Institut für Experimentelle Innere Medizin
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VL 1 (Dr. Dawadschargal Dubiel)

Immunoproteasome (The Ubiquitin Proteasome System) (UPS)

Outlines

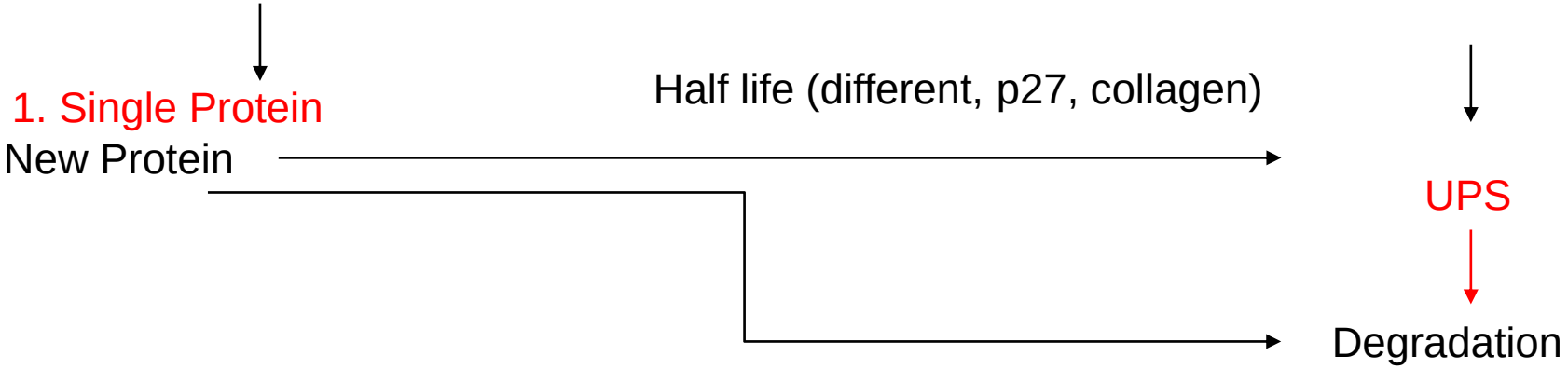
1. Protein, Proteinturnover
2. Discovery
3. UPS components:
    - Ubiquitin:
        - Ubiquitin-bond (isopeptide, covalent bond)
        - Ubiquitin-chains
    - E1-E2-E3 enzyme cascade: E3 Ub ligases classes
    - 26S Proteasome: 20S Proteasome
        - 19S Regulator
        - Another regulators
    - DUBs: Deubiquitinating enzymes
    - Immunoproteasome
4. Functions:
    1. Antigene presentation (MHC class I)
    2. Protein quality control (degradation of misfolded proteins in cytosol)
    3. Signal transduction (NFkB pathway)
5. Ub-like proteins:
    - Features of Ub-like proteins
    - Functions

Protein (Eiweß)_Turnover

Definition: In cell biology, protein turnover refers to the **replacement of older proteins as they are broken down within the cell by new synthesized protein**.

Different types of proteins have very different turnover rates.

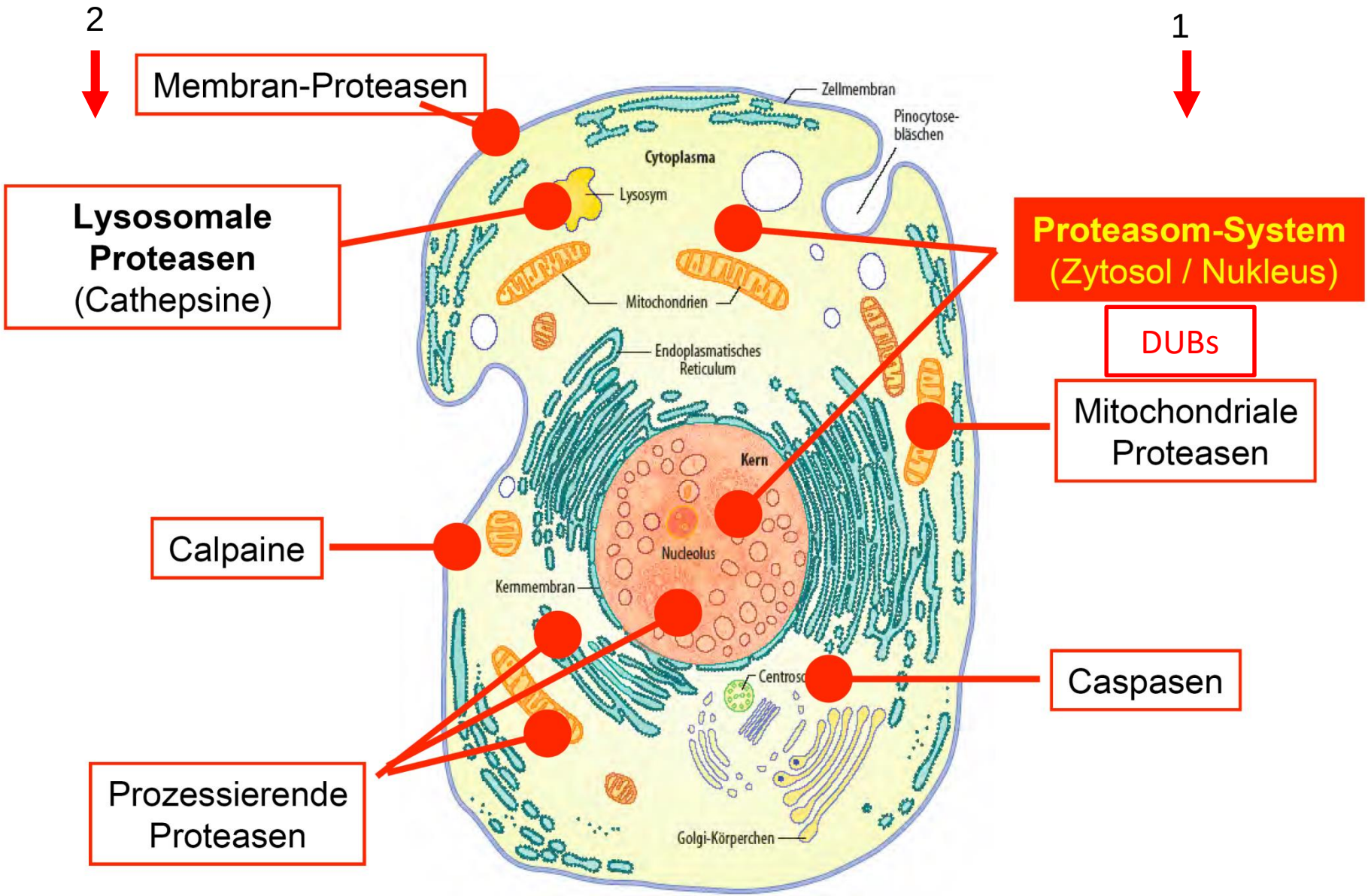
A rate of new synthesized protein is equal to rate of degrading protein: Steady state



Single proteins: from amino acids (peptide bond)

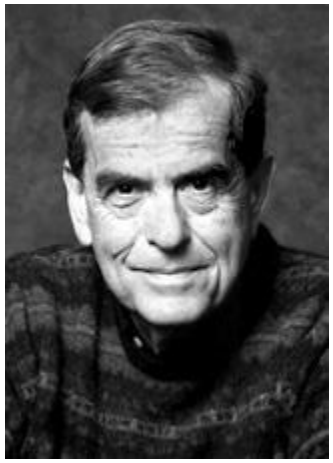
Cleaved by enzymes: proteases

Localization of cellular proteases



The Nobel Prize in Chemistry 2004

"for the discovery of ubiquitin-mediated protein degradation"



Aaron Ciechanover

1/3 of the prize

Israel

Technion – Israel Institute of Technology
Haifa, Israel

b. 1947

Avram Hershko

1/3 of the prize

Israel

Technion – Israel Institute of Technology
Haifa, Israel

b. 1937
(in Karcag, Hungary)

Irwin Rose

1/3 of the prize

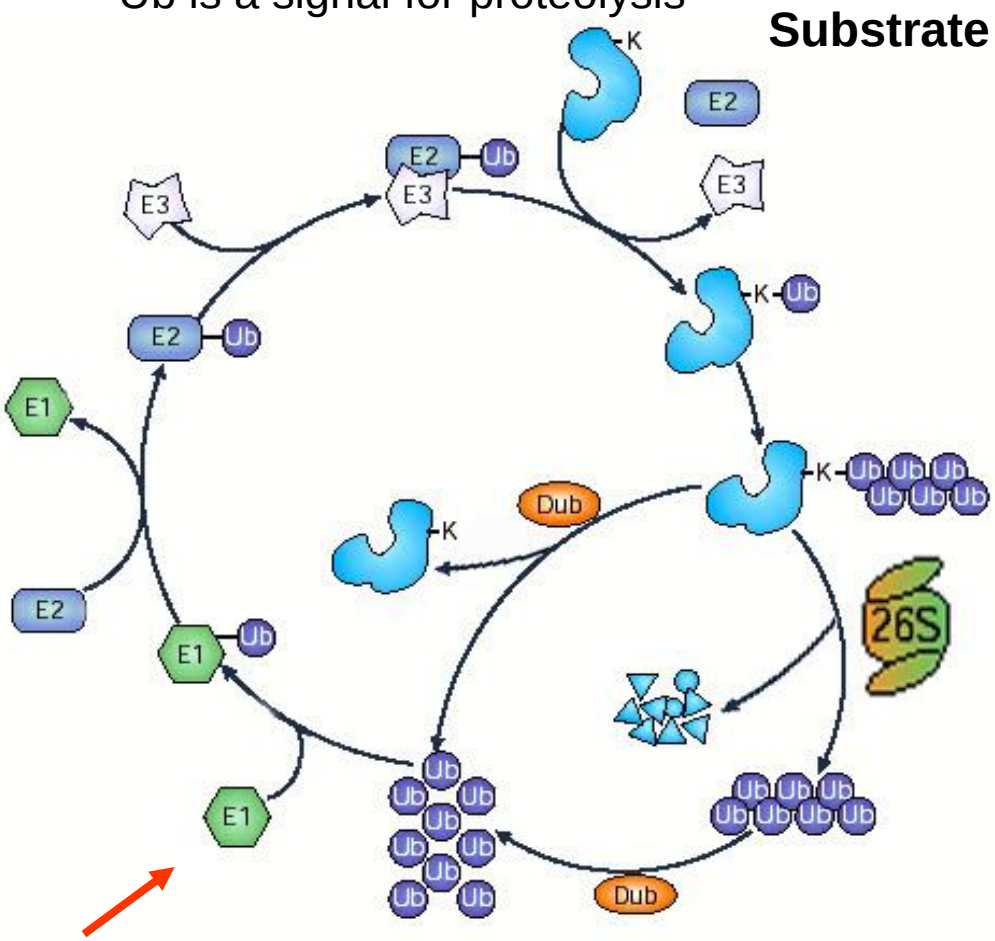
USA

University of California
Irvine, CA, USA

b. 1926

The Ubiquitin (Ub) Proteasome System (UPS)

-Major proteolytic system in eukaryotic cells
-Ub is a signal for proteolysis



Components of the UPS

- E1 – Ub activating enzymes
- E2 – family of Ub conjugating enzymes
- E3 – families of Ub ligating enzymes
- The 26S proteasome
- Family of deubiquitinating enzymes (DUBs)

Modified from Sullivan et al., 2003

Components of the UPS

Ubiquitin: from Latin *ubique* (“everywhere”), from *ubi* (“where”)

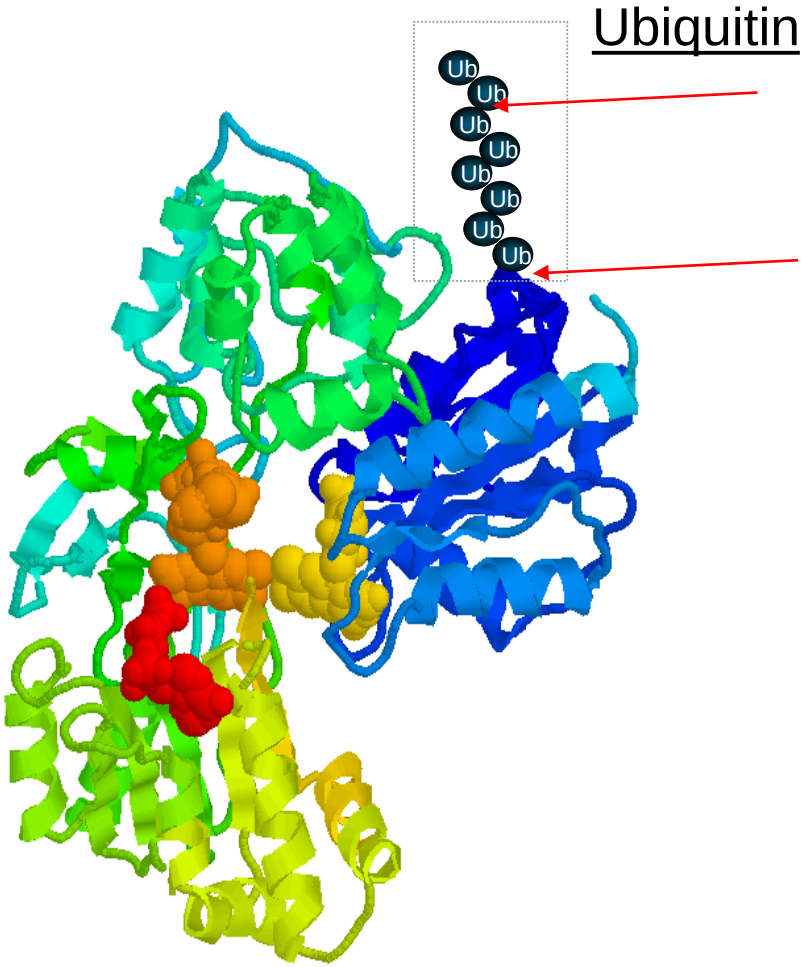
Ubiqutin: Covalent bond between:

Ub-Ub

Ub-Substrates

-Ubiquitination/Ubiquitylation:

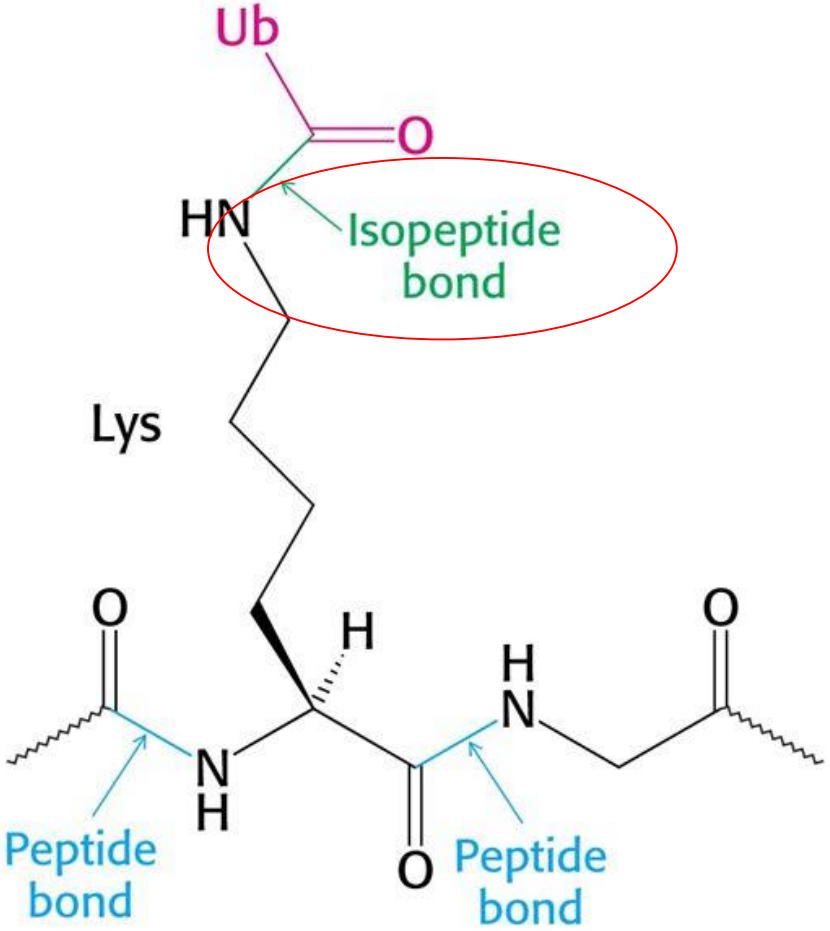
a posttranslational modification



The peptide bond

Ubiquitin covalently binds to ε-amino group of lysine residue on a protein destined to be degraded.

Isopeptide bond is formed.

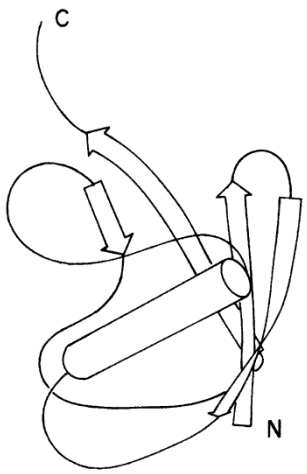


Ubiquitin (Ub)

Amino acid sequence of Ub

```
Met - Gln - Ile - Phe - Val - Lys - Thr - Leu - Thr - Gly -
Lys - Thr - Ile - Thr - Leu - Glu - Val - Glu - Pro - Ser -
Asp - Thr - Ile - Glu - Asn - Val - Lys - Ala - Lys - Ile -
Gln - Asp - Lys - Glu - Gly - Ile - Pro - Pro - Asp - Gln -
Gln - Arg - Leu - Ile - Phe - Ala - Gly - Lys - Gln - Leu -
Glu - Asp - Gly - Arg - Thr - Leu - Ser - Asp - Tyr - Asn -
Ile - Gln - Lys - Glu - Ser - Thr - Leu - His - Leu - Val -
Leu - Arg - Leu - Arg - Gly - Gly
```

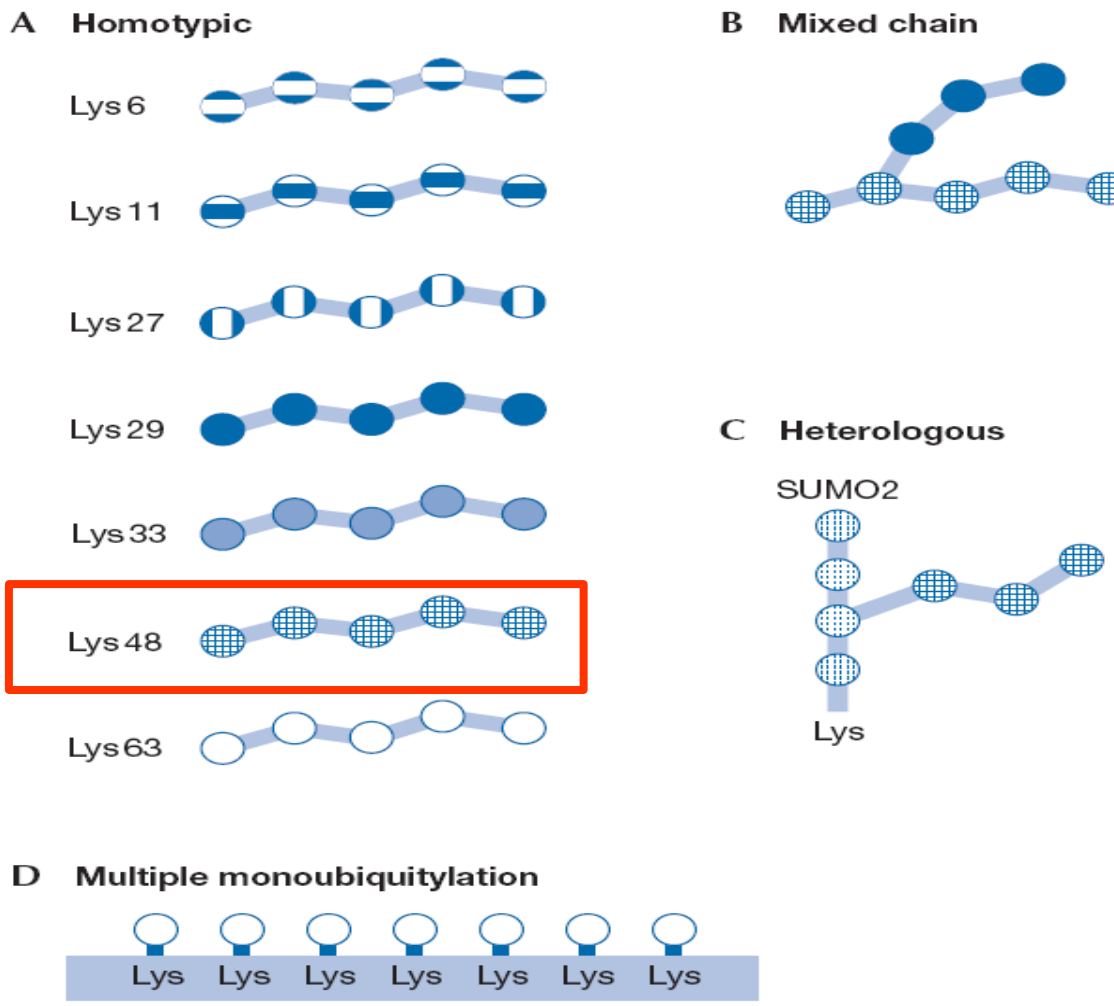
5, 10, 15, 20, 25, 30, 35, 40, 45, 48, 50, 55, 60, 63, 65, 70, 75



