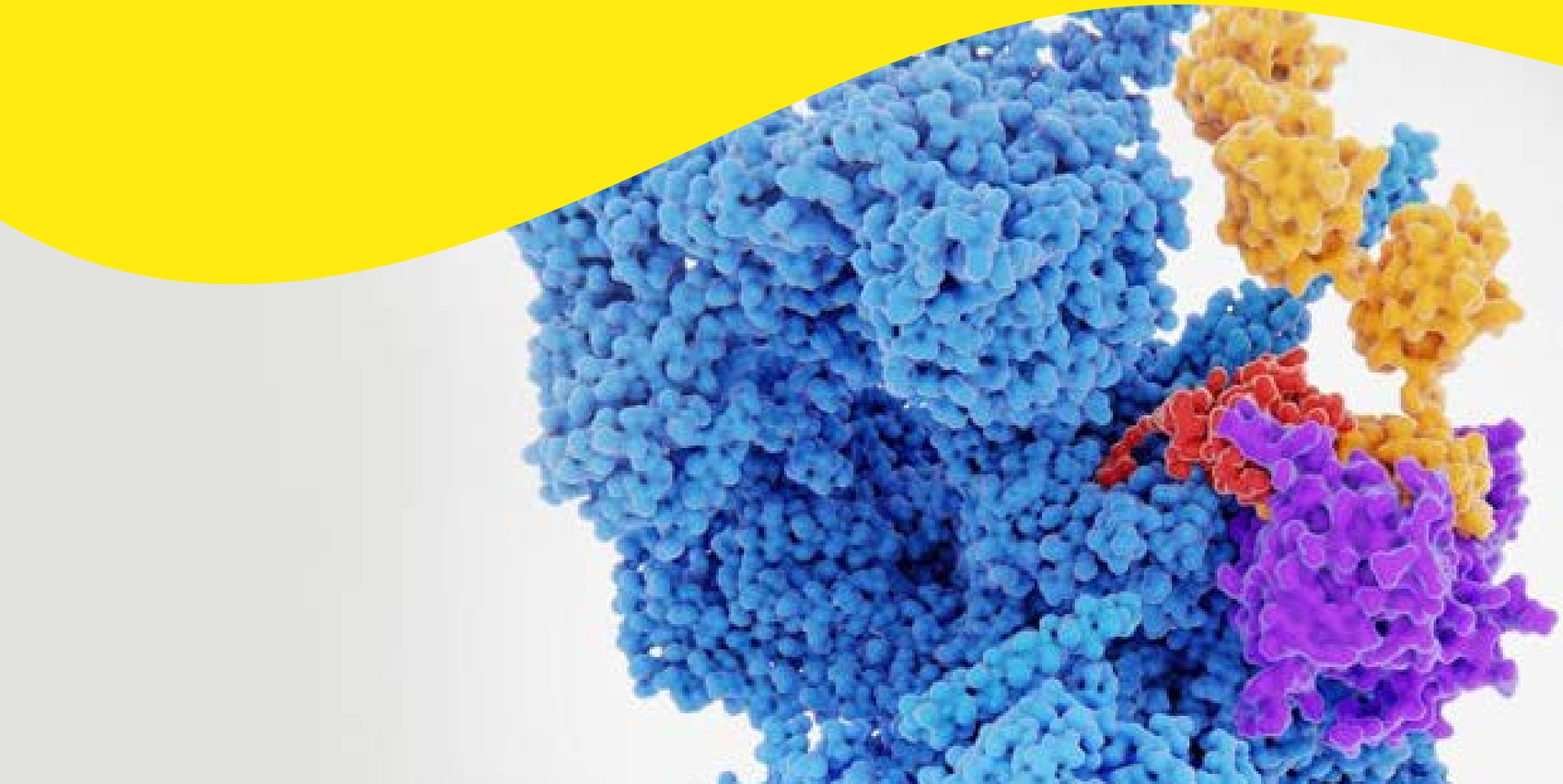
Protein degradation

Guide to the proteasome and autophagy systems



INTRODUCTION
Purpose and scope

WHAT TO EXPECT?

Welcome to this protein degradation booklet, a document that helps scientists and researchers appreciate and navigate the diversity of molecular pathways associated with protein degradation. We hope the visuals provided in this document will shed light on and clarify these otherwise complex mechanisms.

You will find five separate sections:
The first section covers the ubiquitin structure, function, and regulation.

The second to the fourth sections dive more deeply into the cellular and molecular understandings on proteasome and autophagy degradation machineries, as well as their crosstalk.

The last section gives an overview of current therapeutic strategies, as well as the promising PROTAC approach to specifically target protein degradation.

The collection of cellular and molecular basics presented in this document was prepared based on authentic and highly regarded articles and journals. The numbers in brackets indicate the references used, and all pathways have been curated for scientific knowledge and accuracy by Revvity's scientific team.

WHY THIS GUIDE?

This new guide represents a continuum in Revvity's experience in providing a collection of specialized documents dedicated to different therapeutic areas such as immunology, autoimmunity, neurosciences, diabetes, and NAFLD, as well as more practical guides covering the expertise of Revvity's scientists in assay development and performance.

TABLE OF CONTENTS

INTRODUCTION
Proteostasis at a glance

Protein homeostasis or proteostasis refers to the biological mechanisms that control the fate of a protein from synthesis to degradation. The maintenance of proteostasis is ensured by complex and interconnected pathways controlling protein abundance, turnover, folding, functions, subcellular localization, and ultimate degradation.

Impaired proteostasis has been associated with ageing as well as with several pathologies such as cancer, autoimmune diseases, and neurodegenerative disorders, leading to the accumulation of normally degraded proteins, aggregated proteins, damaged organelles, or conversely to excessive protein degradation. Therefore, functional protein degradation mechanisms are essential to maintain overall cellular homeostasis and cell survival.

