquitination Directs Protein to 26S Proteasome

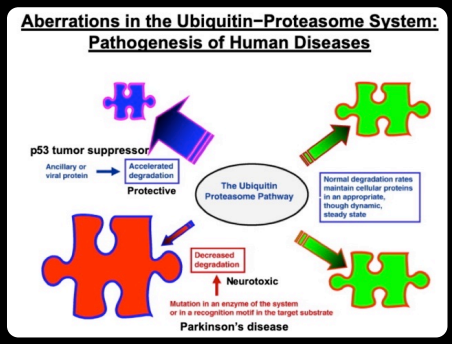


Ub-Proteasome Diseases

Human Papilloma Virus (HPV)

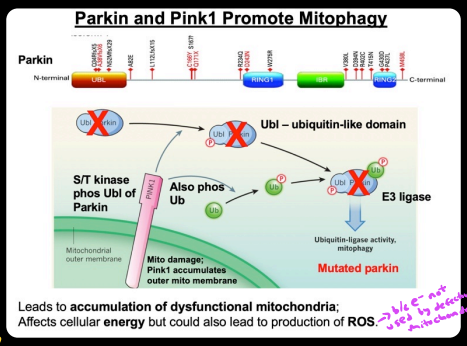
- DNA virus - infects cutaneous or mucosal epithelial cells
- causes most cervical carcinomas
  - HPV 16 & 18 - 70% of cervical cancer
  - HPV 16 - 25% associated w/ head & neck carcinomas
- HPV encodes E6 protein (viral oncoprotein)
  - alters E3 ligase specificity → targets p53 for ubiquitination & degradation via 26S proteasome
    - p53 = tumor suppressor protein
      - binds & inhibits G1 cyclin-CDK complex
        ↳ arrests cells in G1

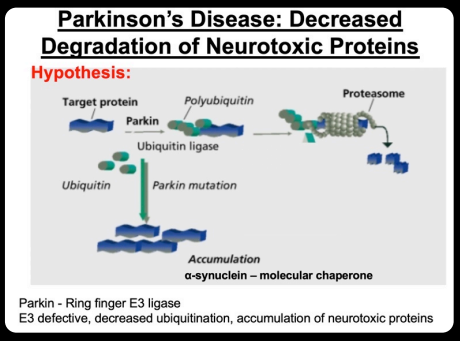




Parkinson's Disease

- Chronic, progressive disorder
- resting tremor, slowed movements, postural instability
- Defective Parkin Protein
  - Parkin = E3 ligase
    ↳ 50% of disease-causing defects in autosomal recessive juvenile onset PD
  - Characterized by mitochondrial dysfunction, oxidative stress, & Lewy Bodies (protein aggregates)
- Normally, Parkin helps degrade dysfunctional mitochondria
  - dysfunctional mitochondria → PINK1 translocated to membrane surface → phosphorylates Ubl domain on Parkin AND phosphorylates Ubiquitin → Parkin-P binds Ub-P → Active complex
    → E3 ligase activity activates → encapsulation & degradation of defective mitochondria (Mitophagy)





Multiple Myeloma

- Malignant plasma cell disorder → overproduction of WBC's
- Excessive NF-κB activity → tumorigenesis
- NF-κB Pathway
  - NF-κB = Txn factor w/ p65 & p50 subunits
    - normally found in cytosol complexed w/ I-κB
      ↳ I-κB = inhibitor of NF-κB, masks NLS sequence
  - ↑ stress → Ikk (Ik kinase) phosphorylates I-κB → I-κB-P detected & degraded by proteasome → NF-κB NLS exposed → translocates to nucleus → ↑ txn of pro survival genes

Tx: Bortezomib - 35% success rate

- Inhibits chymotrypsin-like activity of proteasome
  → prevents degradation of I-κB → ↓ NF-κB activity
  → ↓ txn of pro survival proteins