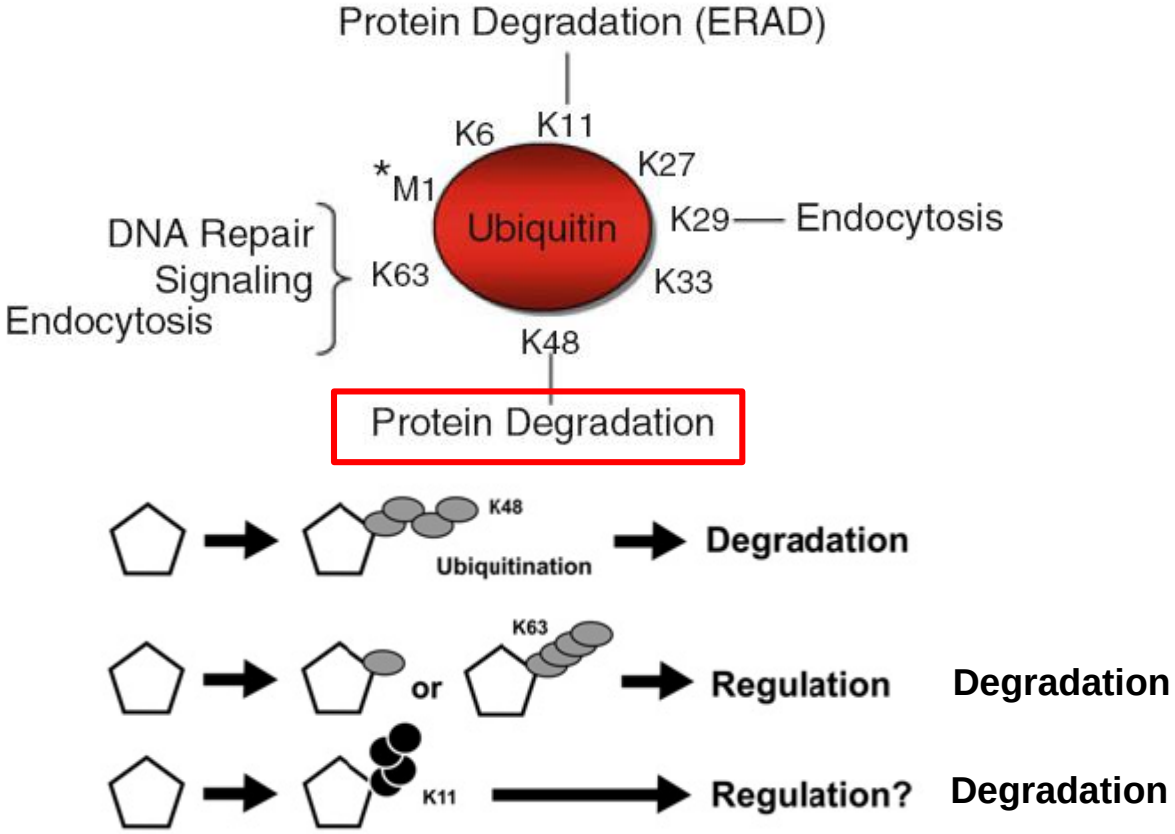
Alpha-helix (cylinders) and beta-strands (ribbons) of Ub structure

Ubiquitin-like proteins:
SUMO - small ubiquitin-like modifier
Nedd8 - neural-precursor-cell-expressed developmentally down-regulated 8

The diversity of possible Ub chains



Different forms of Ubiquitin chains have different functions



Mono-ubiquitination of membrane receptor proteins leads to internalization.

Poly-Ub chains are the signals for proteolysis

In tetra-Ub molecules of Ub are linked via isopeptide bond between the Ub-Gly-COOH and Ub-Lys48-NH2.

Ub conjugates = branched proteins

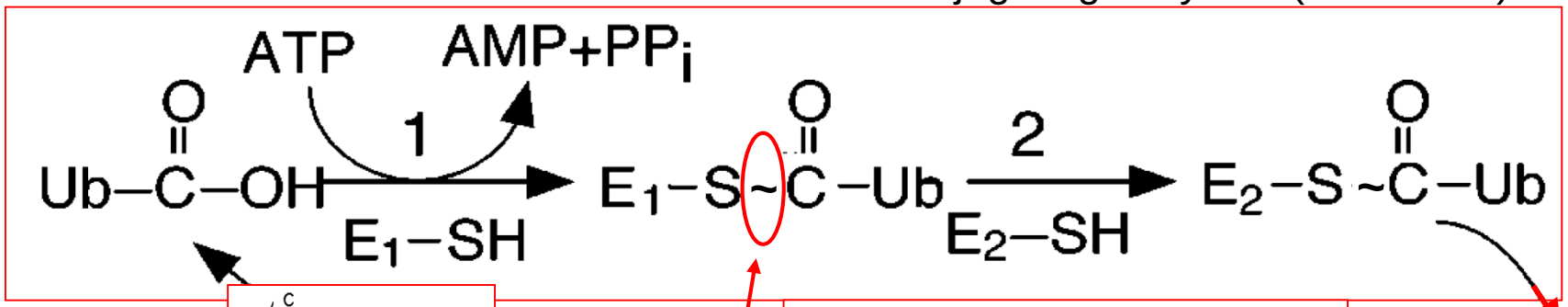
Activation and transfer of Ub

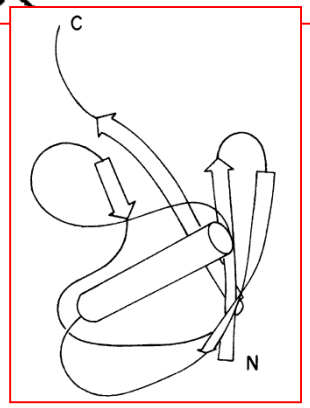
E1 – Ub activating enzyme

There are few Ub activating enzymes in eukaryotic cells

E2s – UBCs – Ub conjugating enzymes

There is a large family of Ub conjugating enzymes (~50 UBCs).



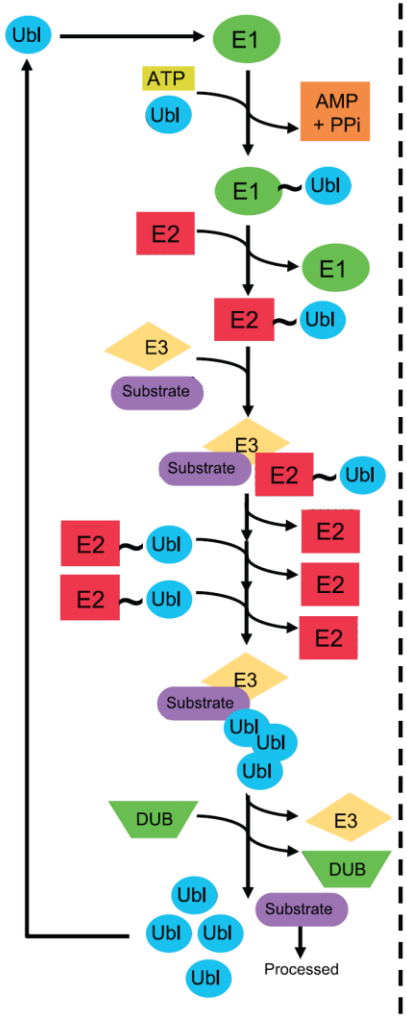


Transthiolation reaction from E1-S-Ub to E2-S-Ub.

Thioester bond

36 kJ/mol

E1-E3 enzyme cascade



Ubiquitin

E1 (2):

Uba1
Uba6

E2 (30):

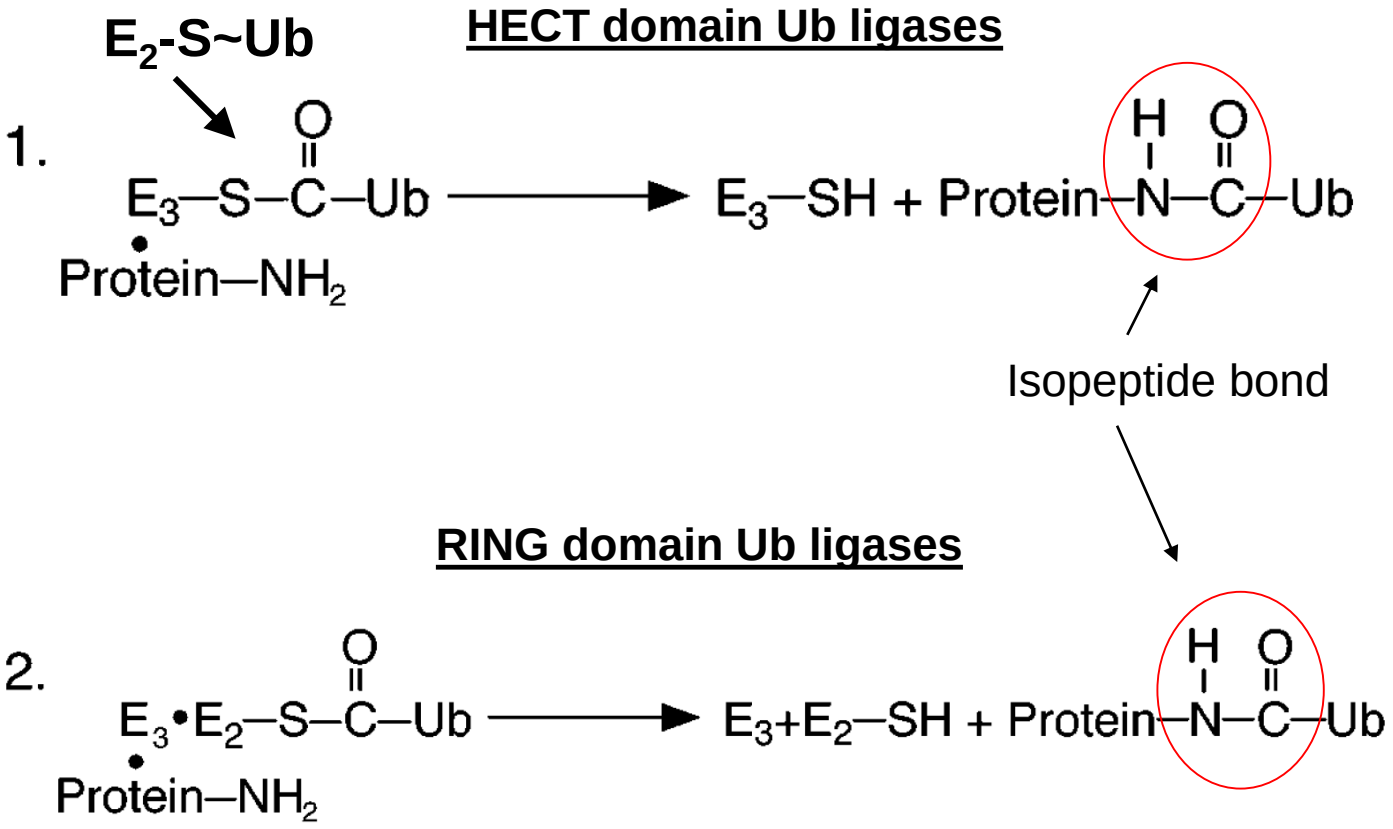
UBE2A(hHR6A)
UBE2B(hHR6B)
UBE2C(UbcH10)
UBE2D1(UbcH5A)
UBE2D2(UbcH5B)
UBE2D3(UbcH5C)
UBE2D4(HBUCE1)
UBE2E1(UbcH6) ±
UBE2E2
UBE2E3(UbcH9)
UBE2G1(UBE2G)
UBE2G2(UBC7)
UBE2H(UBCH)
UBE2J1(NCUBE1)
UBE2J2(NCUBE2)
UBE2K(HIP2)
UBE2L3(UbcH7)
UBE2L6(UbcH8) ±
UBE2N(Ubc13)
UBE2O(E2-230K)
UBE2Q1(NICE-5)
UBE2Q2
UBE2R1(CDC34)
UBE2R2(CDC34B)
UBE2S(E2-EPF)
UBE2T(HSPC150)
UBE2U*
UBE2V1(UEV-1A)
UBE2V2(MMS2)
UBE2W
UBE2Z(Use1)
BIRC6(apollon)

E3 (>1000):

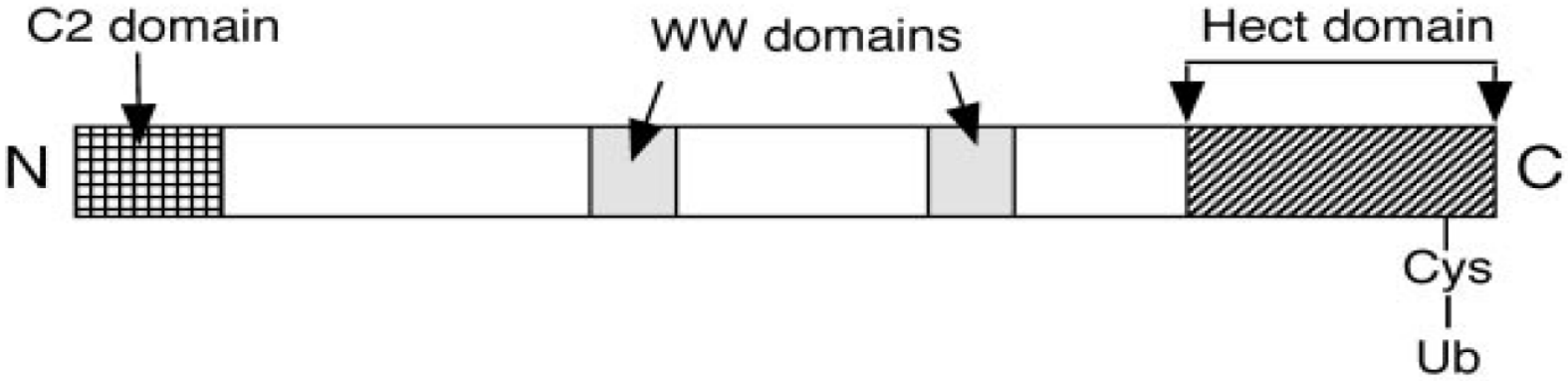
Single/multiple subunit
RING, HECT, U-box, PHD

Ub ligases (E3s)

The Ub ligases (E3s) ligate specifically Ub to protein substrates. They determine the specificity of the UPS.



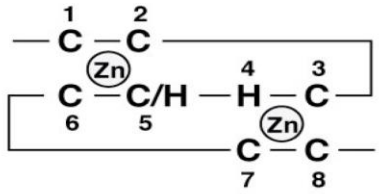
1. HECT domain Ub ligases



HECT – homologous to E6-AP carboxy terminus. Approximately 30 genes.
E6-AP is a typical HECT domain E3, which ubiquitinates p53 in cervical tumor cells.
Cervical carcinoma = Gebärmutterhalskrebs.

These tumor cells are infected with the human papillomavirus (HPV) transmitted by sex, which produces the protein E6. This viral protein binds to cellular E6-AP, activates it and causes the degradation of the tumor suppressor p53. → Vaccination

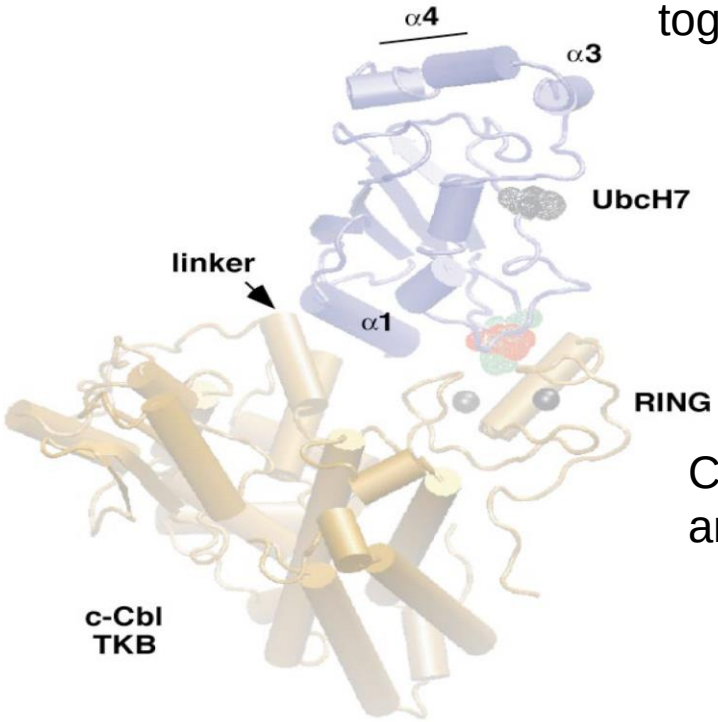
2. RING domain Ub ligases



$CX_2CXnCX_{1-3}HX_{2-3}C/HX_2CX_nCX_2C$

1 2 3 4 5 6 7 8

RING domain – RING=Really Interesting New Gene
-Hundreds of genes
-Zinc-binding domain
-Functions as molecular scaffolds that bring UBCs and substrate proteins together and support the transfer of Ub



Complex of the RING domain CBL and the UBCH7 enzymes.

Metal-Ion???

2. RING domain Ub ligases as protein complexes

Protein complex functions:
-Energy saving
-Specificity
-Regulation
-Time saving: quick reaction

UPS

Inactivation, Activation:

Protein complexes

Half life

New subunits

Coordinated assembly

Autophagy

Degradation

Cullin-RING Ub ligases (CRLs)

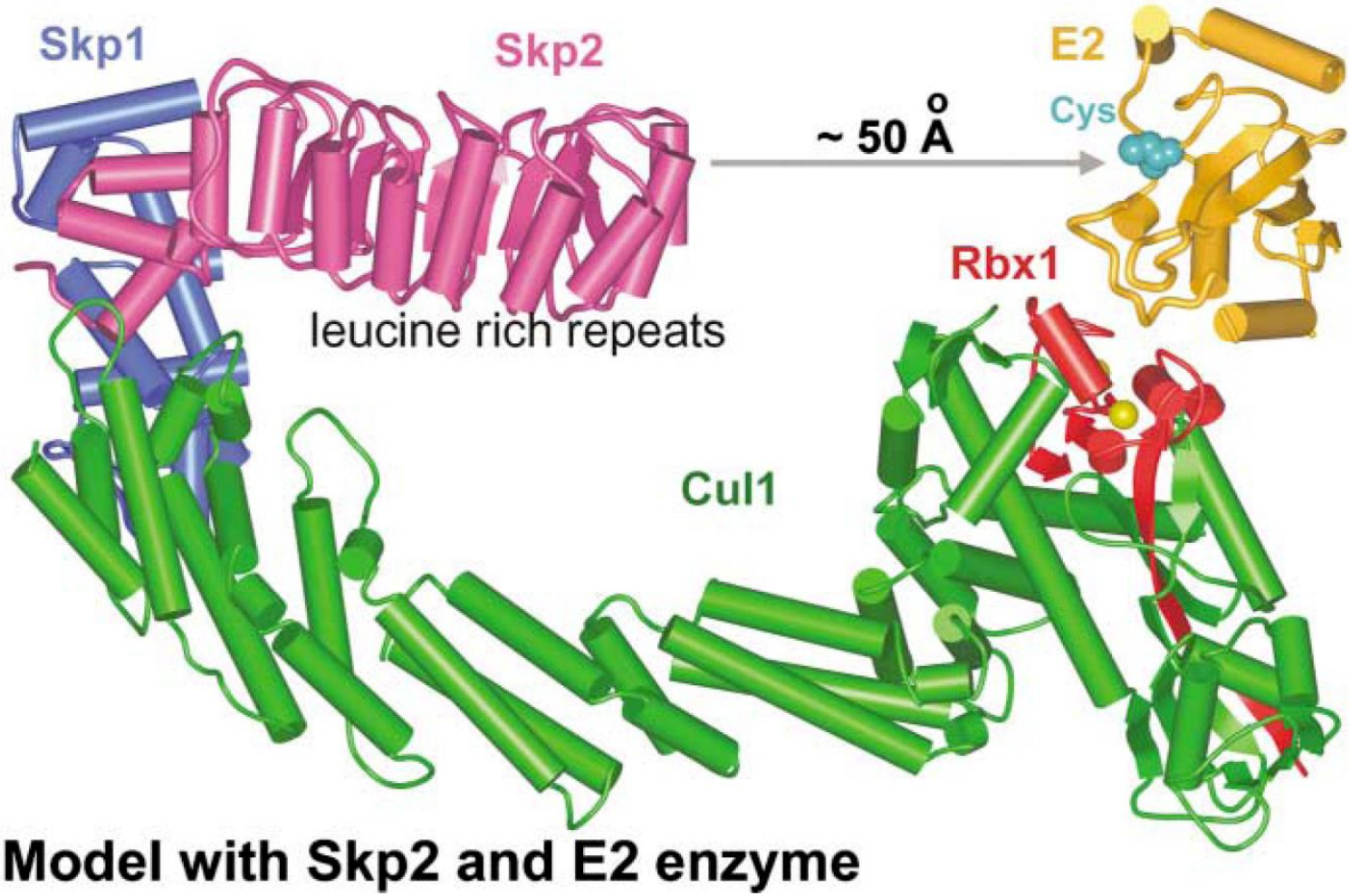
There are nearly 600 Ub ligases encoded in the human genome and up to 240 are CRLs.