Two major intracellular proteolysis disposals, the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system (ALS), play critical roles for the maintenance of cellular homeostasis. They both rely on a small 'ubiquitous' protein, ubiquitin, working as a posttranslational tag and controlling the stability of almost all proteins in a highly specific and regulated manner. Whereas the UPS is the principal proteolytic mechanism responsible for degradation of short-lived proteins as well as damaged and misfolded proteins, the ALS is involved in the clearance of long-lived proteins and organelles, and in the recycling of amino acids. Both systems regulate essential cellular functions such as cell growth and apoptosis.

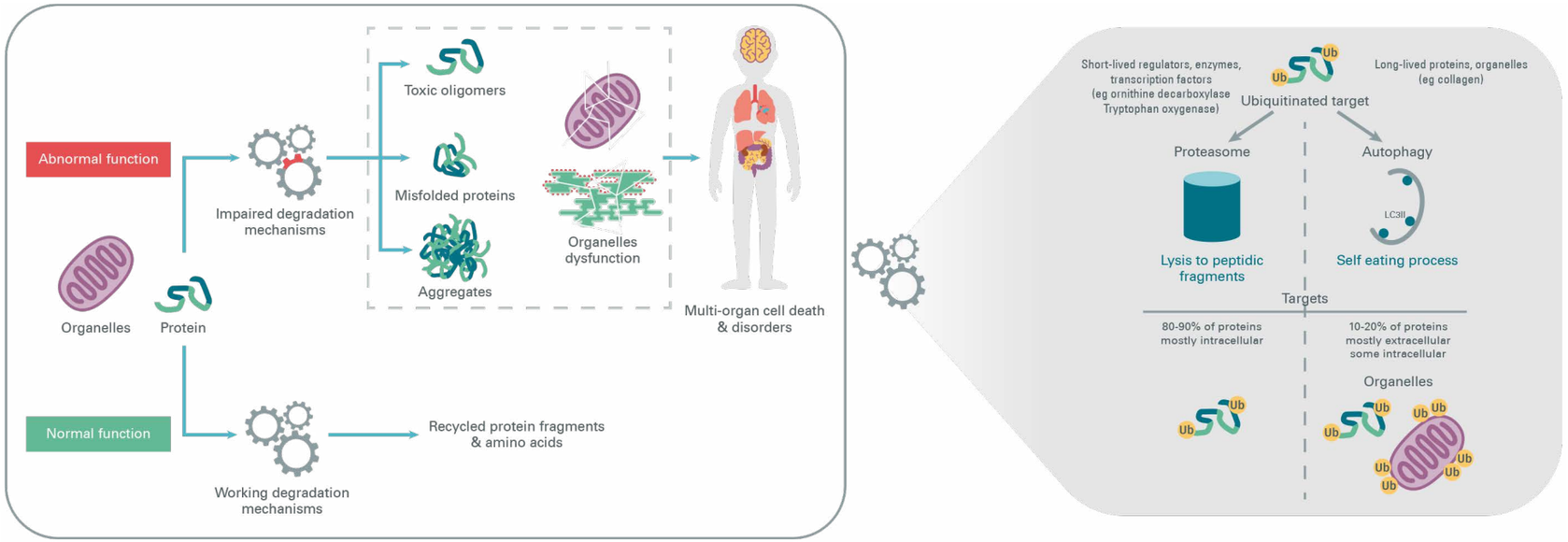


TABLE OF CONTENTS

GENERAL KNOWLEDGE

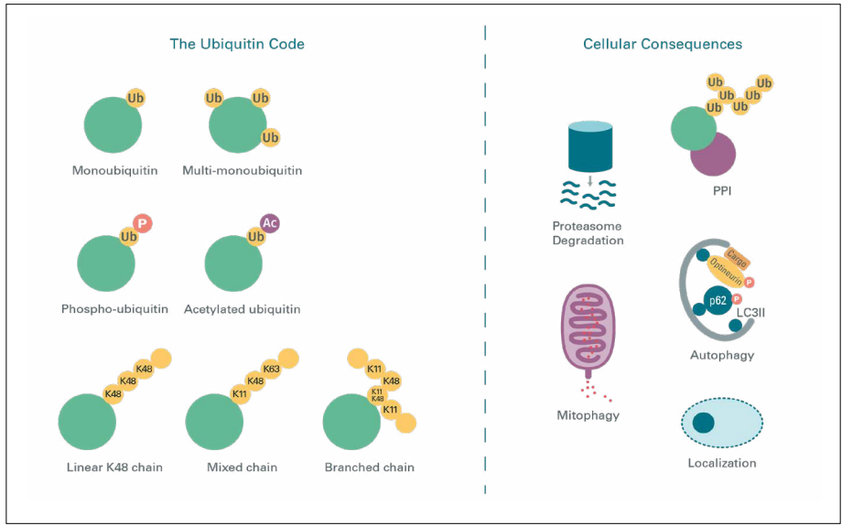
It all starts with ubiquitin

Ubiquitin is a small regulatory protein of only 76 amino acids that is virtually expressed in every cell type. It is encoded by four genes: UBB, UBC, UBA52, and RPS27A. Ubiquitin is one of the most prevalent post-translational modifications, notably for signaling proteasomal degradation. However, this modification can encompass other cellular consequences including mitophagy, autophagy, protein-protein interactions, and localization. [1]

Ubiquitin contains seven lysine residues, which can be ubiquitinated to create ubiquitin-linked chains. These chains can be linear or complex branched structures, yielding a wide variation in the chain topology. These chains and linkages create a unique ubiquitin code which can be recognized by proteins containing ubiquitin binding domains (UBDs). The UBDs of effector proteins recognize a specific ubiquitin motif on a target protein to signal a downstream biological response. An additional level of complexity arises as ubiquitin linkages not only signal specific UBD proteins, but their cellular context and localization can dictate how effector proteins interact with a ubiquitin target. [1,2]