About 20% of the proteasome-dependent degradation is dependent on CRLs.

Composition of CRLs:

- Cullin, scaffold, member of the cullin family (cullins 1 -7)
- RING domain proteins, Rbx1 or Rbx2
- Substrate recognition subunits, F-box proteins, BTB-proteins
- In mammalian cells exist 69 genes for F-box proteins and hundreds of BTB proteins

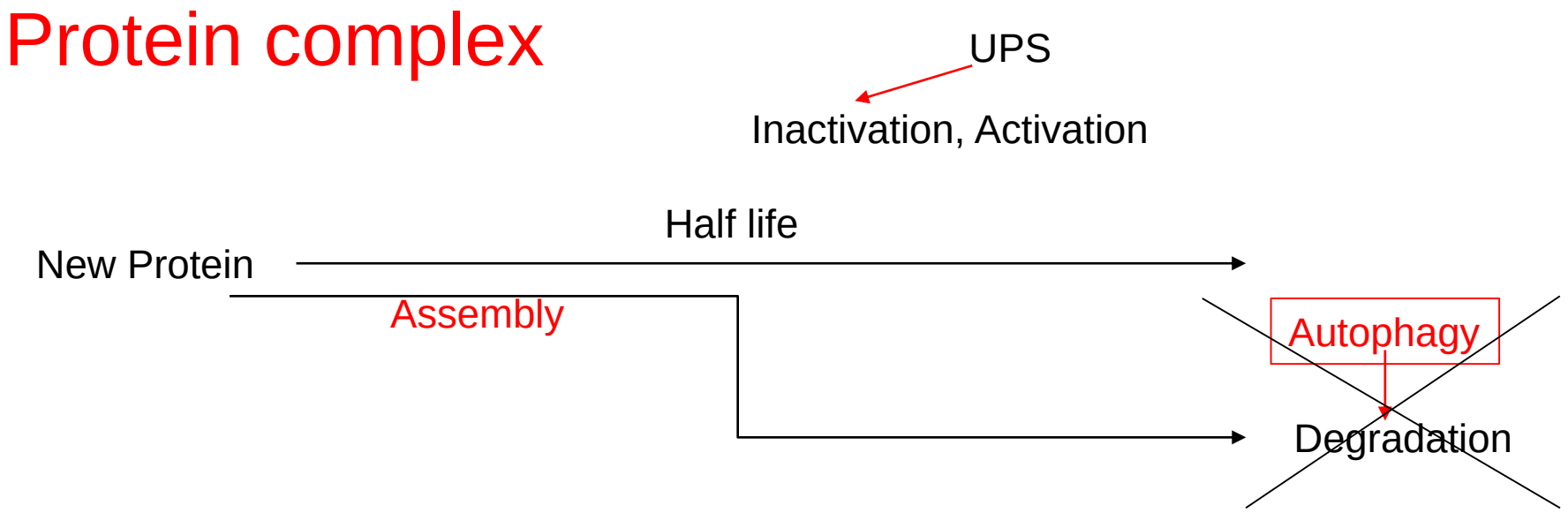
Multisubunit RING domain ligases
Cullin-RING Ub Ligases (CRLs)



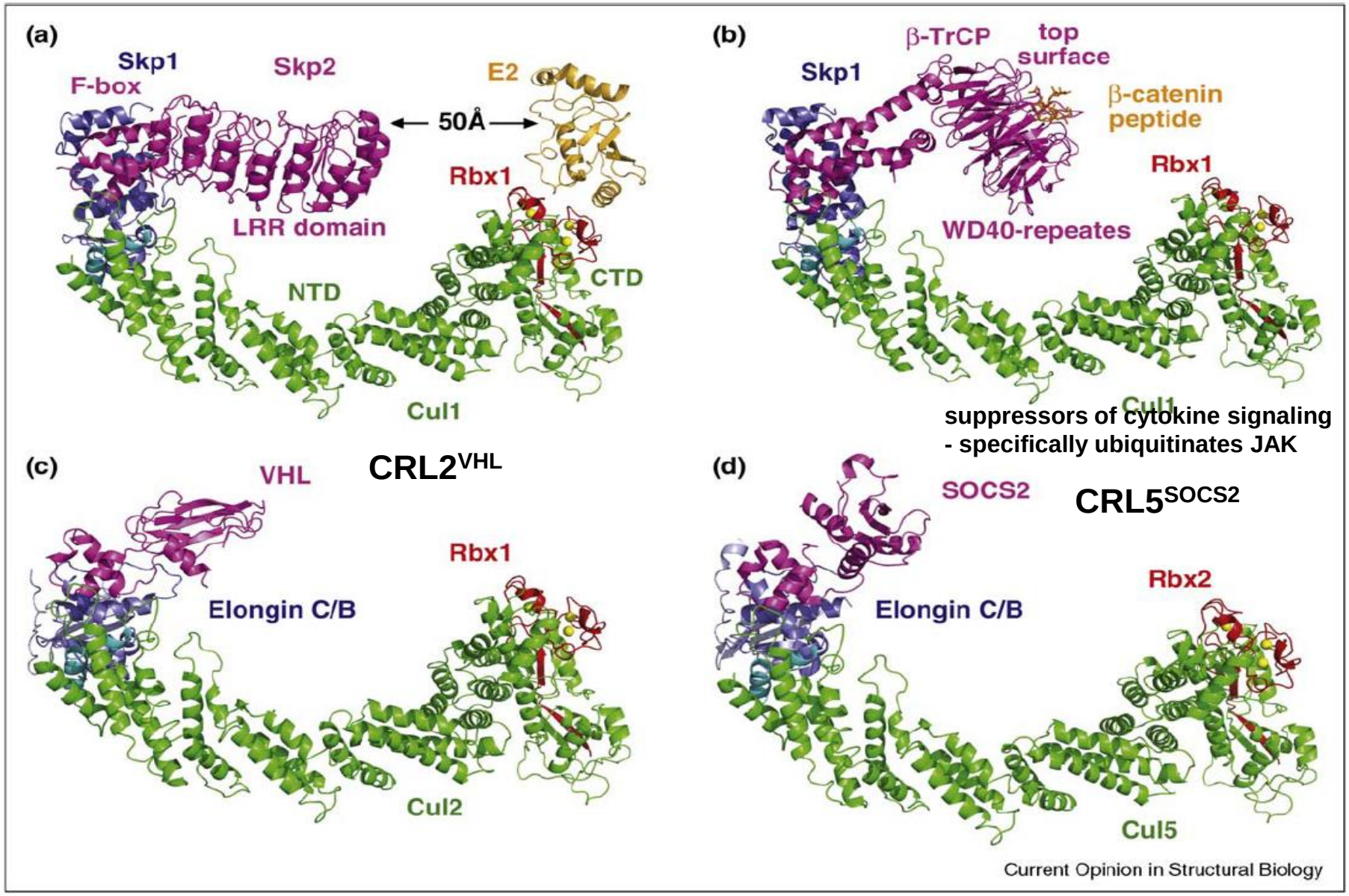
Model with Skp2 and E2 enzyme

Protein complex activation/inactivation:

Protein complex:
-Energy
-Time
-Complex: Specificity

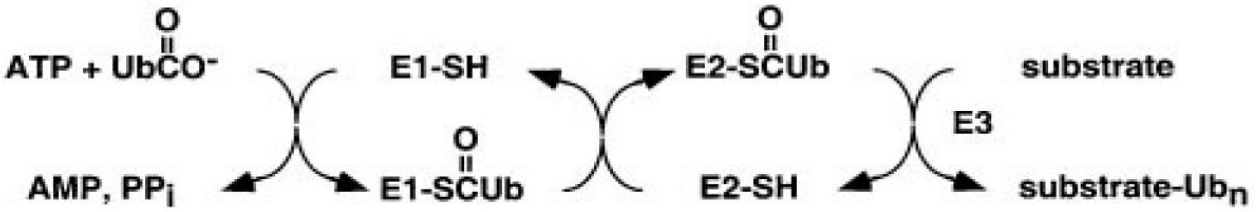


Structure of CRLs



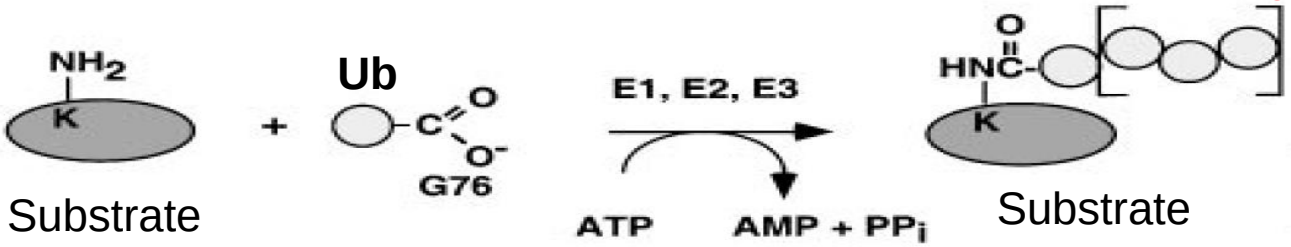
The cascade of E1, E2 and E3 enzymes activates and transfers Ub to protein substrates

Formation of poly-Ub conjugates



Poly-ubiquitination of substrate proteins

Ub moieties linked through lysine 48



The E3 enzymes determine the specificity of the UPS. They select proteins at a certain time, at a certain place for ubiquitination and degradation. Binding of substrates to the Ub ligases can be regulated by phosphorylation etc.

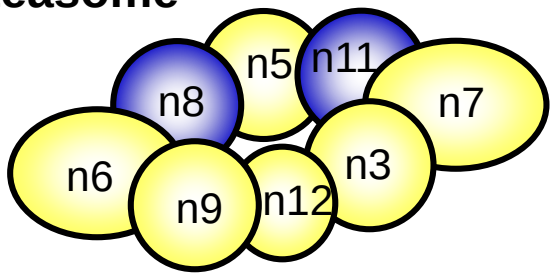
Poly-ubiquitinated proteins are recognized and degraded by the 26S proteasome

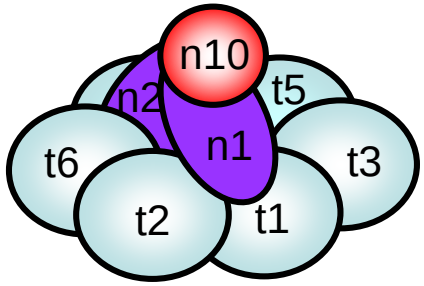
Composition of the 26S proteasome

19S regulatory complex

Lid

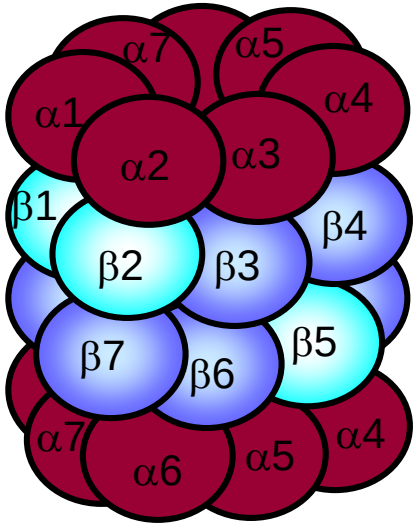
Base



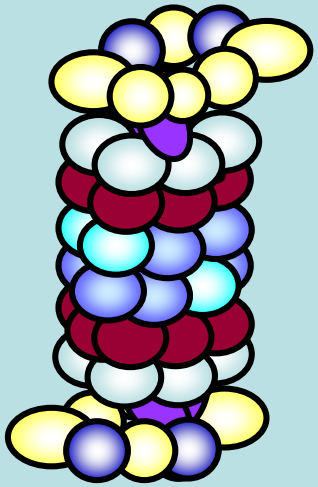


n = Rpn
t = Rpt

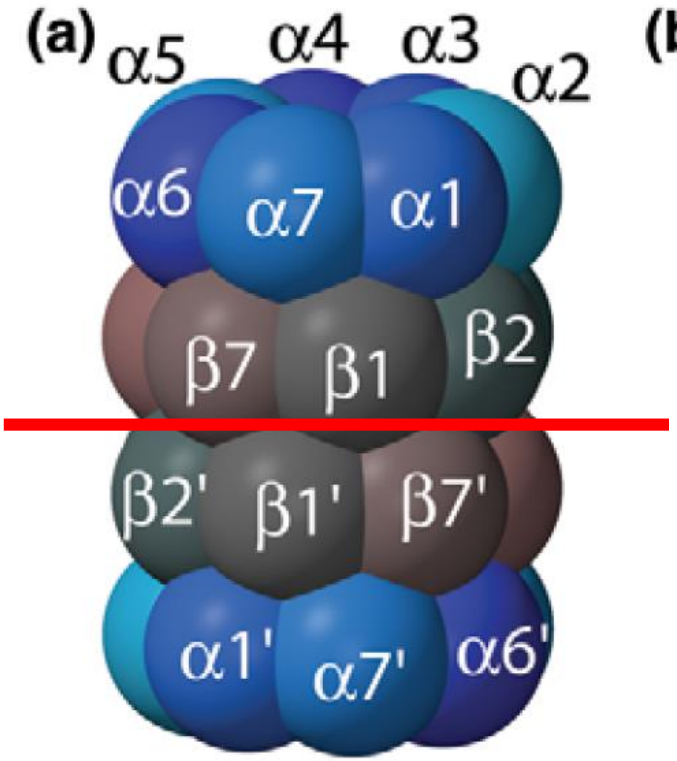
20S proteasome

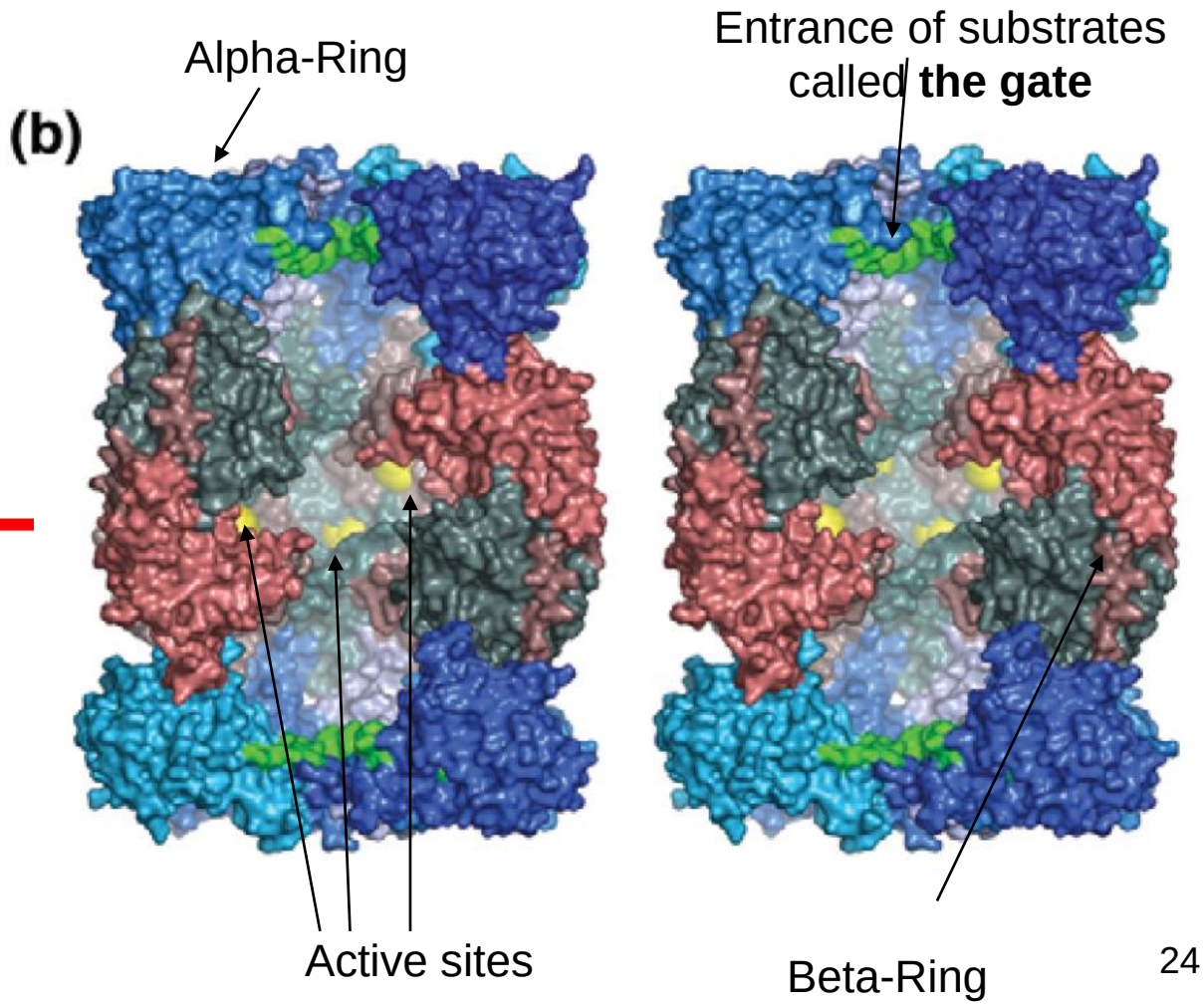


26S proteasome



Structure of the 20S core proteasome





Horizontal symmetry

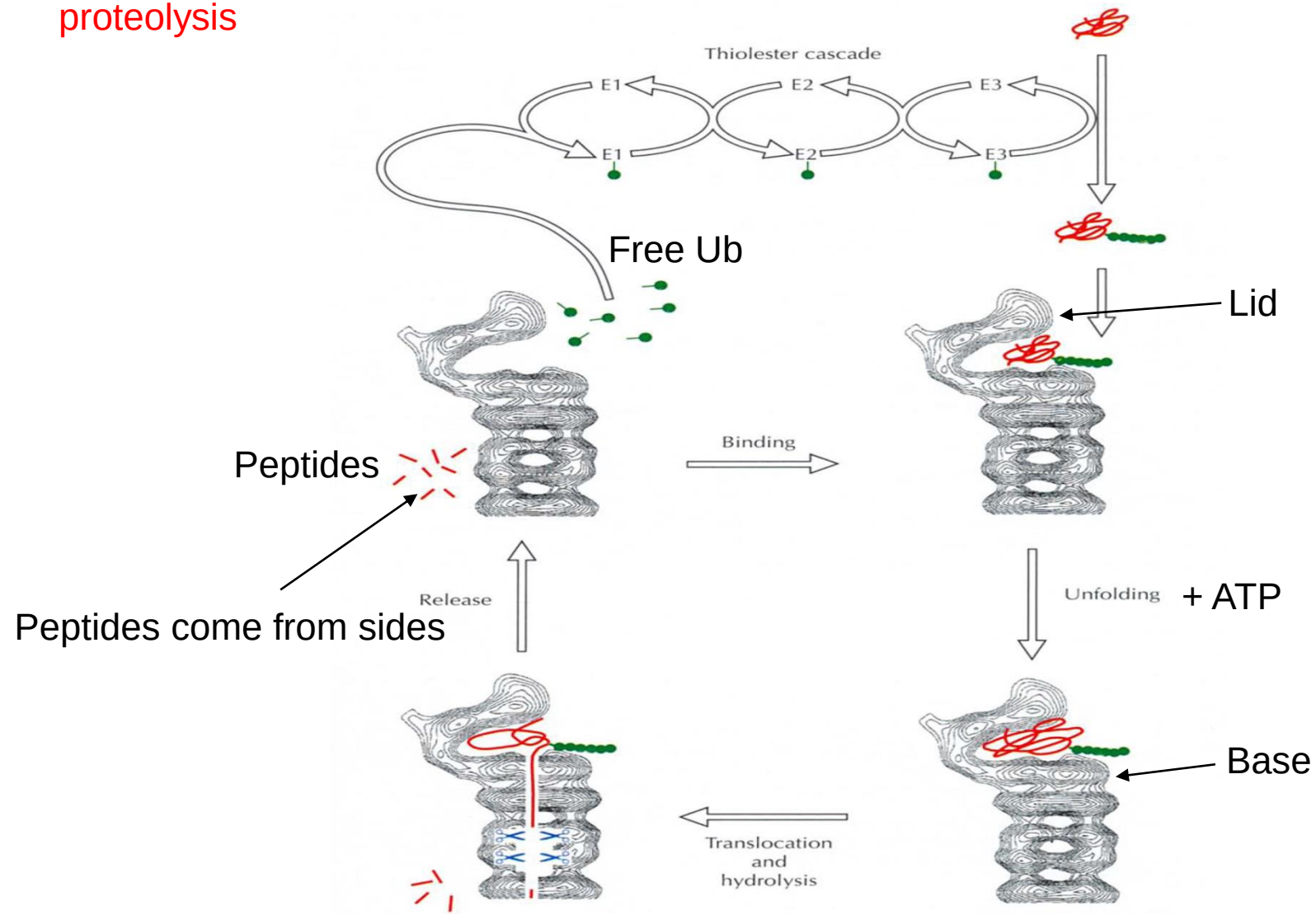
Active sites of the 20S proteasome (Threonine (Thr) protease)

- Caspase-like activity (ß1): cleaves after Glu/Asp residues
- Trypsin-like activity (ß2): cleaves after the basic amino acids (Lys, Arg)
- Chymotrypsin-like activity (ß5): cleaves after hydrophobic amino acids (Ala, Val, Leu, Isoleu)

One molecule of 20S Proteasome contains 6 active sides

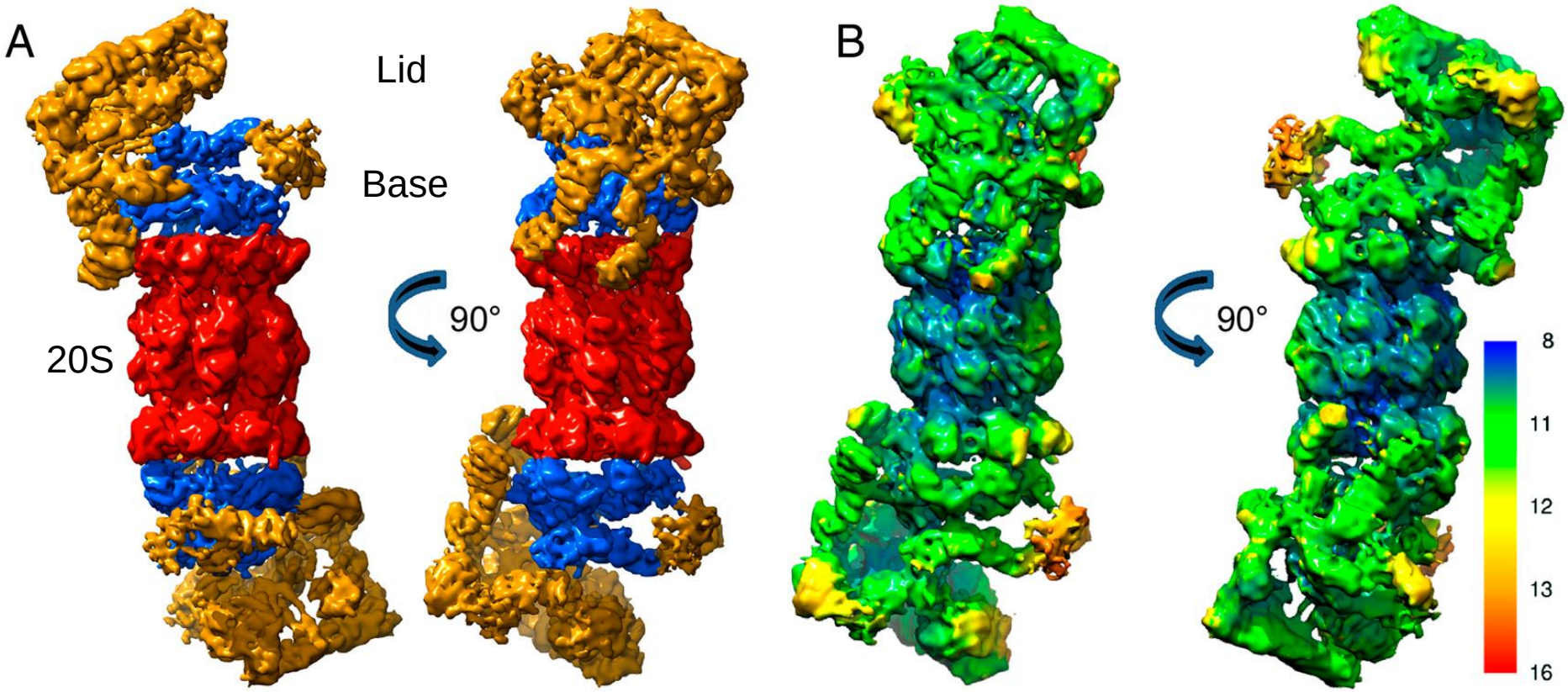
Function of the 26S proteasome

proteolysis



Cryo-electron microscopy of the 26S proteasome

Lasker K, Förster F, Bohn S, Walzthoeni T, Villa E, Unverdorben P, Beck F, Aebersold R, Sali A, **Baumeister** W.
Proc Natl Acad Sci U S A. 2012 Jan 31;109(5):1380-7.

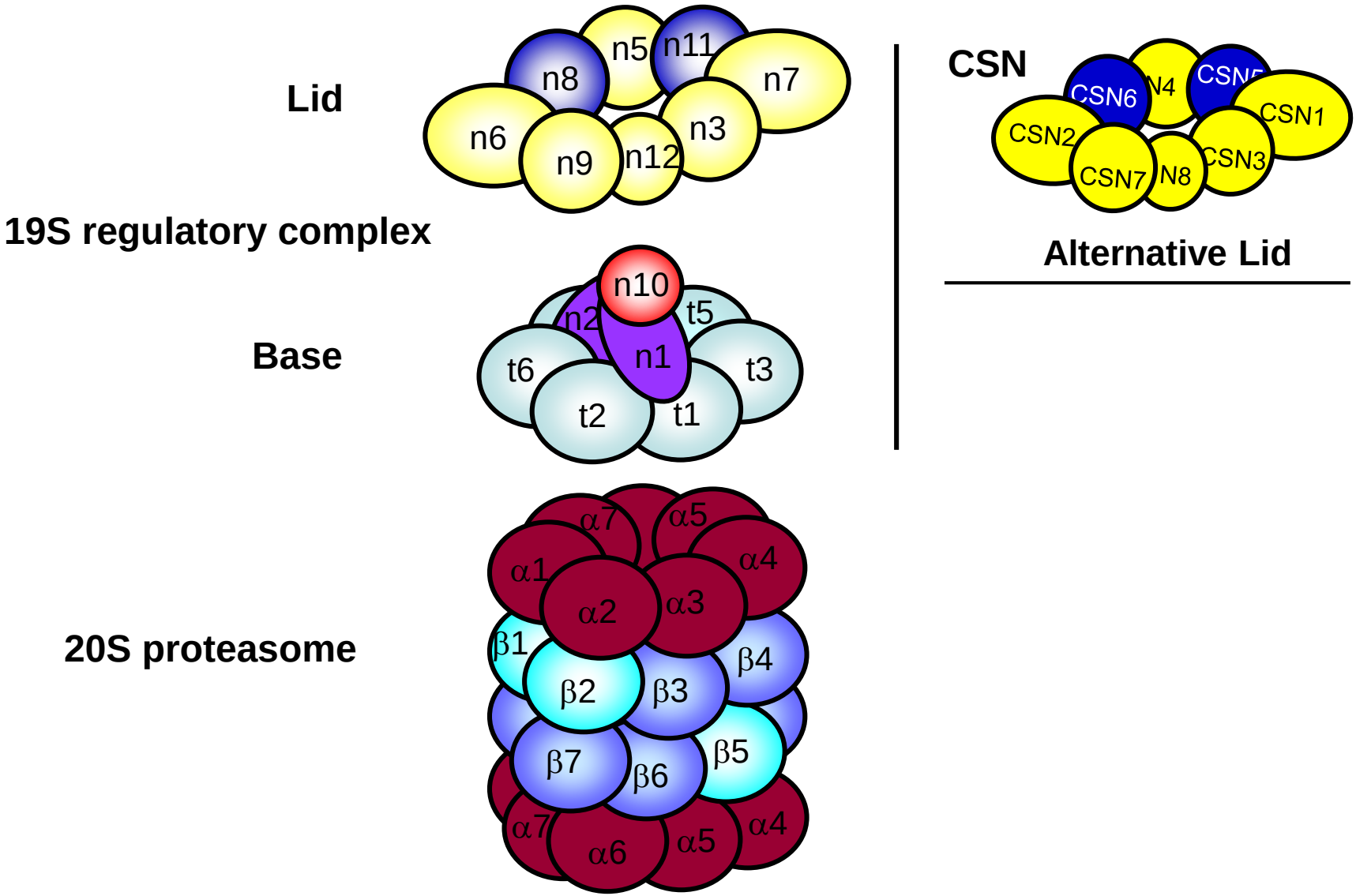


Cryo-EM map of the S. pombe 26S proteasome. (A) The single-particle cryo-EM density map of the 26S proteasome from S. pombe at 8.4-Å resolution is shown in two views, related by a 90° rotation around the pseudo-sevenfold axis of the CP (CP: red; AAA-ATPase hexamer: blue; Rpn subunits: gold). (B) The isosurface of the cryo-EM map is colored according to the local resolution in Å, as specified in the color bar.

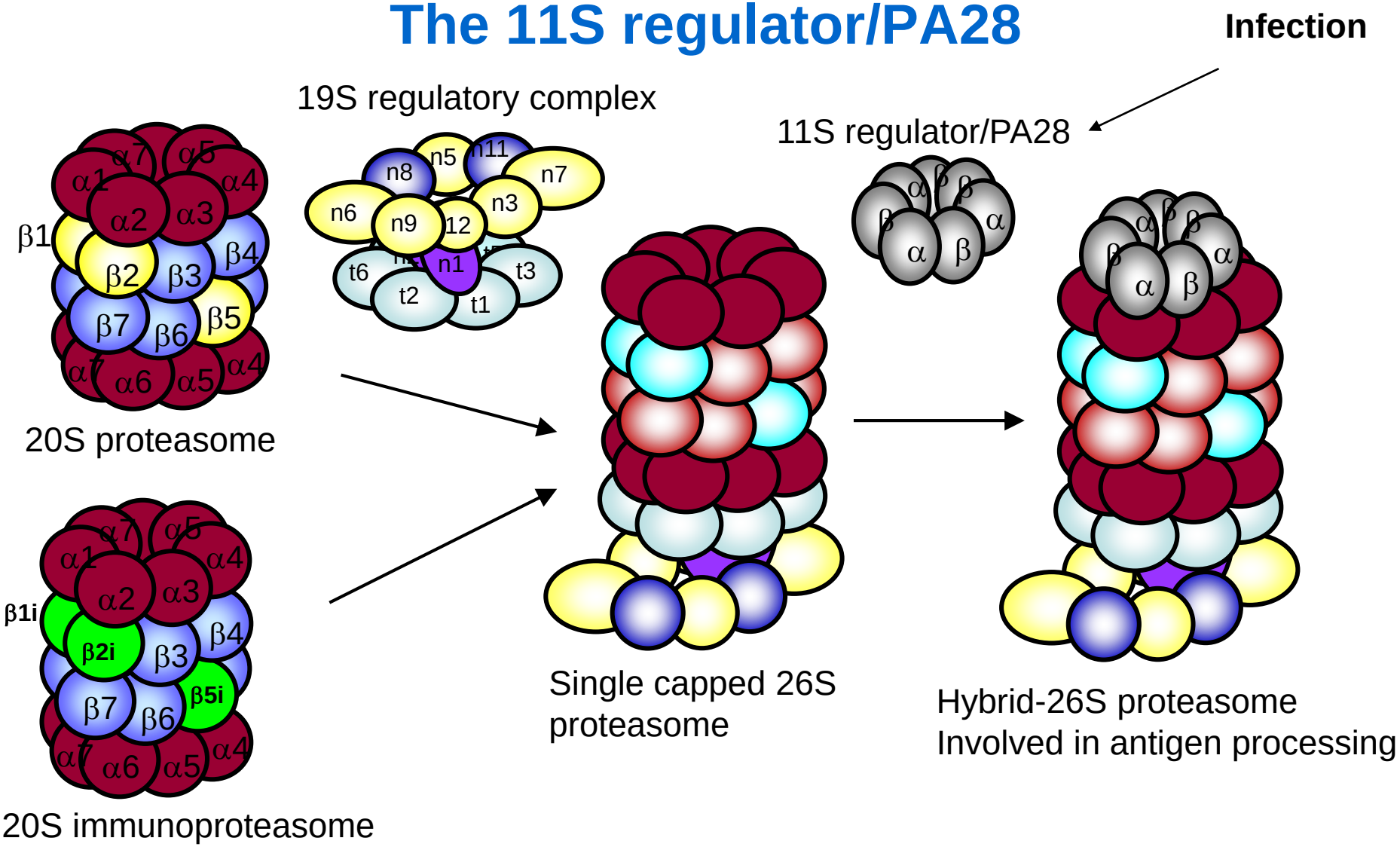
Regulatory complexes of the proteasome

- The 19S regulatory complex
- The COP9 signalosome (CSN)?
- The PA28 or 11S regulator

Composition of the 26S proteasome, 19S regulator and the COP9 signalosome (CSN)

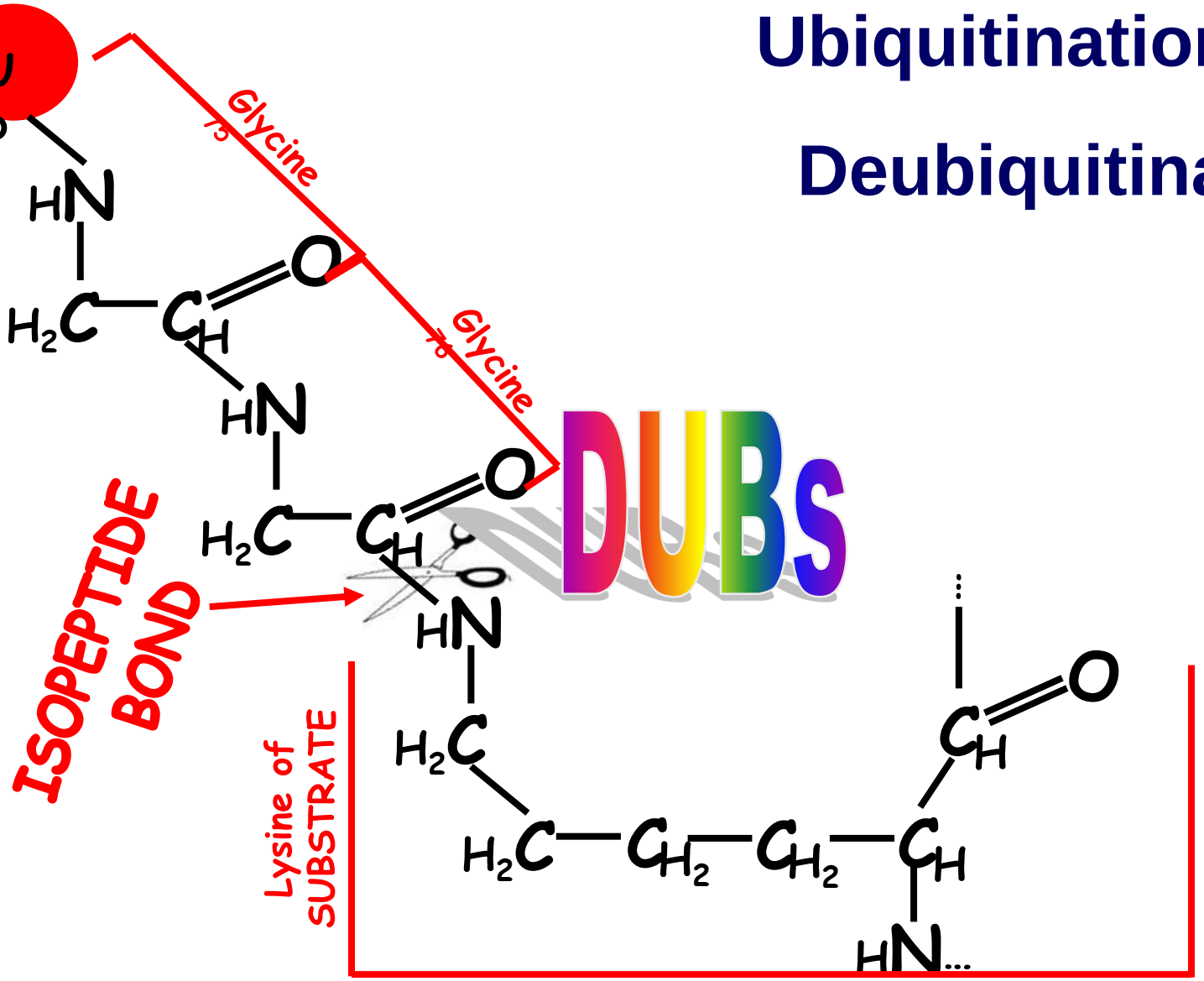


Different forms of the proteasome
The 11S regulator/PA28



Replacement by ß1i, ß2i and ß5i ← **Infection**

Ubiquitination and Deubiquitination



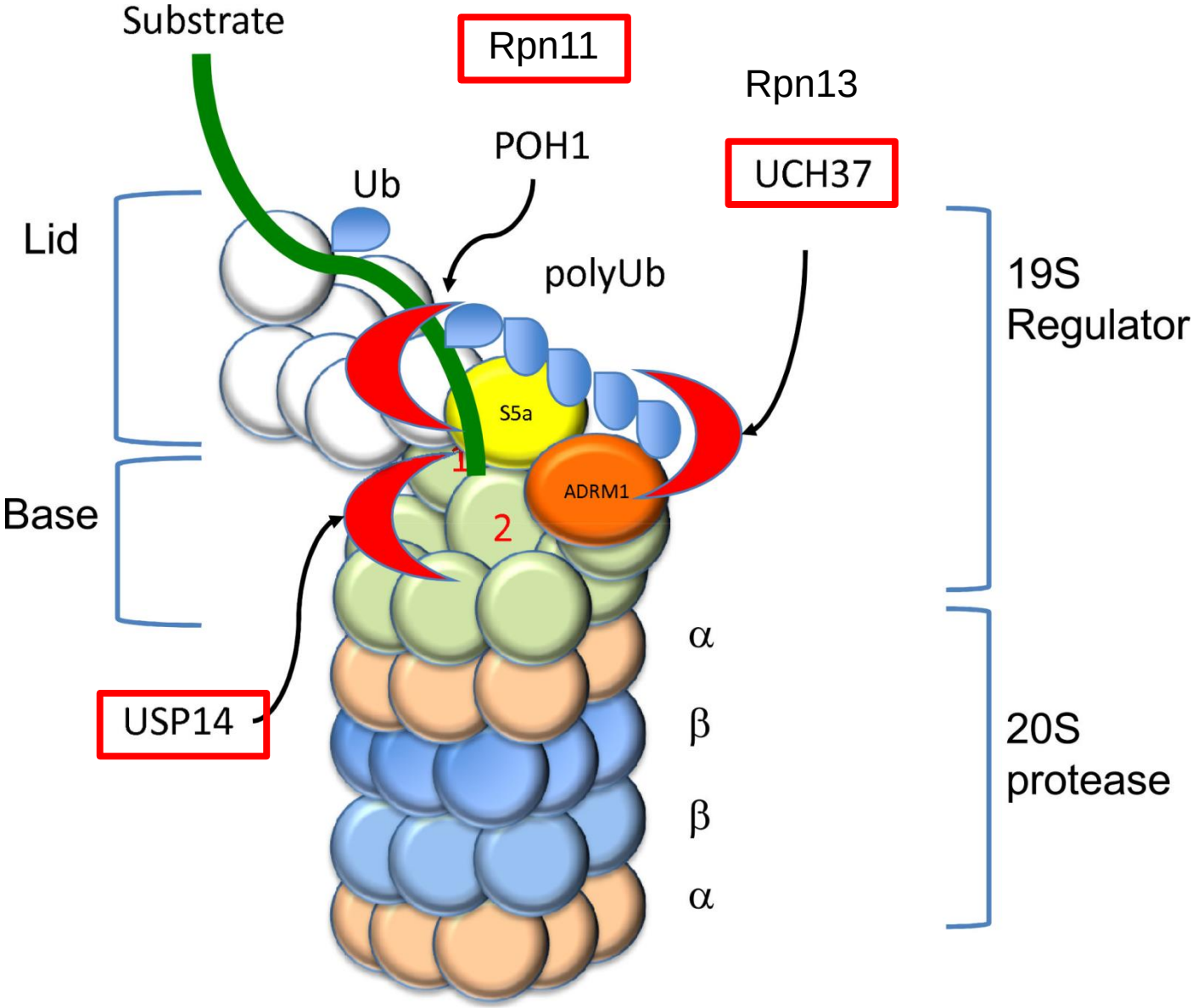
Deubiquitinating enzyme (DUB) families

USP = Ub-specific protease; UCH = Ub-carboxy-terminal hydrolases; OTU = ovarian tumor enzymes; JAMM-domain enzymes; MJD = Machado-Josephin domain superfamily; SENP = SUMO proteases

Substrate-specificity of protease families acting on ubiquitin-like proteins

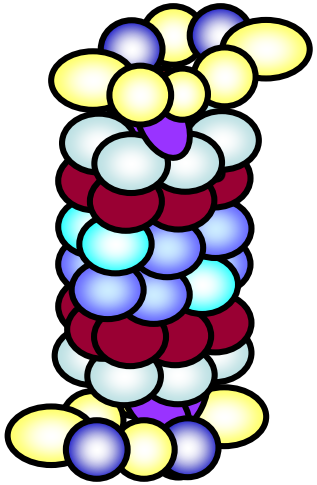
Activity	DUB protein family					
	USP	UCH	OTU	JAMM	MJD	SENP
Deubiquitylating (isopeptidase)	Ub PolyUb	Ub	Ub PolyUb	PolyUb	PolyUb	
Deneddylating	Nedd8	Nedd8		Nedd8		Nedd8
DeISGylating	ISG15					
Desumoylating						SUMO

DUBs are necessary for the 26S proteasome



Functions of the ubiquitin proteasome system (UPS) in cells

26S proteasome



Cell cycle

Transcription regulation

3.