Further intricacies in the ubiquitin code are seen through post-translational modifications of ubiquitin. Modifications such as phosphorylation at Serine 65 is found in neurodegeneration cellular models, while acetylation at Lysine 48 inhibits polyubiquitin chain elongation. It is thought these modifications affect the charge and surface properties of the ubiquitin, creating a conformational change to alter the downstream signaling response. In totality, the ubiquitin code, which is composed of linkage location, chain length, and post-translational modifications, affects the biological outcome of a ubiquitinated substrate protein. [2]

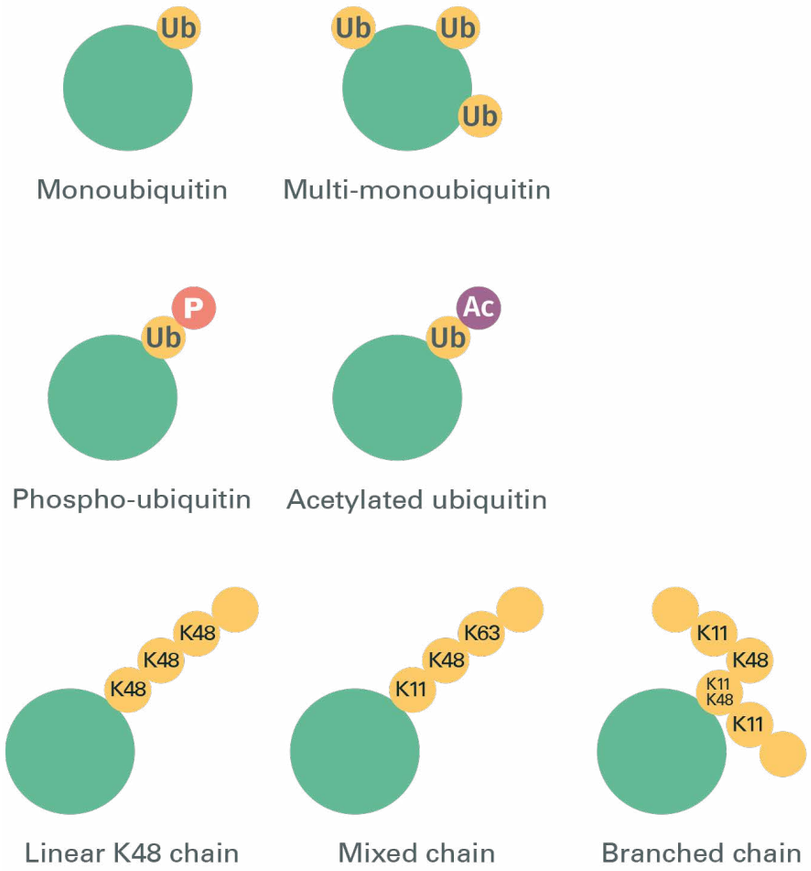
The most well studied ubiquitin modifications are through linkages at Lysine 48, Lysine 29, and Lysine 11. These lysine-linked ubiquitin chains are a recognition motif for the ubiquitin proteasome system (UPS), a multidomain complex responsible for degrading proteins into single amino acids. Autophagy is another protein destruction mechanism, where long-lived proteins and protein aggregates are enclosed in a vesicle called an autophagosome which is then fused with the lysosome for degradation. Proteins associated with creating the autophagosome and the lysosome are regulated by ubiquitination to alter their stability and activity. The regulation of UPS and autophagy is thought to play an important role in many diseases, including neurodegeneration and metabolism-linked disorders. [3,4]



Click to Enlarge

TABLE OF CONTENTS

The Ubiquitin Code



Cellular Consequences

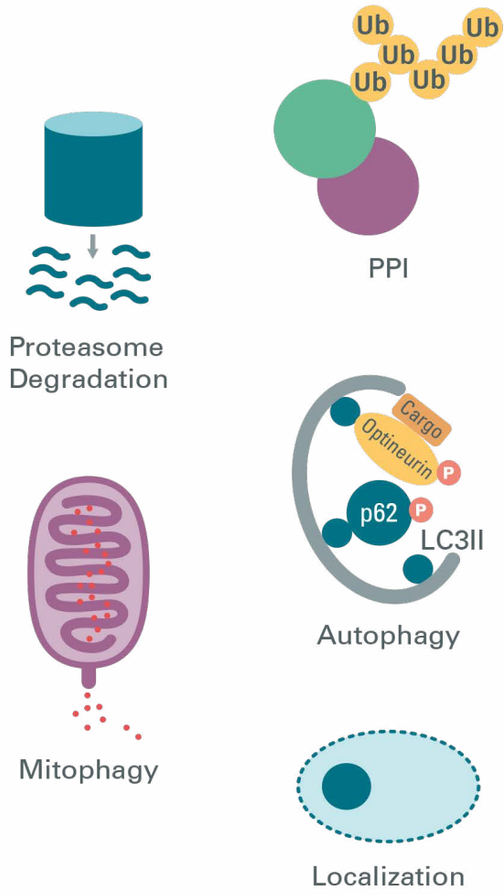


TABLE OF CONTENTS

GENERAL KNOWLEDGE

It all starts with ubiquitin

The process in which a substrate undergoes ubiquitination is a multi-enzyme cascade that begins with ubiquitin activating enzyme or E1. This enzyme uses two molecules of ATP to link ubiquitin to the E1 enzyme. The ubiquitin from E1 is then transferred to a thiol group of a cysteine amino acid in ubiquitin conjugating enzyme or E2. Finally, E3 or ubiquitin ligase is responsible for bringing the protein substrate into proximity so the ubiquitin can be transferred from E2 to the target protein. [5]

This highly coordinated pathway has been mechanistically and structurally characterized to examine how it can distinguish between various ubiquitin linkages and specific target proteins. Many studies have shown these enzymes are regulated through protein-protein interactions. Enzymes in the cascade have been found to be activated through various post-translational modifications such as phosphorylation and poly (ADP-ribosylation), also known as PARylation. Further, substrate recruitment and specificity are carried out through specific structural motifs on each enzyme. [5]

The interplay between the E2 and E3 proteins to transfer the ubiquitin moiety to a target protein demonstrates the diverse mechanisms that have evolved within this process. While the E2 protein is responsible for determining the unique ubiquitin linkage, it is the E3 ligase that is responsible for localizing and positioning the target protein for ubiquitination. There are two structurally distinct classes of E3 ligases, RING and HECT, which have allowed these ligases to function under various cellular responses to accommodate a wide variety of substrates. Together, the E2 and E3 ligases work in tandem to promote ubiquitin processivity, whether through a single ubiquitination transfer of a polyubiquitin chain or through multiple iterations of a single ubiquitin transfer. [5]

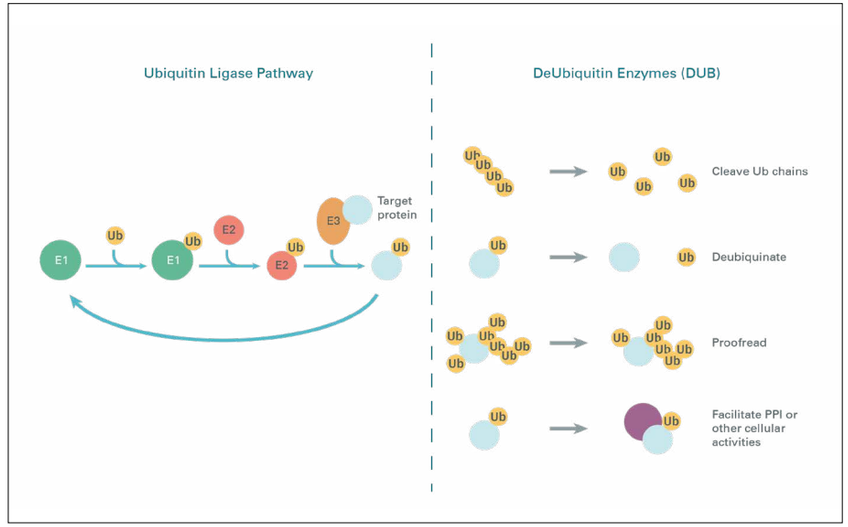
The class of enzymes responsible for removing ubiquitin from a substrate is called deubiquitinating enzymes (DUBs). This family of nearly 80 unique enzymes consists of two main classes: cysteine proteases and metalloproteases. It is thought that DUBs work in partnership with a ubiquitin ligase or scaffold protein to recognize a ubiquitin-linked substrate and aid in removing any mismatched ubiquitin linkages. DUBs can disassemble an entire ubiquitin chain from a target protein or simply remove or trim a misplaced ubiquitin. Since ubiquitination is a dynamic process, DUBs play an important role in determining which ubiquitin chains are created on specific proteins. [6]

TABLE OF CONTENTS

GENERAL KNOWLEDGE

It all starts with ubiquitin

DUB dysfunction or mutations have been found in many diseases, including cancer, neurological diseases and microbial pathogenesis, as DUB function is critical for DNA repair checkpoints, regulating the cell cycle, cytokine signaling, and apoptosis. DUBs may provide therapeutic intervention possibilities for many diseases, as DUBs are highly selective for their substrates and have been shown to be critical in many cellular functions. [6]