Signal transduction

2.

Protein quality control

Apoptosis

Stress response

Angiogenesis

1.

Antigen processing

Adipogenesis

Tumor growth

Bortezomib – inhibitor of the 20S proteasome used in tumor therapy

Functions of the UPS:

THE ROLE OF THE UPS IN ANTIGEN PROCESSING

What is the immune system?

The **immune system** is a host defense system comprising many biological structures and processes within an organism that protects against disease.

It protects against pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.

In Mammalia the immune system can be classified into subsystems, such as the innate immune system versus the adaptive immune system, or humoral immunity versus cell-mediated immunity.

Innate immune system

The **innate immune system**, also known as the **non-specific immune system or in-born immunity system**.

The cells of the innate system recognize and respond to pathogens in a generic way, but, unlike the adaptive immune system, the system does not provide long-lasting immunity to the host.

Innate immune systems provide immediate defense against infection, and are found in all classes of plant and animal life.

The innate immune system is an evolutionarily older defense strategy.

Major functions of the innate immune system

- Recruiting immune cells to sites of infection, through the production of chemical factors, including specialized chemical mediators, called cytokines

- Activation of the complement cascade to identify bacteria, activate cells, and promote clearance of antibody complexes or dead cells

- Identification and removal of foreign substances present in organs, tissues, blood and lymph, by specialized white blood cells

- Activation of the adaptive immune system through a process known as antigen presentation

- Acting as a physical and chemical barrier to infectious agents.

What are antigens?

An **antigen** is a molecule capable of inducing an immune response (to produce an antibody) in the host organism

An immunogen is an antigen substance (or adduct) that is able to trigger a humoral (innate) or cell-mediated immune response. It first initiates an innate immune response, which then causes the activation of the adaptive immune response. An antigen binds the highly variable immunoreceptor products (B-cell receptor or T-cell receptor) once these have been generated.

Antigen presenting cells present antigens in the form of peptides on histocompatibility molecules. The T cell/T lymphocyte (a subtype of white blood cell), of the adaptive immune system, selectively recognize the antigens. Depending on the antigen and the type of the histocompatibility molecule, different types of T cells will be activated. For T-Cell Receptor (TCR) recognition, the peptide must be processed into small fragments inside the cell and presented by a major histocompatibility complex (MHC).

Major histocompatibility proteins

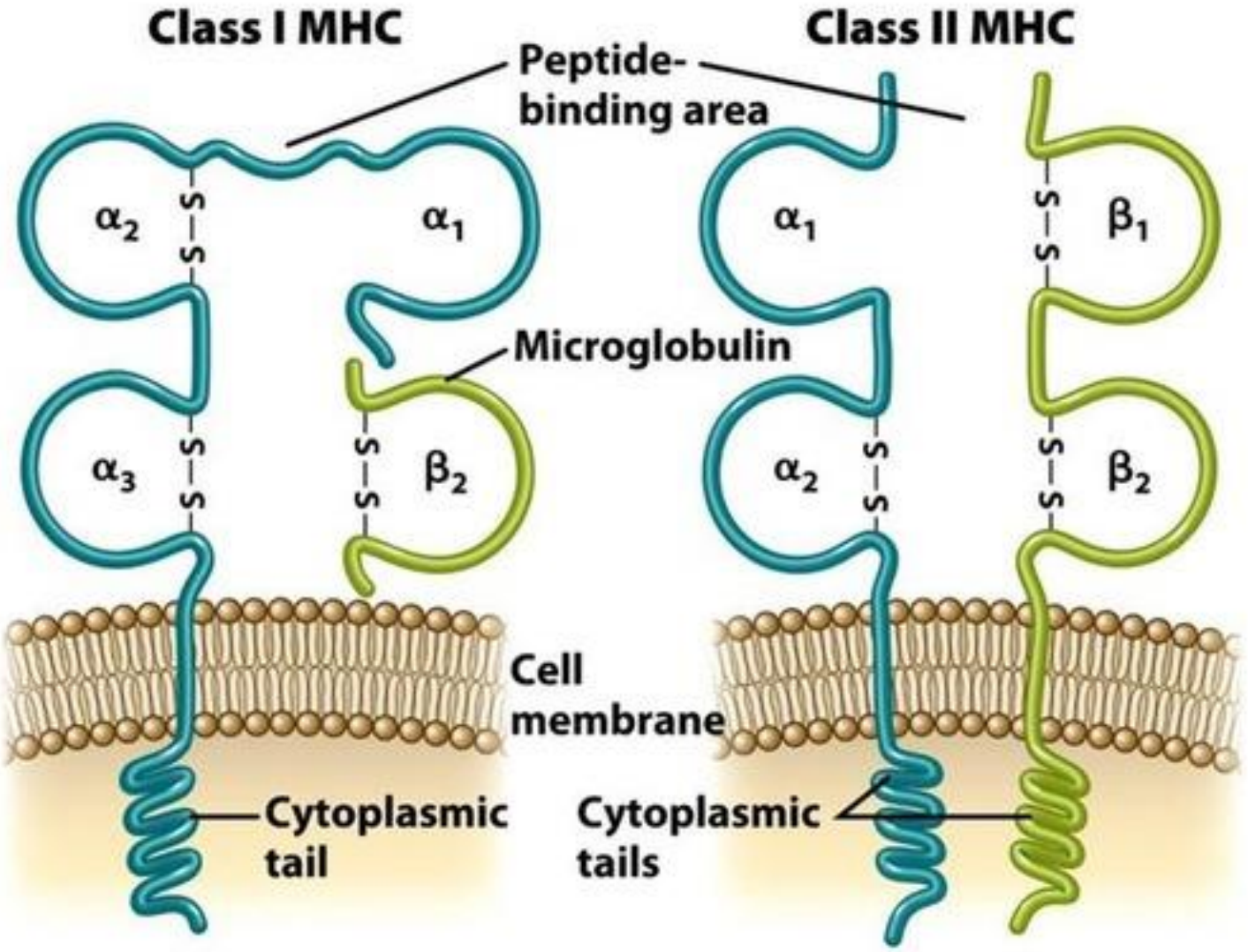


Figure 24.16a Microbiology: An Evolving Science

Antigenic peptides

MHC class I

~8-10 amino acids long, generally SMALLER because the antigenic peptide binding domain is CLOSE-ENDED (like a pita). Anchor residues are at position #2 and #9.
Nonamers bind strongest!!!

Endogenous antigens

Why self-peptides are not recognized by TCRs???

MHC class II

~18-20 amino acids long, generally LARGER because the Antigenic peptide binding domain is OPEN-ENDED (like a hotdog).

Exogenous antigens

Which cells are involved in antigen presentation?

Which cells express MHC class I molecules?

All nucleated cells, all somatic cells.

Which cells express MHC class II molecules?

All professional antigen presentig cells including macrophages, Dentritic cells and B cells.

Antigen presentation and the creation of „Self“

Antigen processing by the proteasome and presentation via MHC class I is important:

-self recognition

-for the recognition of mutated/tumor cells by the immune system

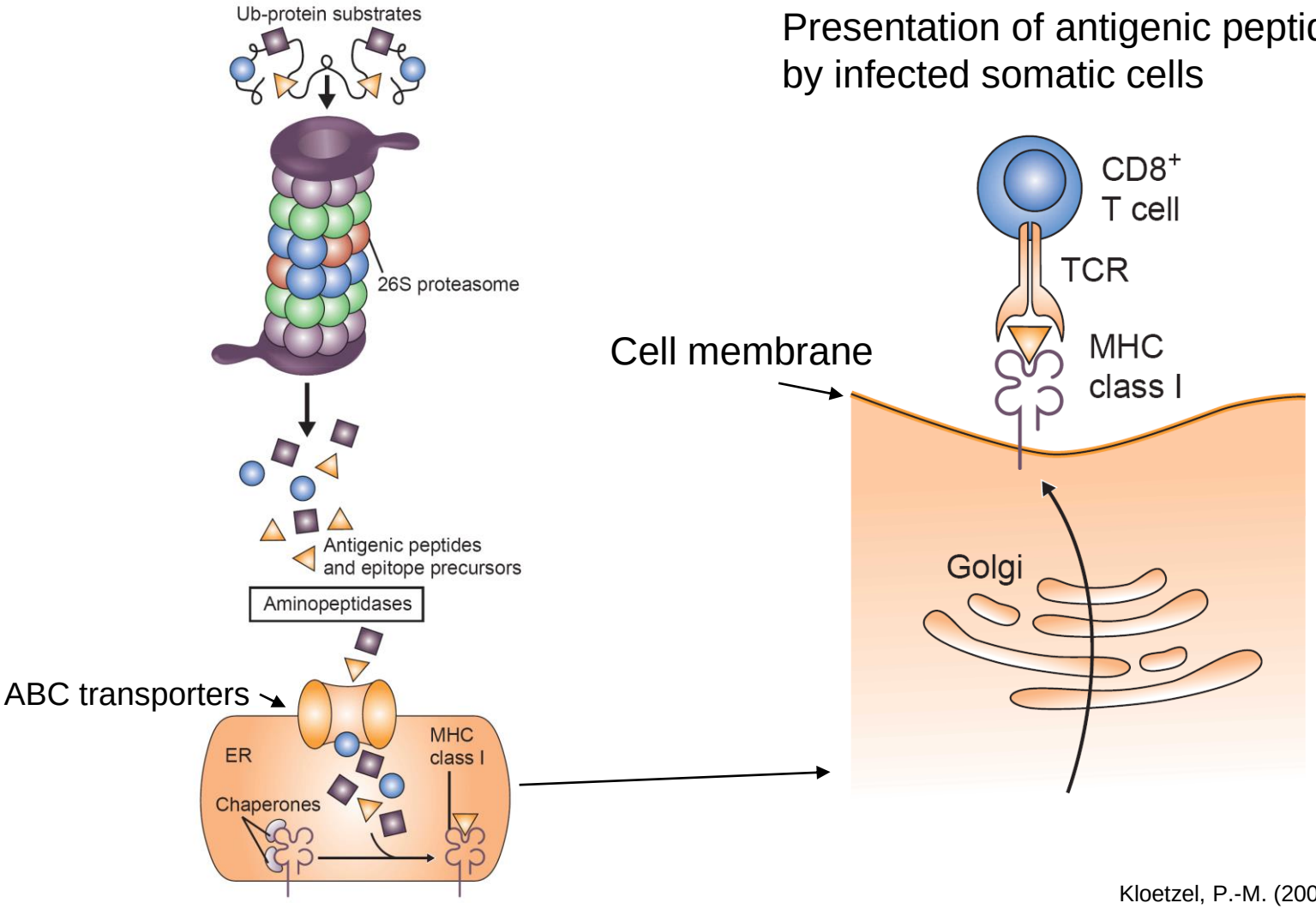
-for the recognition of antigenes drived from viral proteins

Bad consequence:

-for rejection of organs during transplantation

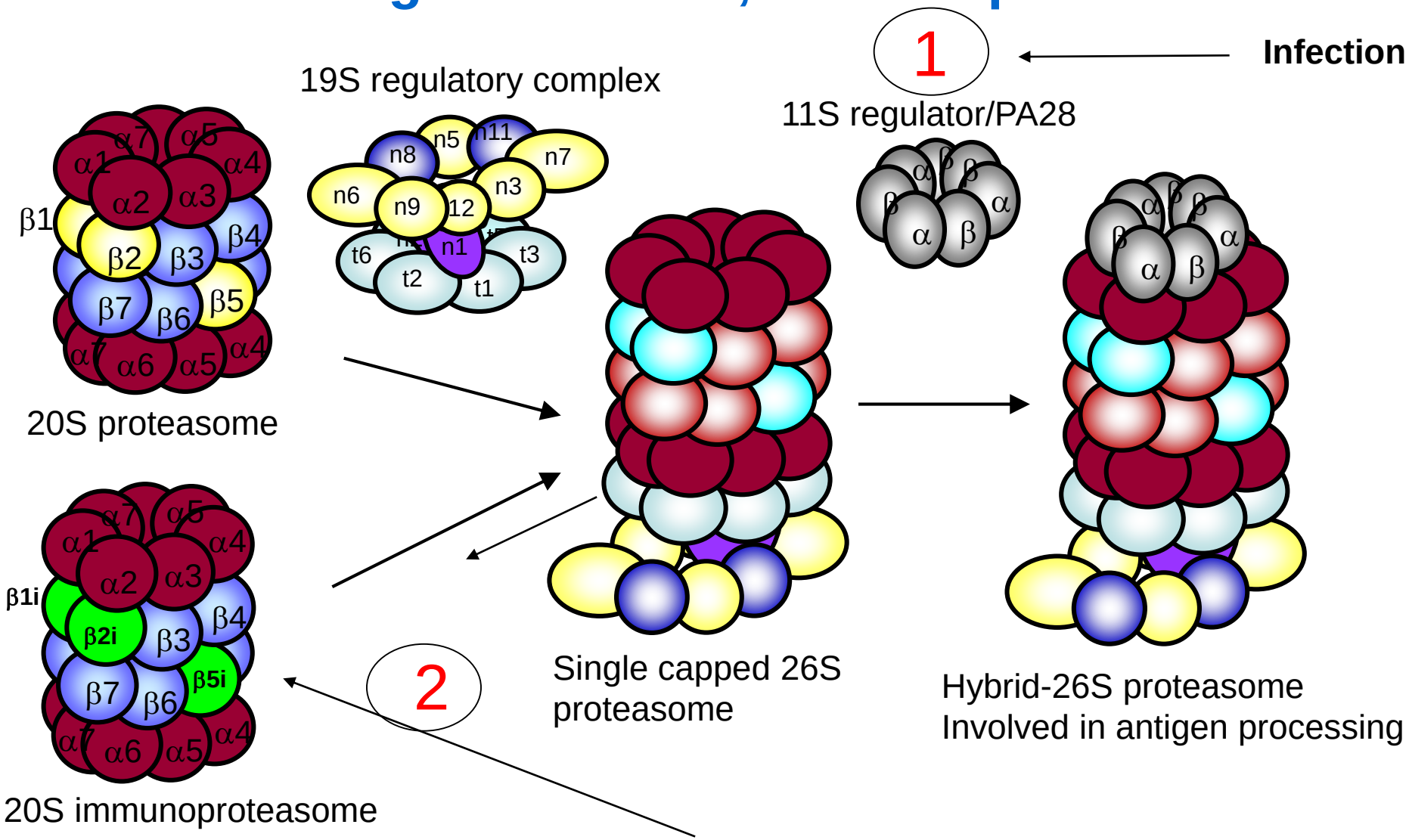
1. Antigen processing by the 26S proteasome

Processing of viral proteins

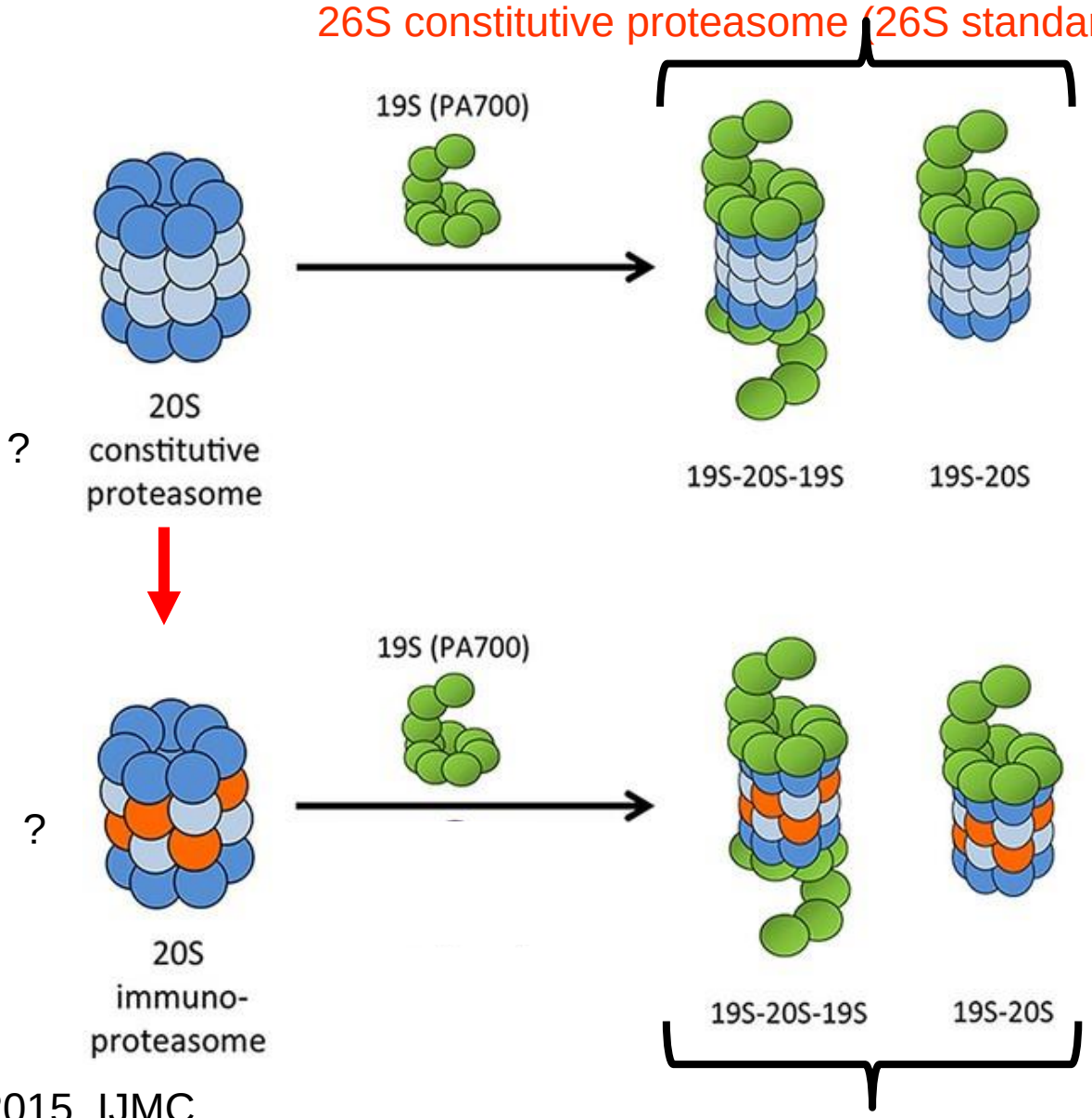


Kloetzel, P.-M. (2004) Nature Immunology

Different forms of the proteasome
The 11S regulator/PA28, immunoproteasome



Proteasome populations in infected or cytokine stimulated cells

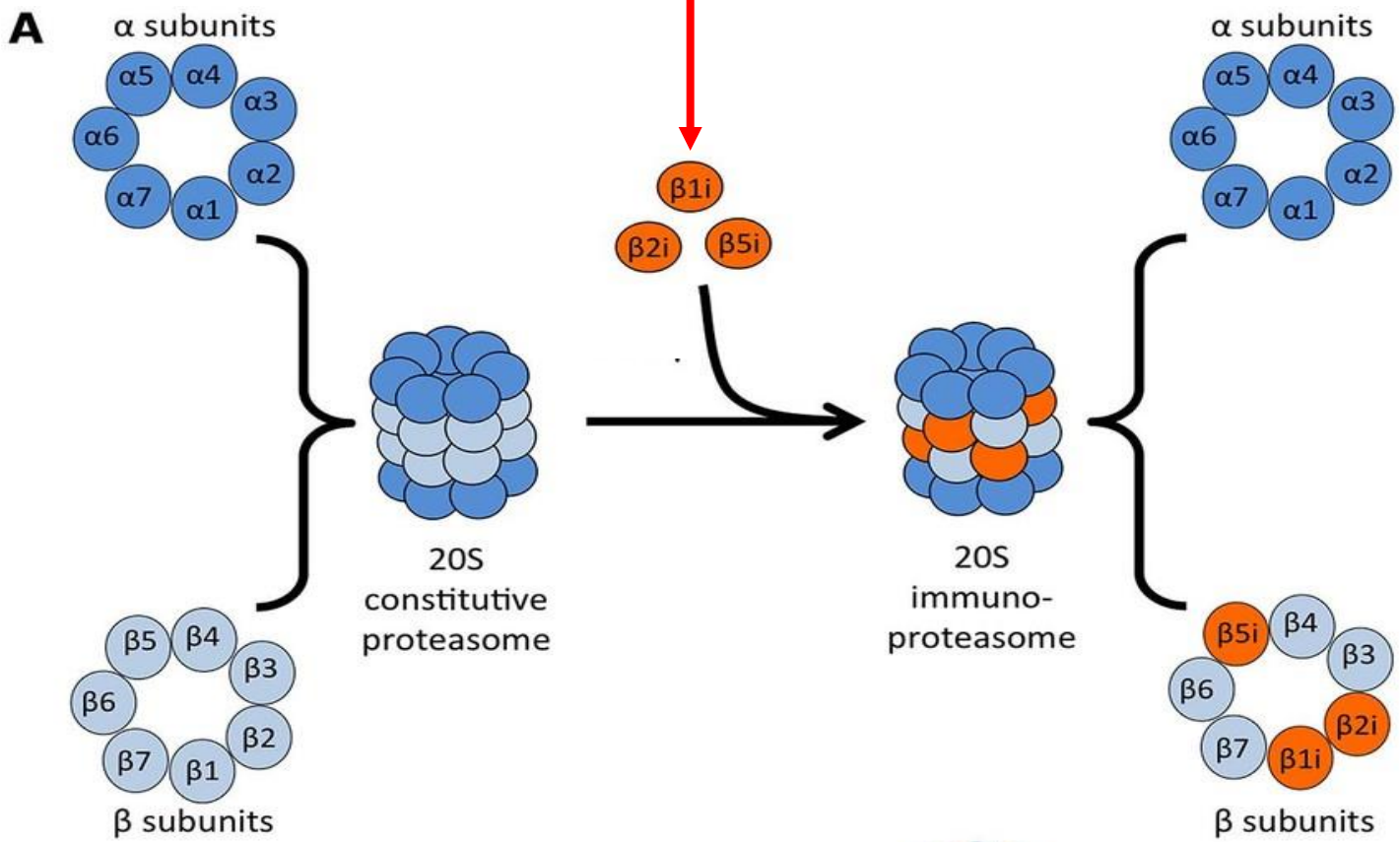


McCarthy et al. 2015, IJMC

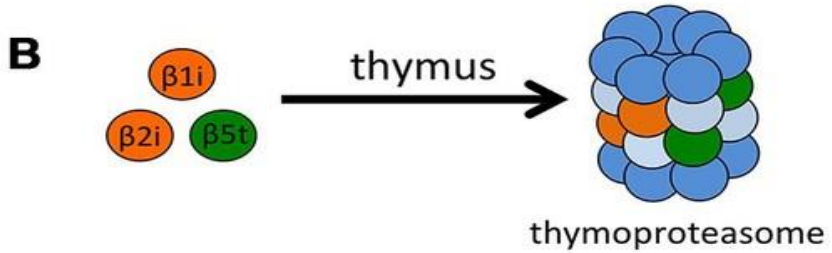
20S immunoproteasome

Stimulis ⟶ INFγ, TNFα, viral infection, oxidative stress

In all cells apart from red blood cells



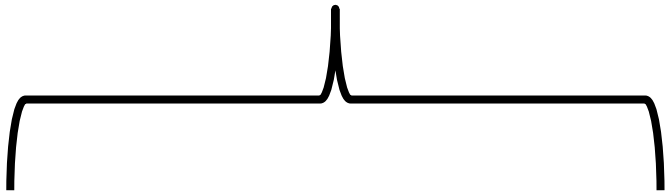
Thymus



McCarthy et al. 2015, IJMC

20S constitutive- and immuno-proteasome subunits:

70-80% amino acid sequence similarities



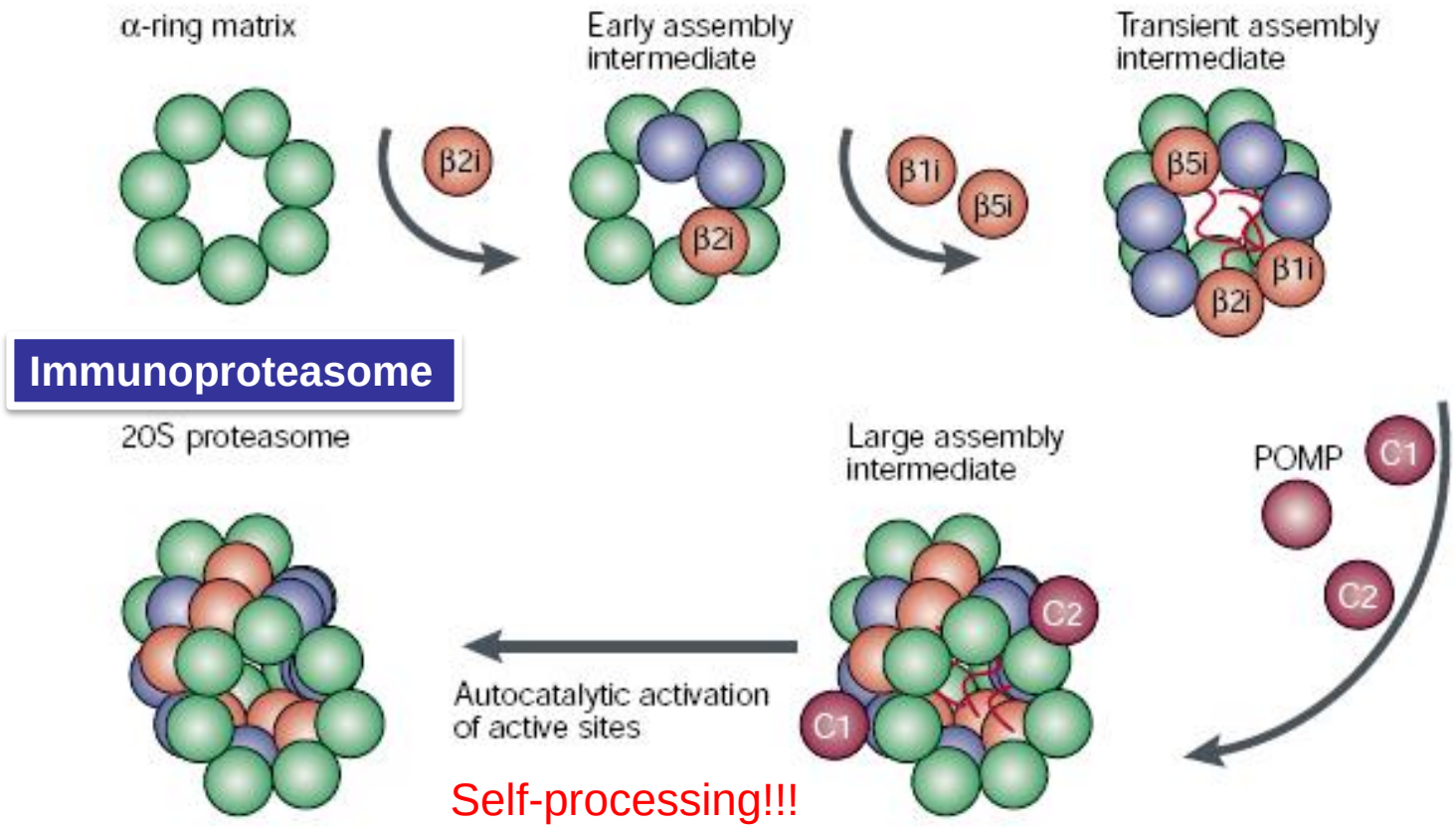
ß1:	ß1i, LMP2
ß2:	ß2i, MECL-1
ß5:	ß5i, LMP7

Active sites of the 20S constituve- and immuno-proteasome
(Threonine (Thr) protease)

- Caspase-like activity (ß1, ß1i): cleaves after Glu/Asp residues
- Trypsin-like activity (ß2, ß2i): cleaves after the basic amino acids (Lys, Arg)
- Chymotrypsin-like activity (ß5, ß5i): cleaves after hydrophobic amino acids e.g. Met, Val, Trp, Leu, Ile, Phe; Tyr, Pro

One molecule of 20S Proteasome contains 6 active sides

Formation of the 20S immunoproteasome is induced by interferon γ



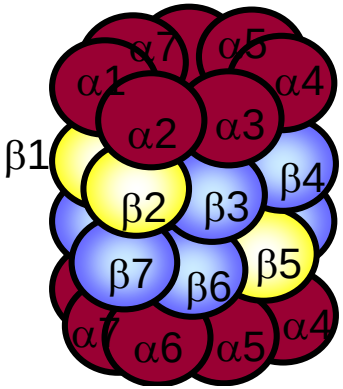
Self-processing!!!

Coordinated assembly!

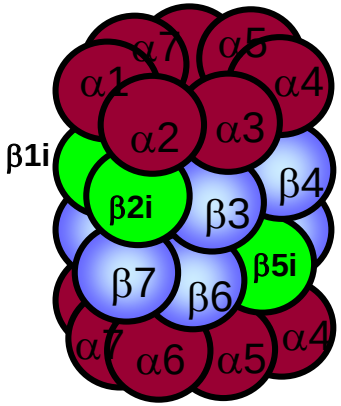
Kloetzel, P.-M. (2004) Nature Immunology

Function of immunoproteasomes

- Efficient MHC class I antigen presentation
- Degradation of oxidized proteins



20S constitutive proteasome



20S immunoproteasome

Reasons:

Production of epitops:

1. some preferences e.g. more digestion after hydrophobic amino acids, high binding affinity to MHC class I molecule, more nanomers (qualitative change), but no formation of neoantigens!
2. Immunoproteasome is more active?. (quantitative change)!!!!

Viral escape mechanisms

HIV-1 Tat protein

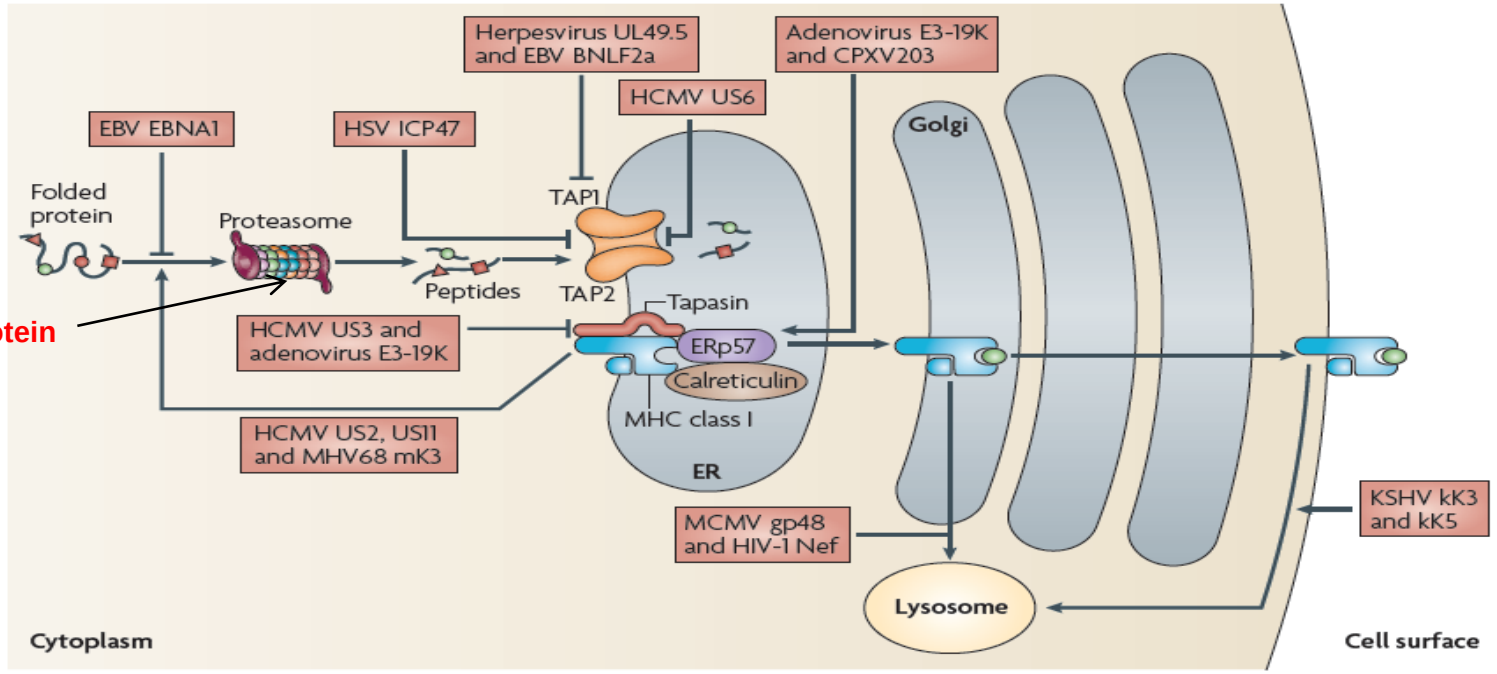
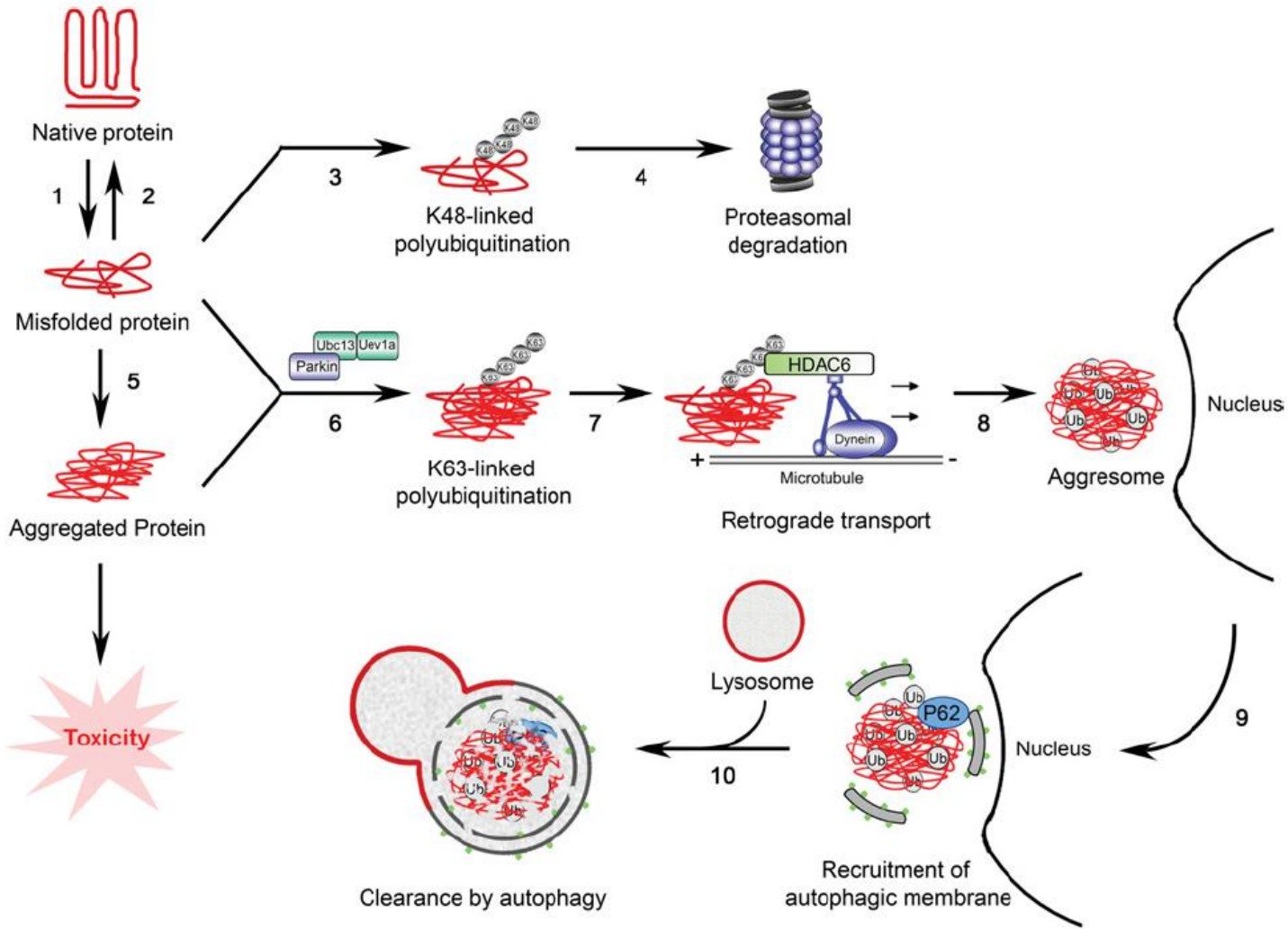


Figure 1 | **The MHC class I antigen presentation pathway is targeted by viral immune evasion proteins.** The degradation of proteins by the proteasome generates peptides that are translocated into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP). Nascent MHC class I molecules associate with calreticulin, tapasin and ERp57 to form the peptide-loading complex, which facilitates the loading of peptides into the MHC class I peptide-binding groove. Kinetically stable MHC class I molecules then transit to the cell surface. Key stages of the pathway are targeted by immunomodulatory proteins. Proteasomal processing inhibitors, such as Epstein–Barr virus (EBV) nuclear antigen 1 (EBNA1), escape processing by the proteasome. TAP function inhibitors, such as herpes simplex virus (HSV) protein ICP47 and human cytomegalovirus (HCMV) protein US6, block peptide and ATP binding, respectively. Herpesvirus protein UL49.5 and EBV protein BNLF2a also inhibit TAP-mediated peptide transport. Tapasin function inhibitors, such as HCMV protein US3 and adenovirus protein E3-19K, inhibit the peptide optimization and recruiting functions of tapasin, respectively. ER retainers or retrievers of MHC class I molecules, such as adenovirus protein E3-19K and coxpox virus protein 203 (CPXV203) retain MHC class I molecules in the ER. ER-associated degradation inducers, such as HCMV proteins US2 and US11 and mouse herpesvirus 68 (MHV68) protein mK3, target MHC class I molecules for proteasomal degradation. Sorters, such as murine CMV proteins gp48 and HIV-1 protein Nef, divert the trafficking of MHC class I molecules from the Golgi to a lysosomal compartment. Finally, the Kaposi's sarcoma-associated virus (KSHV) proteins kK3 and kK5 induce rapid endocytosis of cell surface MHC class I molecules, leading to lysosomal degradation.