Click to Enlarge

TABLE OF CONTENTS

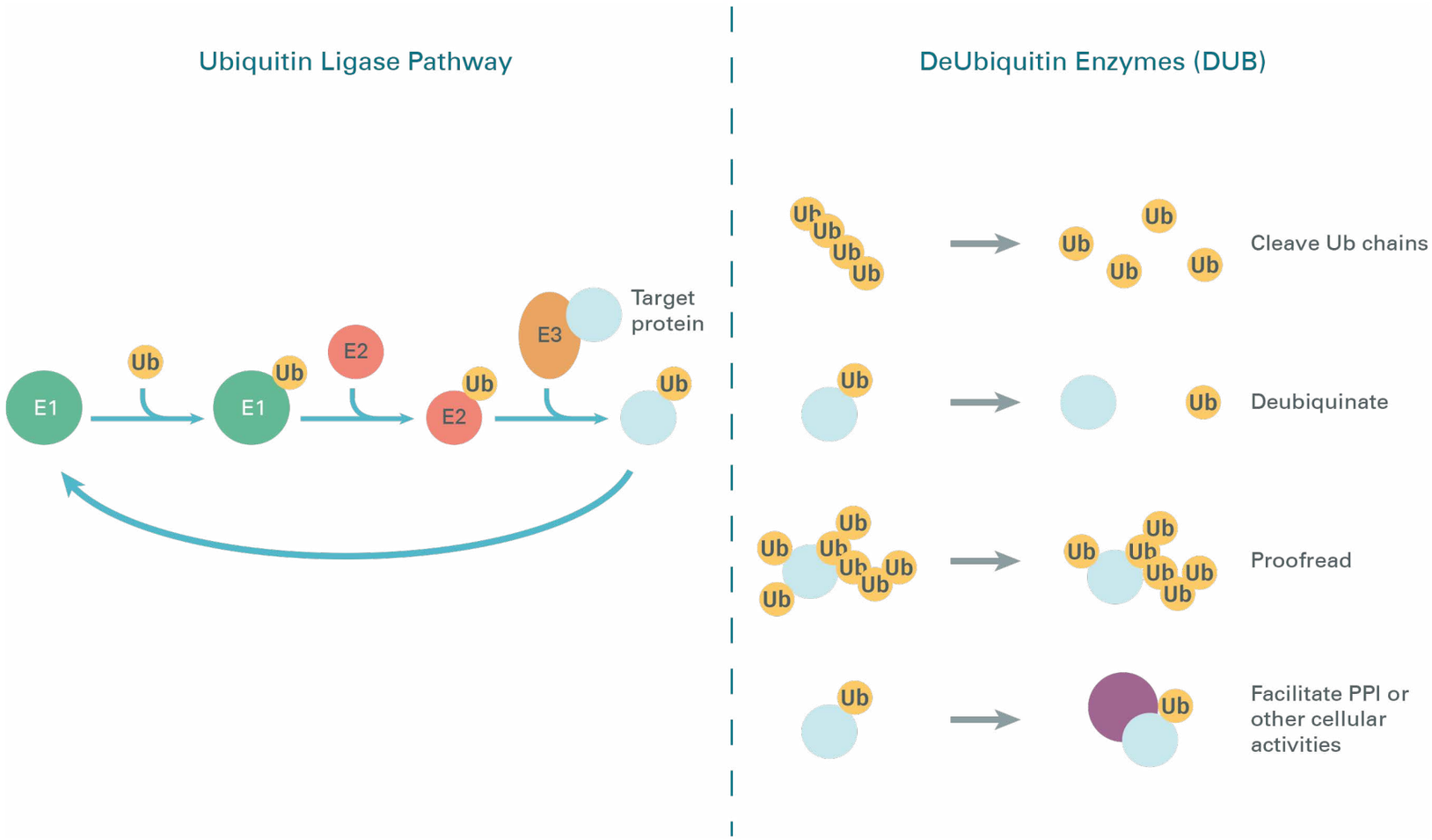


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

At the center of the UPS, the 26S proteasome is the principal proteolytic machine responsible for ubiquitin/ATP-dependent degradation of thousands of short-lived proteins and regulator proteins, as well as damaged and misfolded proteins, in order to regulate various cellular functions including cell cycle, DNA repair, apoptosis, immune response, signal transduction, cellular metabolism, and protein quality control [7].

STRUCTURE, ASSEMBLY, AND FUNCTION OF THE 26S PROTEASOME

The 26S proteasome is a large multi-subunit protease complex of 2.5 MDa that is located in the cytosol and nucleus of all eukaryotic cells. It is divided into two main components: the 20S core particle (CP) that houses the protease activities, capped at one or both ends by the 19S regulatory particle (RP), also called PA700, that recognizes and prepares protein substrates for degradation [8].

The 20S CP has a barrel structure formed by 4 stacked rings (each containing 7 subunits): two outside α-rings forming the proteasome gate, and two inner β-rings with catalytic sites provided by the β1, β2, and β5 subunits responsible for the cleavage of a broad array of polypeptides. As substrate entrance into the proteolytic core is physically blocked by the α-subunits in absence of the 19S RP, the free 20S proteasome is described as a latent complex. Therefore, passage through the gate is the rate-limiting step and prevents unregulated protein degradation [8].

The 19S RP is composed of two sub-complexes: the base and the lid. The base of the cap is formed by 6 regulatory ATPase particles (RPT1–6) involved in substrate translocation and unfolding, as well as in 20S gate opening, and by 4 regulatory non-ATPase particles (RPN1, 2, 10, and 13) involved in ubiquitin binding. Finally, the lid of the cap contains 9 regulatory non-ATPase particles (RPN3, 5-9, 11, 12, and 15) in which RPN11 possesses a DUB activity [8].

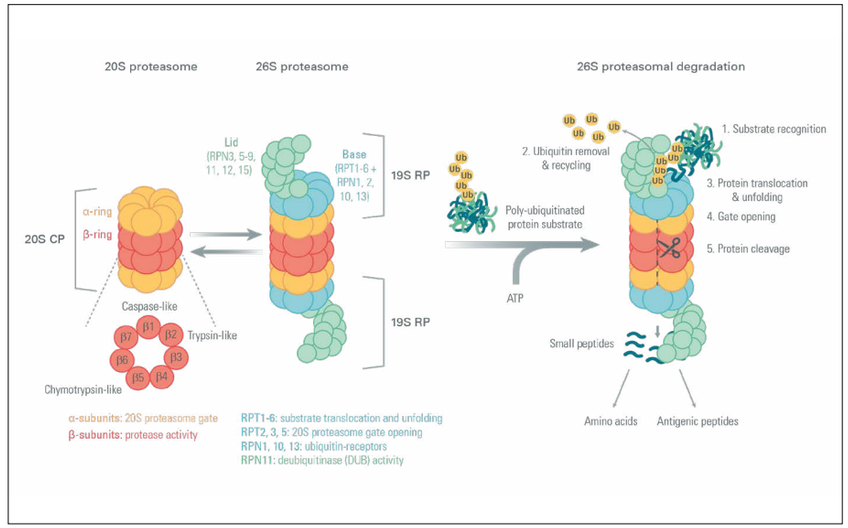
The complex structure of the proteasome requires precise assembly for the generation of a functional unit. The CP and RP are constructed separately with the assistance of specific assembly chaperones. During assembly of the CP, the two complexes PAC1/PAC2 and PAC3/PAC4, as well as the protein UMP1, assist in the formation of the α- and β-rings. Assembly of the RP is mediated by p27, p28, S5b, and PAAF1 [9].

TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

The 26S proteasome degrades proteins through an ATP-dependent multistep process. The 19S RP first recognizes and binds polyubiquitinated protein substrates, stimulating the DUB activity of RPN11 and resulting in the removal and recycling of ubiquitins. The ATP hydrolysis by RPT1-6 then drives protein translocation through the 20S gate, which forces protein unfolding. RPT2, 3, and 5 finally induce the α-ring gate opening through binding between the α-subunits to allow substrate entry into the proteolytic core and degradation by catalytic β-subunits. The small peptides (3 to 25 amino acids) released by the proteasome are degraded into amino acids by peptidases to be reused where needed or used as antigenic peptides [10].



Click to Enlarge

TABLE OF CONTENTS

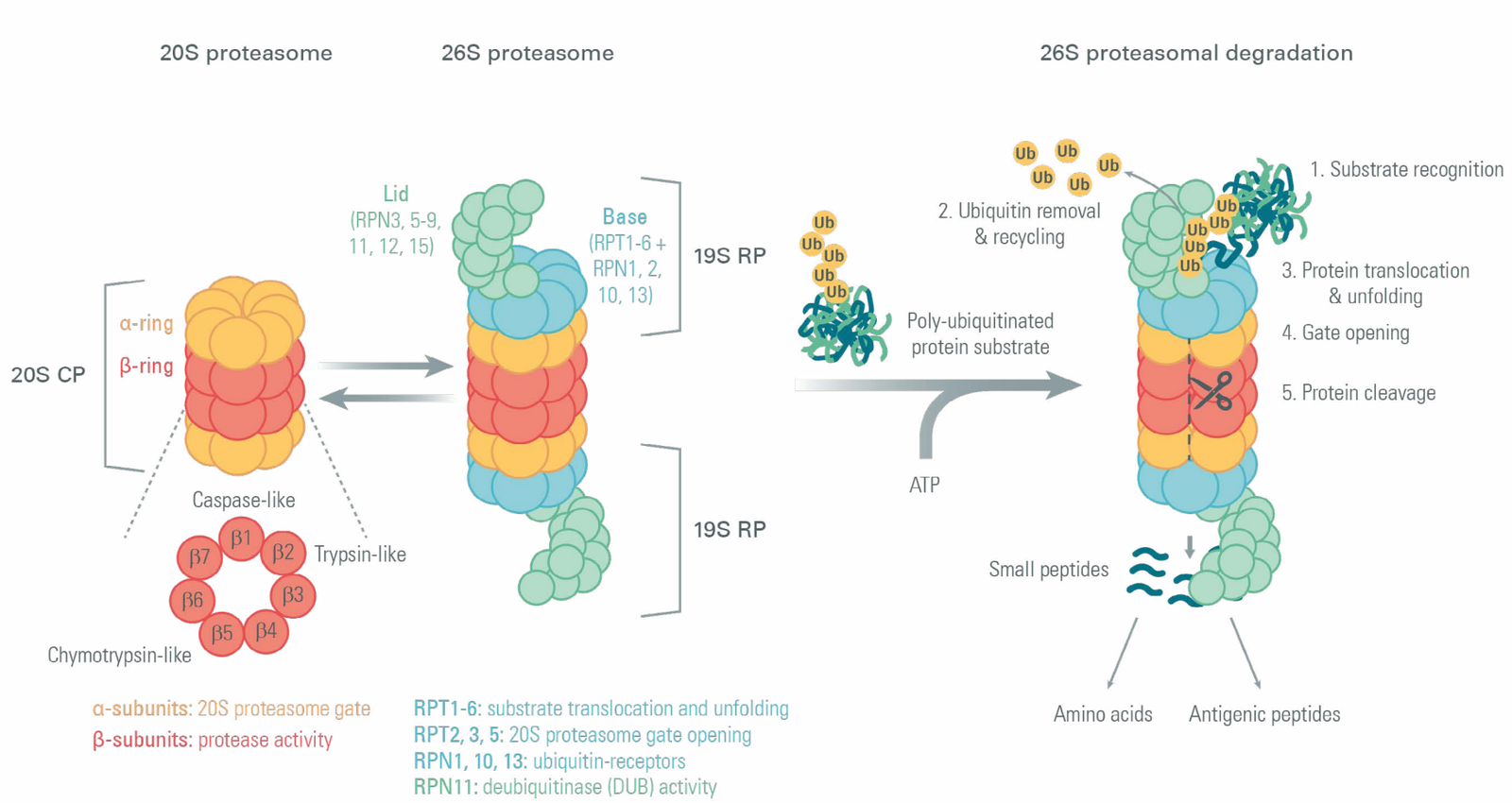


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

REGULATION OF PROTEASOME ACTIVITY UNDER DIFFERENT CONDITIONS

In order to maintain adequate proteasome activity, cells have mechanisms to inhibit or activate the proteasome under different conditions. These proteasome modulations include changes in proteasome composition, transcriptional regulation, and post-translational modifications (PTMs) [11].

CHANGES IN PROTEASOME COMPOSITION

The proteasome is not a static complex, and its activity can be modulated by the binding of different proteasome activators (PAs). The 20S CP can thus also interact at one or both sides with PA28 (also called 11S) or PA200 to form proteasomes different from the 26S. These proteasomal complexes may thereby facilitate the degradation of certain substrates under different physiological conditions, and/or of those that are degraded less efficiently by the 26S proteasome. A variety of hybrid proteasomes can also be formed when the 20S CP is capped with two different regulators. These proteasomes produce different patterns of peptides and play special roles, such as in the DNA damage response [12].