Functions of the UPS:

2. THE ROLE OF THE UPS IN THE DEGRADATION OF MISFOLDED PROTEINS

2a. Stress-induced misfolded proteins can be degraded by the proteasome or by autophagy



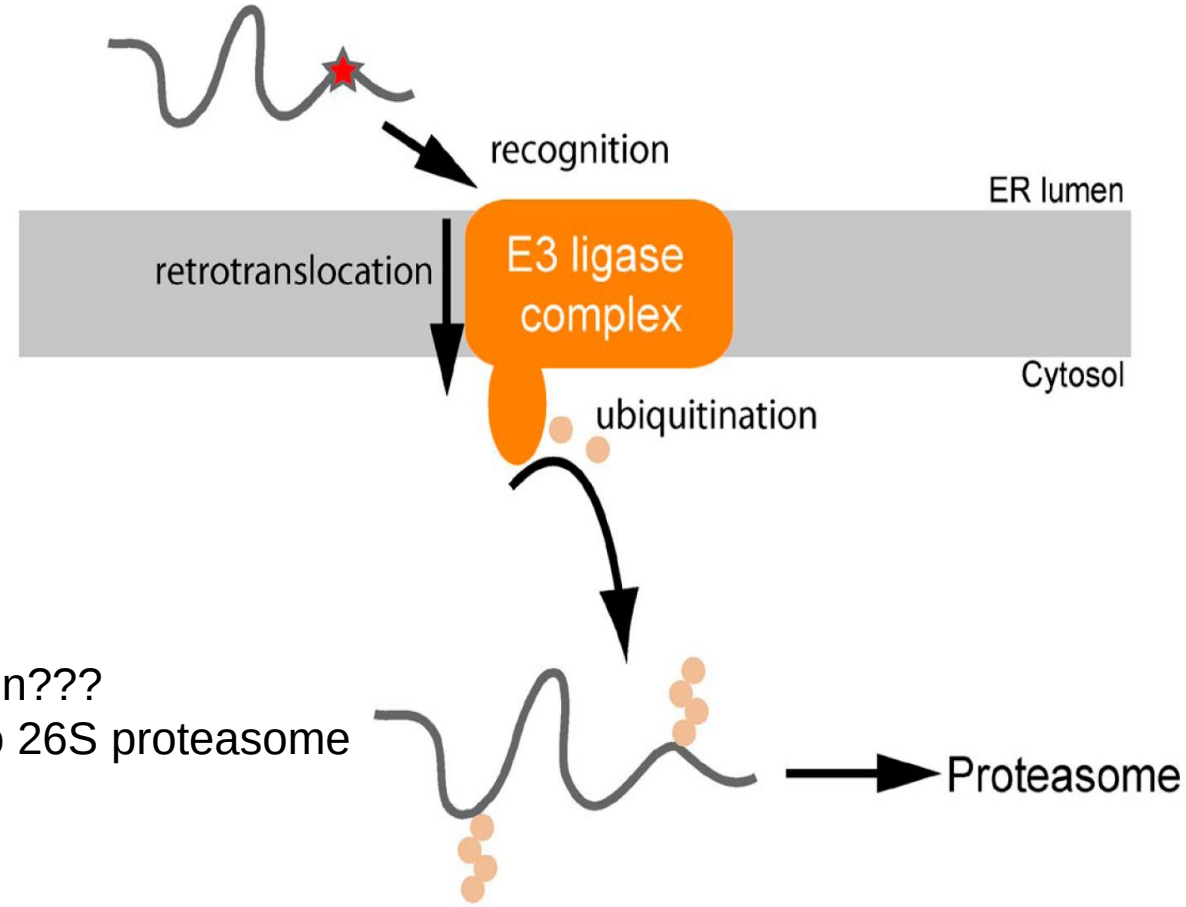
Mutation of proteins implicated in aggresome formation lead to neurodegenerative diseases

Table 1. Proteins Implicated in Aggresome Formation

Protein	Function	Wild-type protein localized to inclusion bodies	Mutations associated with disease	Ref.
Histone deacetylase 6	Deacetylase, adaptor protein	Lewy bodies	Unknown	[46]
Parkin	E3 ubiquitin-protein ligase	Lewy bodies	Parkinson's disease	[34, 38]
Ataxin-3	Deubiquitinating enzyme	SCA type-1 and 2 DRPLA intranuclear inclusions	SCA type-3	[37]
Dynein motor complex	Retrograde microtubule motor	Unknown	Motor neuron degeneration	[87, 88]
Ubiquilin-1	Folding of amyloid precursor protein (APP)	Lewy bodies and neurofibrillary tangles	Alzheimer's disease (potential risk factor)	[147, 154]

SCA, spinocerebellar ataxia; *DRPLA*, dentatorubral-pallidoluysian atrophy; ALS, amyotrophic lateral sclerosis.

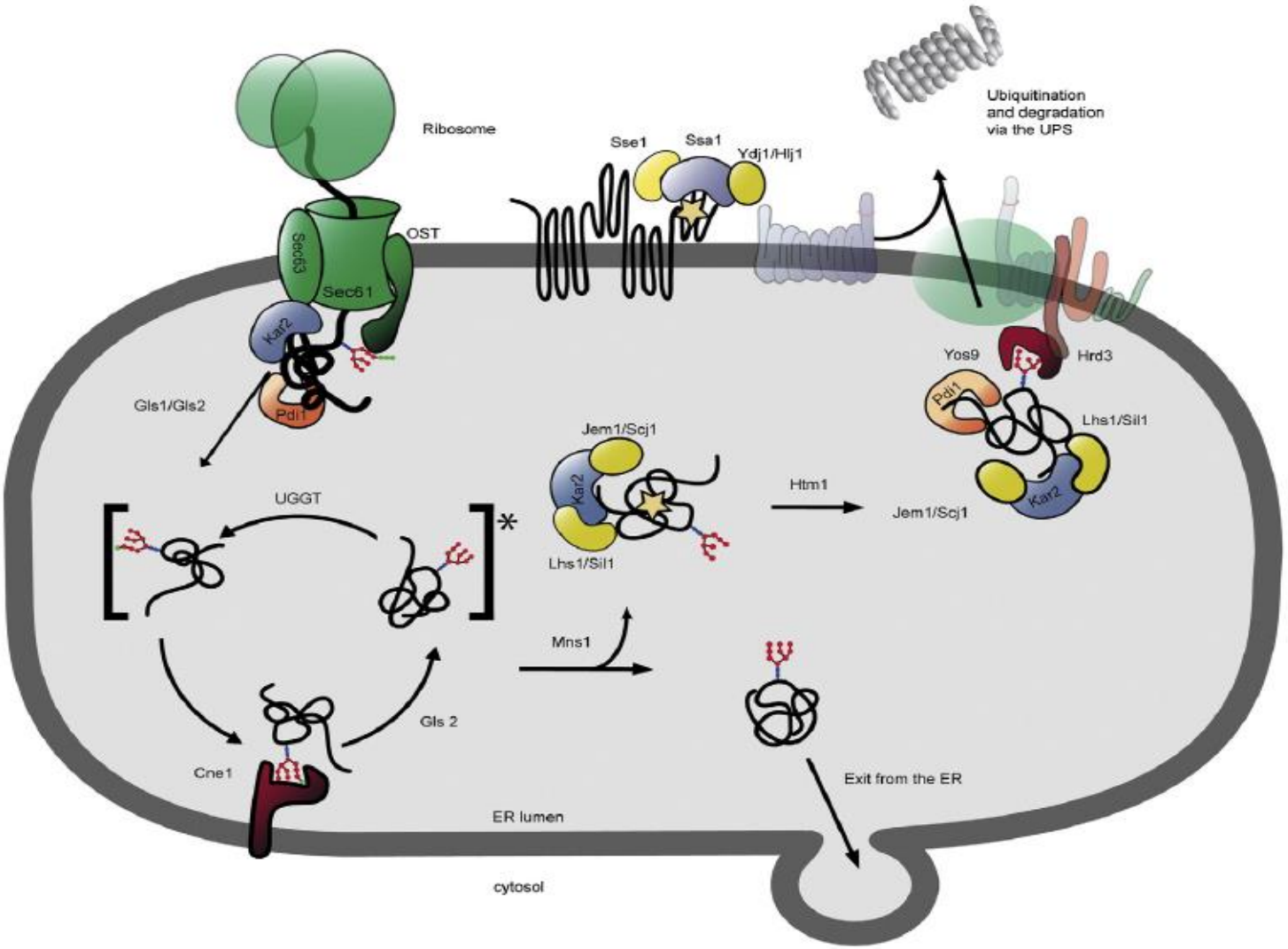
2b. The endoplasmatic reticulum-associated degradation (ERAD) pathway



ER function???
ER has no 26S proteasome

The endoplasmatic reticulum-associated degradation (ERAD)
Protein quality control in the ER

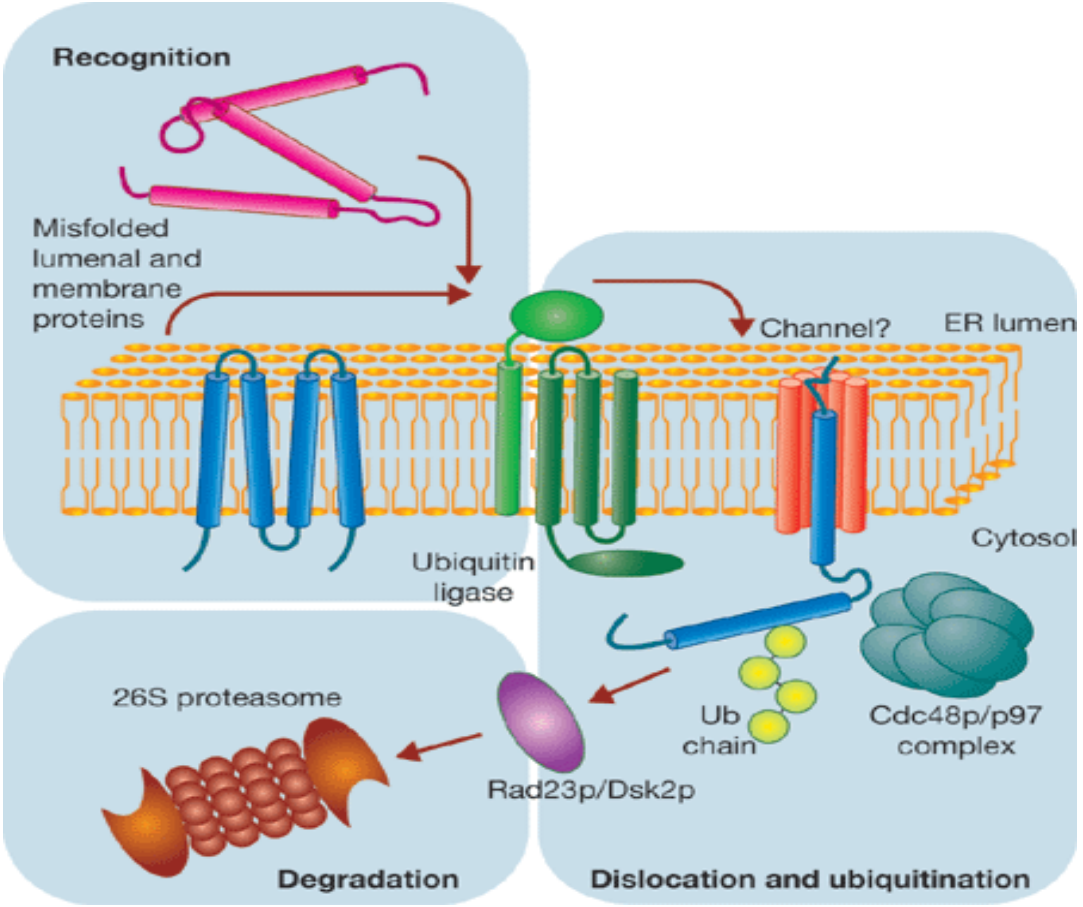
A. Stolz, D.H. Wolf / Biochimica et Biophysica Acta 1803 (2010) 694–705



Degradation of misfolded proteins from the ER

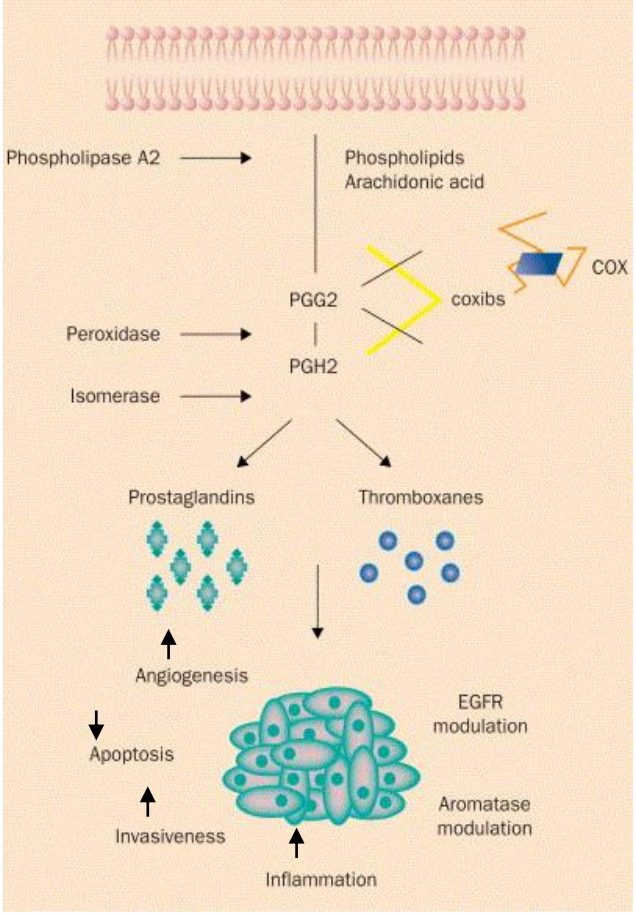
The cyclooxygenase-2 (COX-2) is a substrate of the ubiquitin proteasome system associated with the ER (ERAD)

COX-2 is localized to the luminal surface of the ER and degraded by the ERAD pathway



COX-2 is induced under conditions of inflammation and tumor growth

Gasparini et al. 2003 The Lancet Oncology 4, 605-615



Premalignant or malignant lesion	COX2 expression (%)
Colorectal	80–90
Gastric	80
Oesophageal	70
Hepatocellular (liver cirrhosis)	54 (81)
Pancreatic	67
Head and neck	80
Non-small-cell lung cancer	70
Breast (ductal carcinoma-in-situ)	40 (60)
Prostatic	83–93
Bladder	86
Cervix	43
Endometrial	37
Cutaneous basal cell	25
Cutaneous squamous cell	80
pPNET	100
Glioblastoma multiforme	71–74
Anaplastic astrocytoma (low grade)	44 (30)

References available at http://image.thelancet.com/extras/03oncl205webfr.pdf

Coxibis: inflammation inhibitors e. g. paracetamol, aspirin

Specific inhibition or degradation of COX-2 has high anti-tumor therapeutic significance

Functions of the UPS:

3. THE ROLE OF THE UPS IN THE SIGNAL TRANSDUCTION

3. The NF-κB pathway depends on the UPS

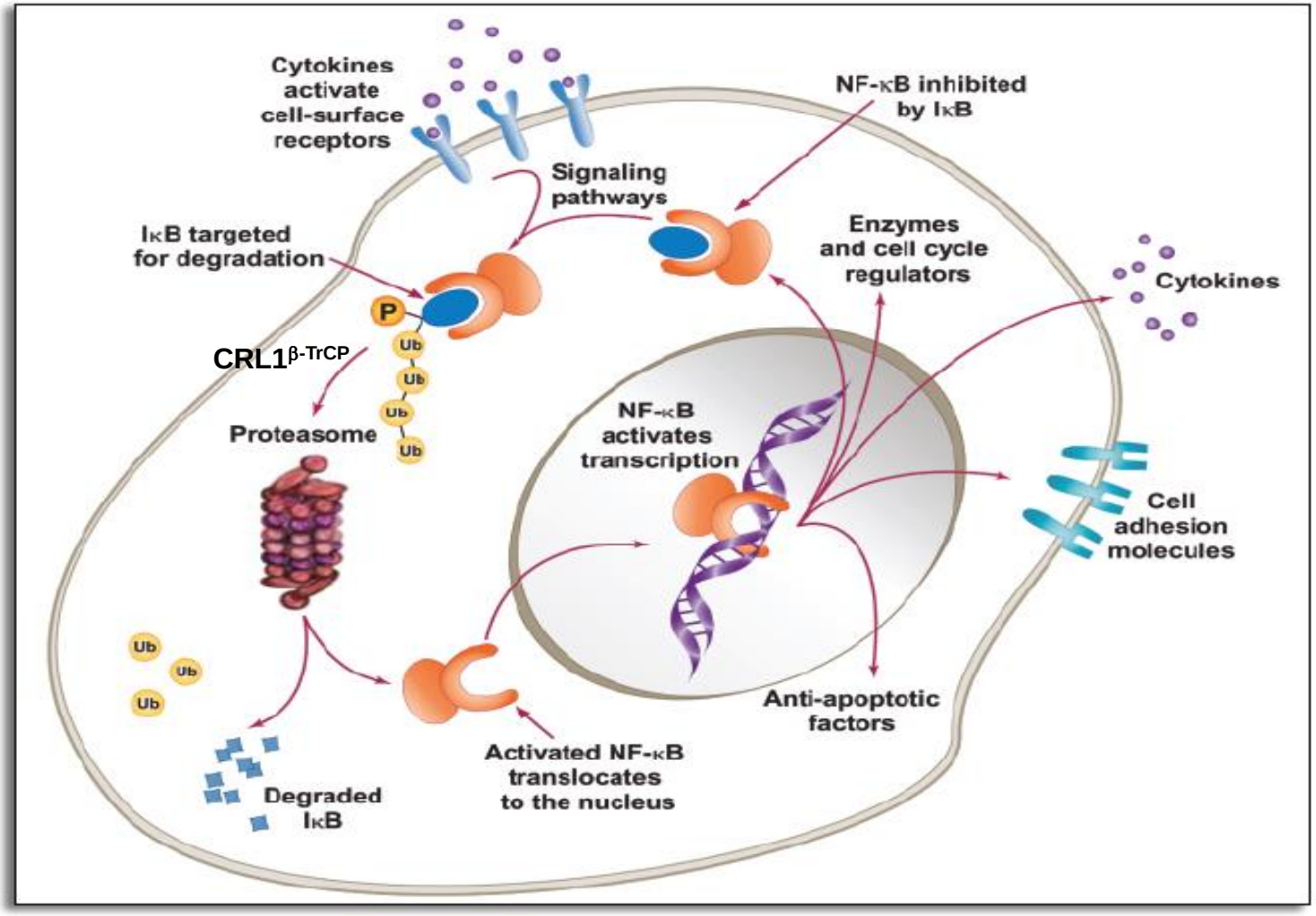
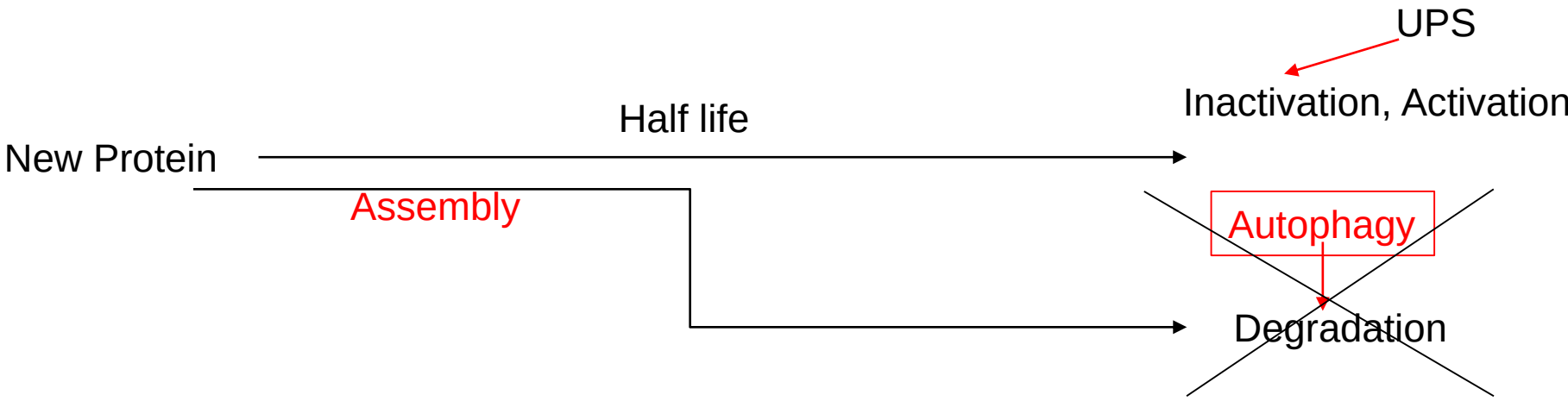


Figure 1. NF-κB initiates a number of survival pathways, including activation of a number of genes, including growth factors, angiogenesis factors, cell adhesion molecules, and anti-apoptotic mechanisms. In response to factors such as stress, growth factors, and radiation, IκB is degraded by the proteasome, freeing NF-κB to activate transcription. Copyright Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts.