Along with the standard 20S CP, mammalian cells have two more subtypes of 20S proteasomes containing more specialized β-subunits:

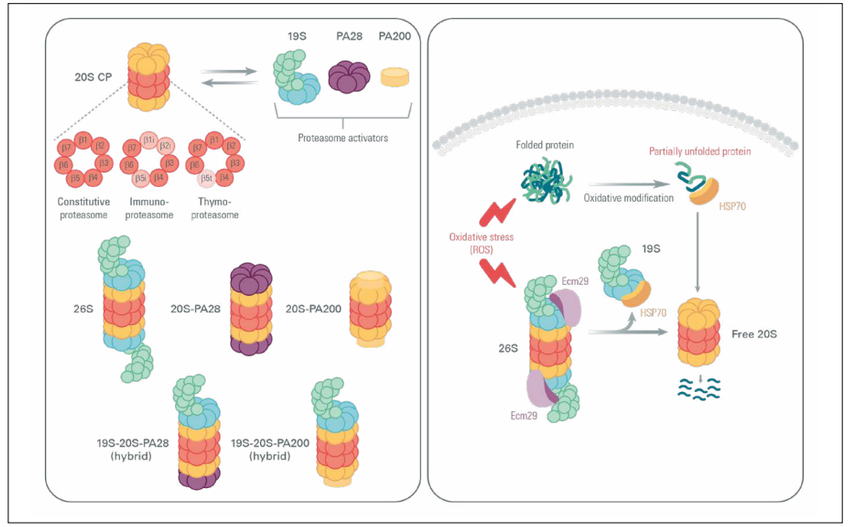
- The immunoproteasome has immune subunits β1i, β2i, and β5i specialized in the processing of antigens allowing the release of peptides for MHC class I antigen presentation. This proteasome is enriched in a variety of immune system-related tissues, antigen-presenting cells, and its expression is also induced in non-immune tissues during infections and inflammation, mainly in response to IFN-γ.
- The thymoproteasome is found only in cortical thymic epithelial cells and contains a specific subunit β5t which is thought to play a pivotal role in positive selection of CD8+ T cells [13].

TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

Additionally, many studies have shown that free 20S proteasome is the major machinery involved in the degradation of oxidized proteins. Oxidative stress caused by environmental toxins or cellular stresses induces 26S proteasome disassembly, increasing the pool of free 20S proteasomes and providing a rapid mechanism to degrade oxidized proteins in an ATP/ubiquitin-independent manner. More precisely, ROS induce the dissociation of the 19S RP from the 20S CP, a process that is assisted by the proteasome-interacting protein Ecm29, as well as by HSP70 that binds and preserves the dissociated 19S RP for subsequent reassembly into 26S proteasomes. In parallel, protein oxidation caused by ROS results in the exposure of hydrophobic sites, which are recognized by the 20S proteasome as a degrading signal.
This stimulates the opening of the barrel that then degrades oxidized proteins. Interestingly, HSP70 also interacts with oxidized proteins to increase their degradation, possibly by shuttling the substrates toward the 20S proteasome. It has also been suggested that free 20S proteasome is able to degrade other types of damaged proteins induced by aging or mutations, as well as native proteins with intrinsically disordered regions (IDRs), demonstrating the importance of this process to maintain normal cellular metabolism and respond to various stresses [14].