Protein complex activation/inactivation:

Protein complex:
-Energy
-Time
-Complex: Specificty

Protein complex



The UPS is involved in cancerogenese and is a target for tumor therapy

- Proteasome inhibitors:

  -bortezomib (Velcade) is a drug against myeloma

  -new drugs: carfilzomib (Kyprolis), ixazomib (Ninlaro) with lesser side effects

E3 Ub Ligases or their regulators (more specific):

  -MLN 4924

Ub-like proteins

Posttranslational Modifications

Covalent-conjugated proteins:

-Ubiquitin

-NEDD8

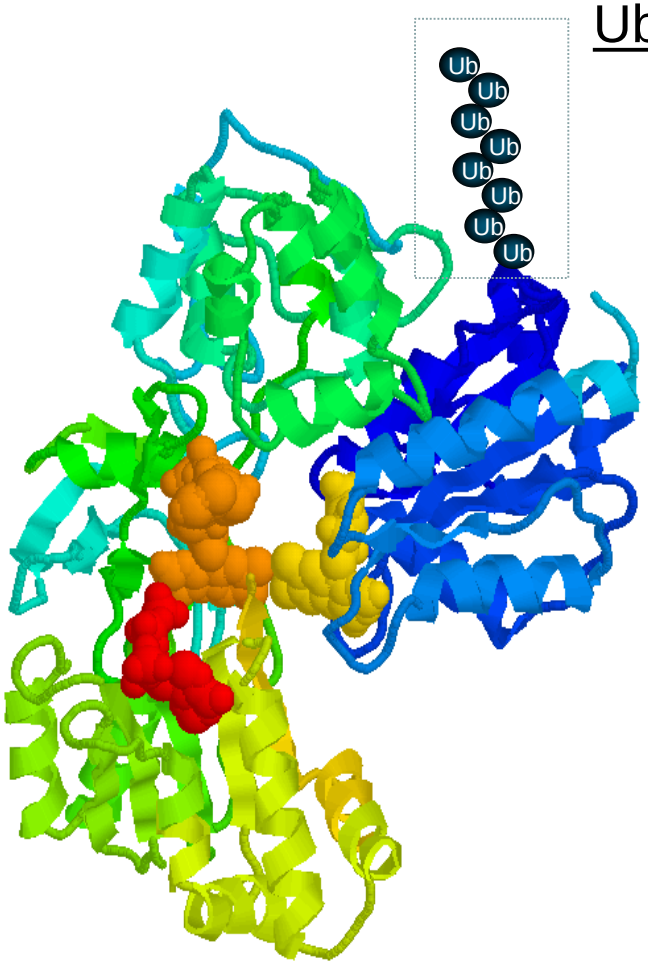
-Sumo

-FAT10

-ISG15

-UFM1

-UBL5



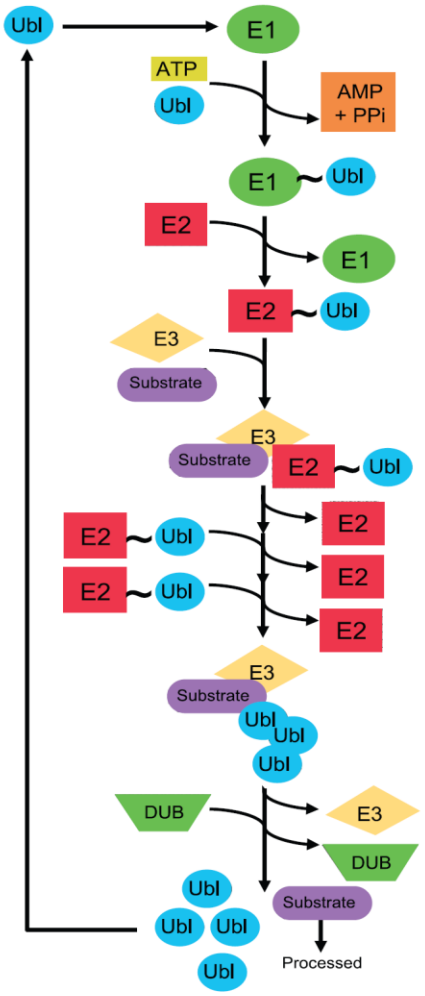
Ubiquitin

INSTITUT FÜR
EXPERIMENTELLE
INNERE MEDIZIN

Why is a protein ubiquitin-like?

- The ability to be conjugated?
- The structure?
- The amino acid composition?

Each ubiquitin-like protein has it‘s own conjugation system



Ubiquitin	SUMO	NEDD8	ISG15
E1 (2): Uba1, Uba6	E1 (1): Aos1/Uba2	E1 (1): APPBP1/Uba3	E1 (1): UBE1L
E2 (30): UBE2A(hHR6A), UBE2B(hHR6B), UBE2C(UbcH10), UBE2D1(UbcH5A), UBE2D2(UbcH5B), UBE2D3(UbcH5C), UBE2D4(HBUCE1), UBE2E1(UbcH6) ±, UBE2E2, UBE2E3(UbcH9), UBE2G1(UBE2G), UBE2G2(UBC7), UBE2H(UBCH), UBE2J1(NCUBE1), UBE2J2(NCUBE2), UBE2K(HIP2), UBE2L3(UbcH7), UBE2L6(UbcH8) ±, UBE2N(Ubc13), UBE2O(E2-230K), UBE2Q1(NICE-5), UBE2Q2, UBE2R1(CDC34), UBE2R2(CDC34B), UBE2S(E2-EPF), UBE2T(HSPC150), UBE2U*, UBE2V1(UEV-1A), UBE2V2(MMS2), UBE2W, UBE2Z(Use1), BIRC6(apollon)	E2 (1): UBE2I(Ubc9)	E2 (2): UBE2M(Ubc12), UBE2F(NCE2)	E2 (3): UBE2L6(UbcH8) :, UBE2E1(UbcH6) :, UBE2E2 ±
E3 (>1000): Single/multiple subunit RING, HECT, U-box, PHD	E3 (4): RanBP2, Pc2, PIAS-proteins, Topors	E3 (2): Rbx1, Rbx2	E3 (2): HERC5, EFP

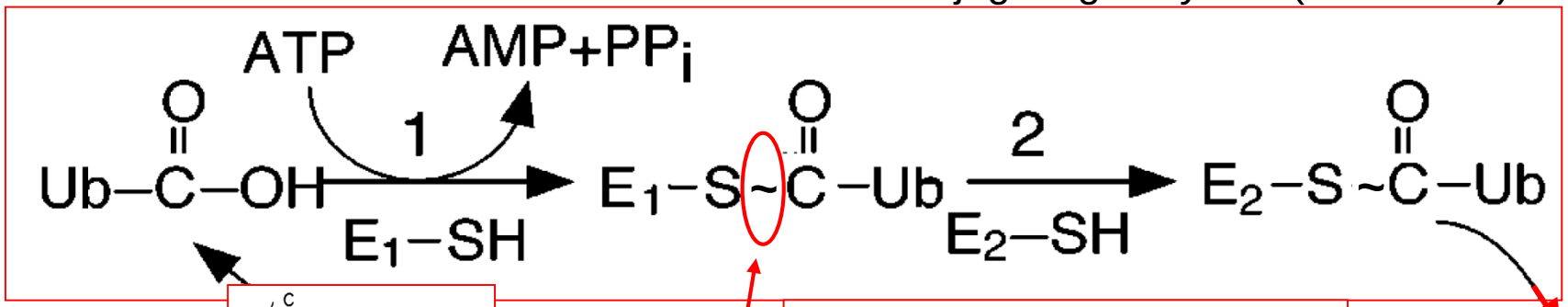
Activation and transfer of UbLs like Ub

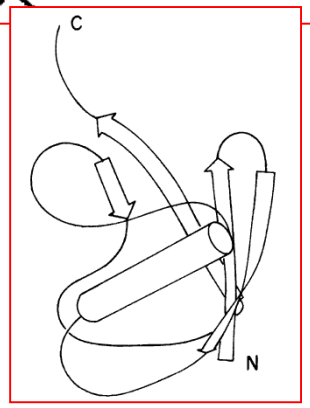
E1 – Ub activating enzyme

There are few Ub activating enzymes in eukaryotic cells

E2s – UBCs – Ub conjugating enzymes

There is a large family of Ub conjugating enzymes (~50 UBCs).

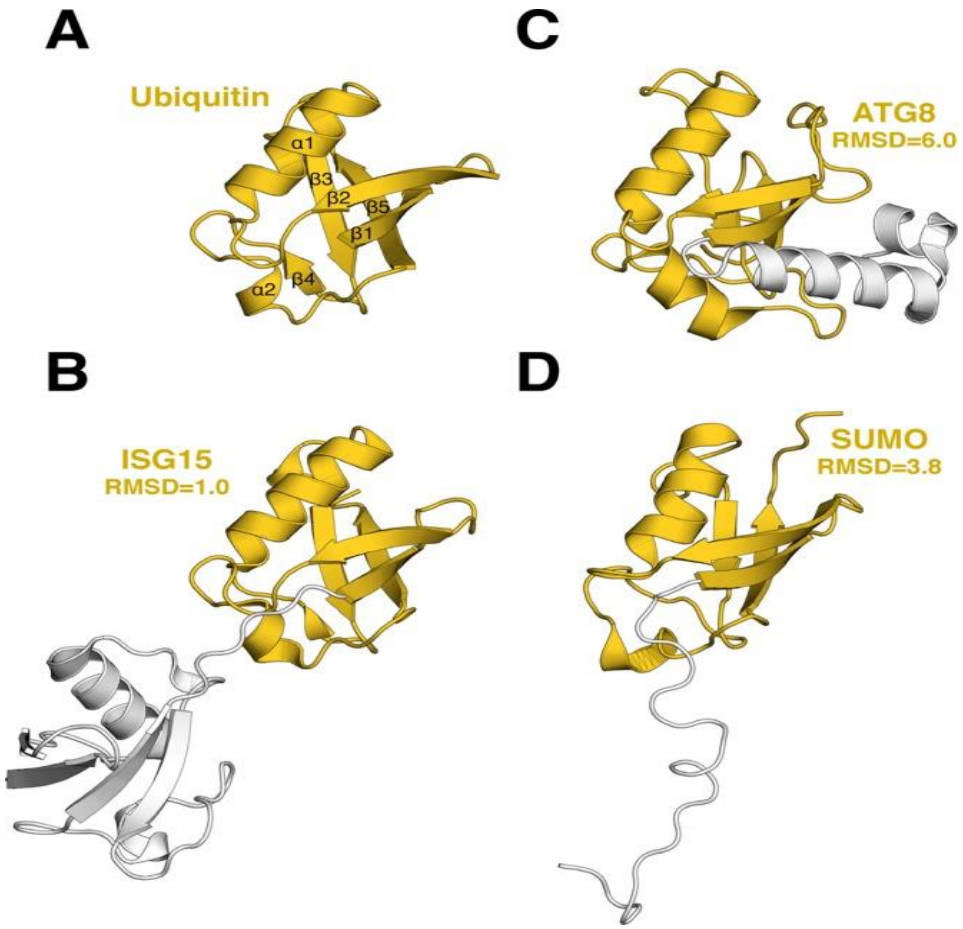




Transthiolation reaction from E1-S-Ub to E2-S-Ub.

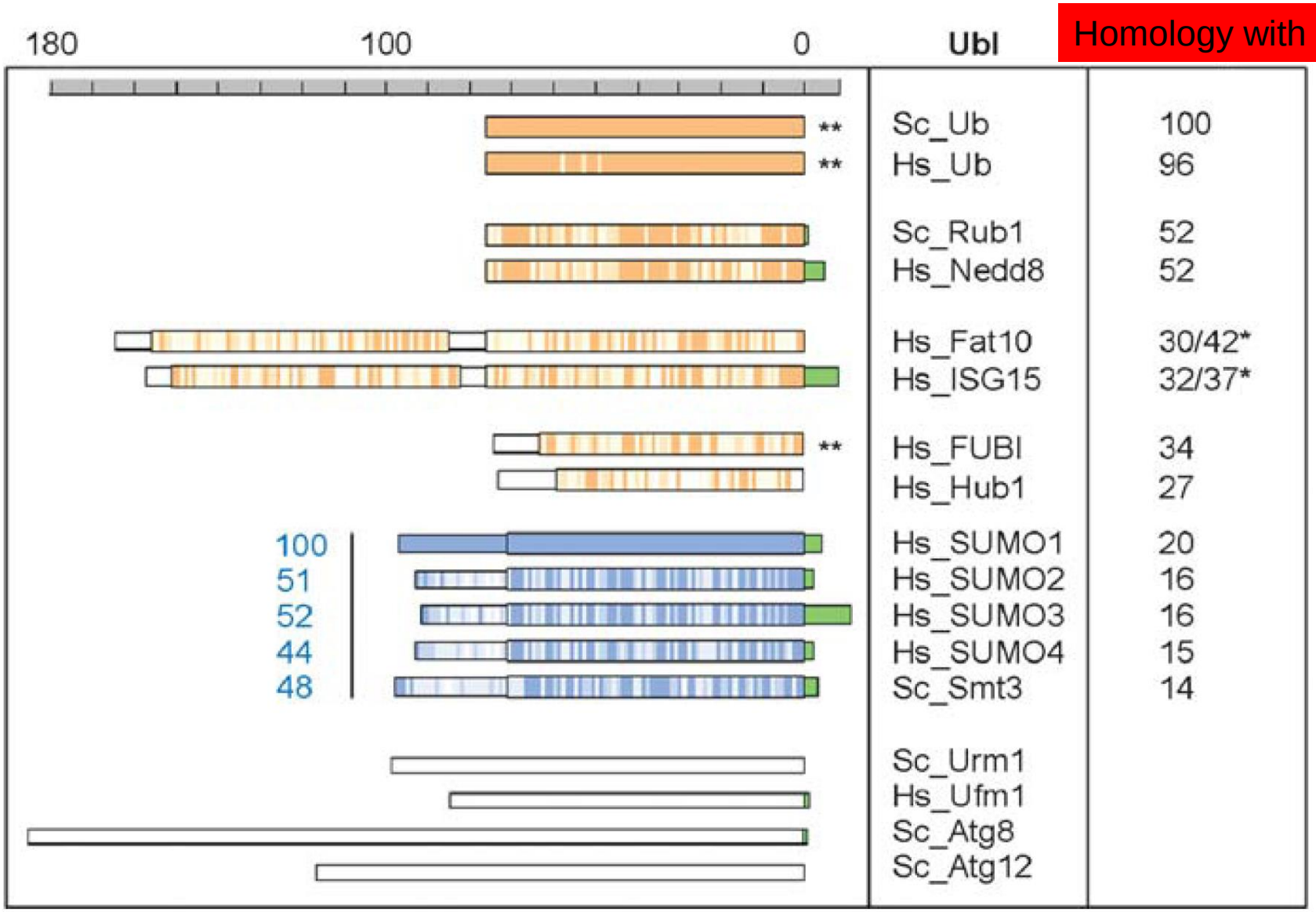
Thioester bond

3D Structures of the Ubiquitin Family Proteins

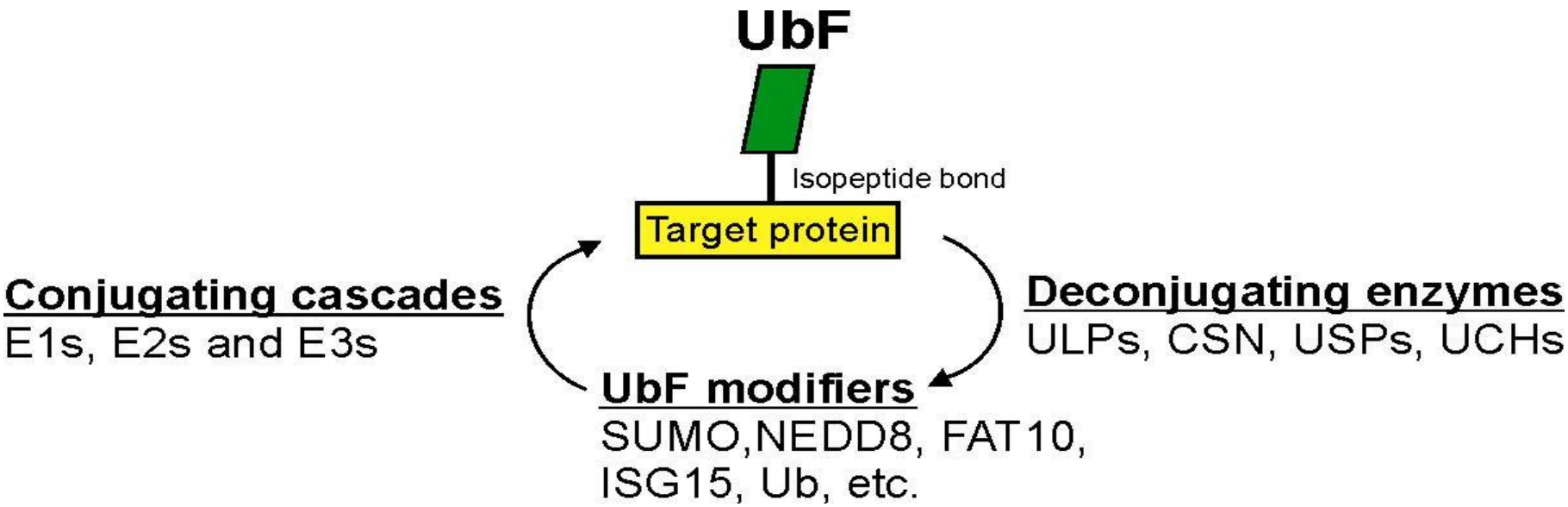


Cappadocia L and Lima CD (2018)

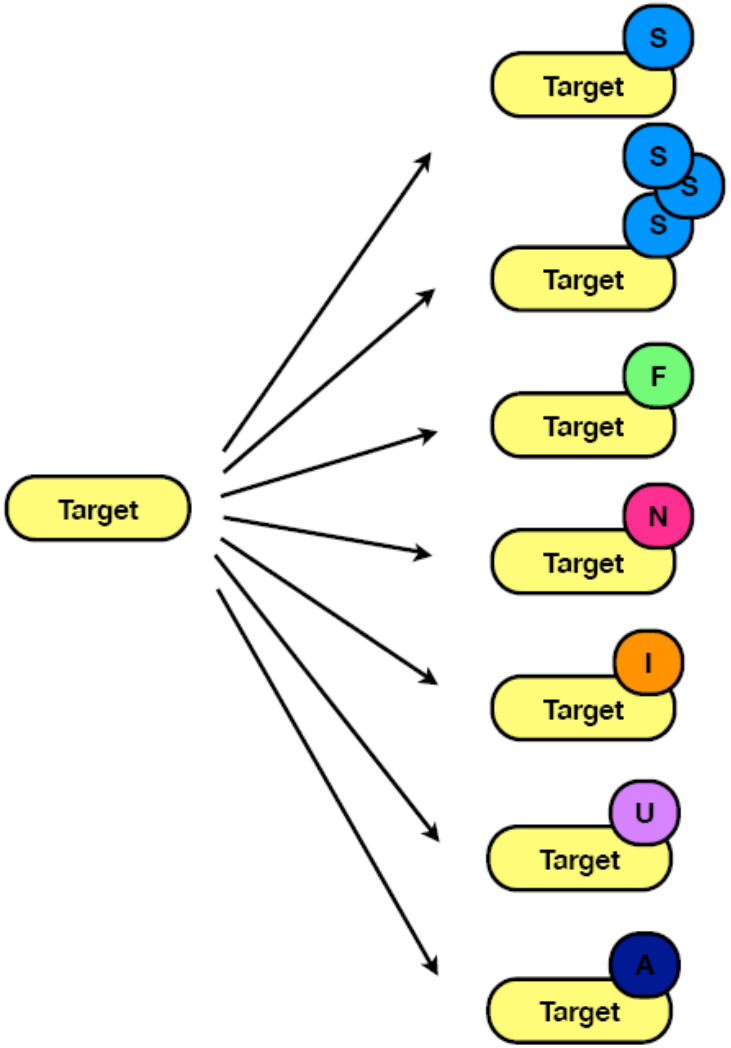
Proteins of the Ubiquitin Family



Conjugation and deconjugation of UbF proteins



Ubiquitin-like signals



Mono-Sumoylierung		nuclear-cytoplasmic transport, DNA repair, chromatin remodeling, transcription regulation
Multiple Sumoylierung		endocytosis
Fatylation		ubiquitin independent proteasomal degradation
Neddylation		Control of E3 ligases
ISGylation		Regulation of immune response
Urmylation		Regulation of redox system
conjugation to	ATG8 ATG12	Autophagy