Click to Enlarge

TABLE OF CONTENTS

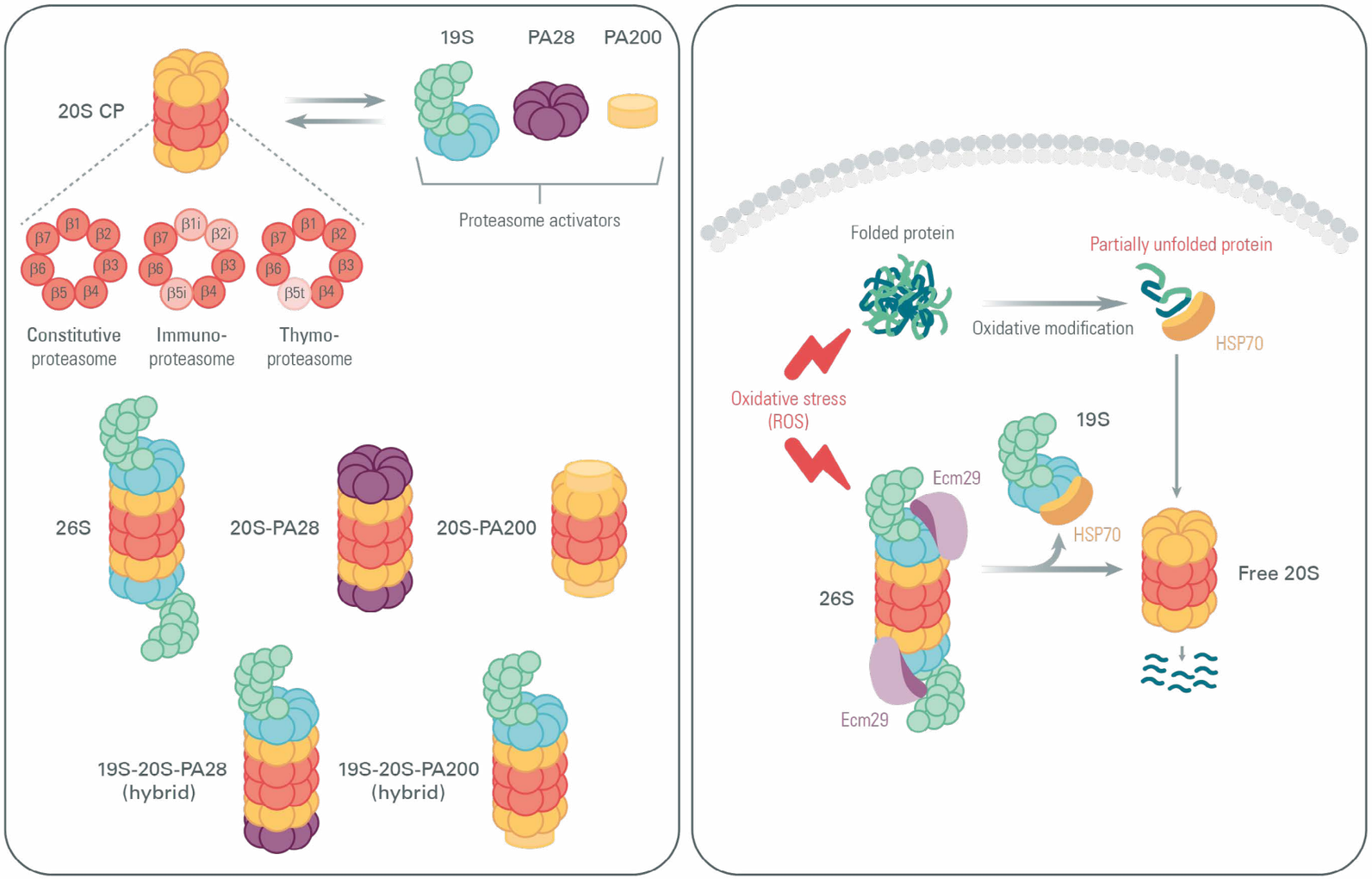


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

TRANSCRIPTIONAL REGULATION

To maintain proteasome function, the expression of proteasome subunits is coordinately regulated at the transcriptional level in order to provide stoichiometric amounts of each polypeptide. Although this regulatory mechanism has not been clearly elucidated, important signaling pathways that affect proteasome gene expression under different conditions have been identified in the last two decades [15].

Nrf1 (also called NFE2L1) is a master regulator of proteasome gene expression in response to proteasome inhibition. This endoplasmic reticulum (ER)-resident transcription factor is inserted into the ER membrane as an N-glycosylated protein. Under normal conditions, Nrf1 is retrotranslocated to the cytosol by the ATPase p97, polyubiquitinated by the ER-resident E3 ligase Hrd1, and continuously degraded via the ER-associated degradation (ERAD) pathway involving the 26S proteasome. The protein is thereby maintained at low basal levels. Upon partial proteasome inhibition caused by an overload of misfolded proteins or by treatment with proteasome inhibitors, retrotranslocated Nrf1 accumulates in the cytosol, where it is deglycosylated by NGLY1 and then cleaved by the protease DDI2. The active processed form of Nrf1 moves to the nucleus and promotes the expression of proteasome subunit genes in partnership with small Maf (sMAF) proteins. This negative feedback mechanism is called a “bounce-back” response. It exerts a compensative effect on proteasome expression when proteasome activity is compromised. Recent studies have also indicated that high nutrient levels, growth factors, and insulin activate mTORC1, which in turn induces the activation of the transcription factor SREBP1 leading to Nrf1 gene transcription and thus proteasome expression [16, 17].

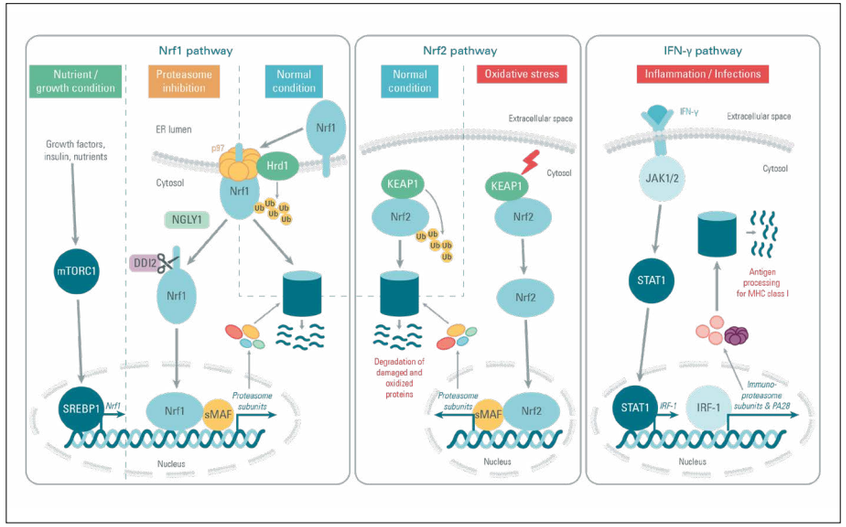
Nrf2 (also called NFE2L2) plays a key role in protection against oxidative stress. Under normal conditions, Nrf2 is found in very low levels due to its constant polyubiquitination by the E3 ligase KEAP1, and subsequent degradation by the 26S proteasome. Upon oxidative stress, highly reactive cysteine residues of KEAP1 are oxidized, which causes dissociation of the KEAP1-Nrf2 complex. The free Nrf2 translocates to the nucleus, where it heterodimerizes with sMAF proteins. This leads to the transcription of various stress response genes, including proteasome subunits. Thus the Nrf2 pathway increases the capacity of the cell to degrade damaged and oxidized proteins [16]. Interestingly, Nrf2 also regulates macro-autophagy via p62. Oxidative stress induces phosphorylation of p62 which is then able to bind to KEAP1, leading to the quick dissociation of active Nrf2 from KEAP1 and the expression of many autophagy genes, such as Atg3, Atg5, Atg7, and p62/SQSTM1 to mediate stress response [19].

TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

IFN-γ is also an important regulator of proteasome gene expression during immune response. Upon inflammation or infections, the binding of IFN-γ to its receptor results in the phosphorylation of JAK1/2. The activated JAKs in turn phosphorylate the transcription factor STAT1, which homodimerizes and translocates into the nucleus to induce IRF-1 gene transcription. IRF-1 in turn promotes the expression of immunoproteasome subunits and PA28, that are involved in the degradation of antigens and the release of peptides for MHC class I antigen presentation [11].



Click to Enlarge

TABLE OF CONTENTS

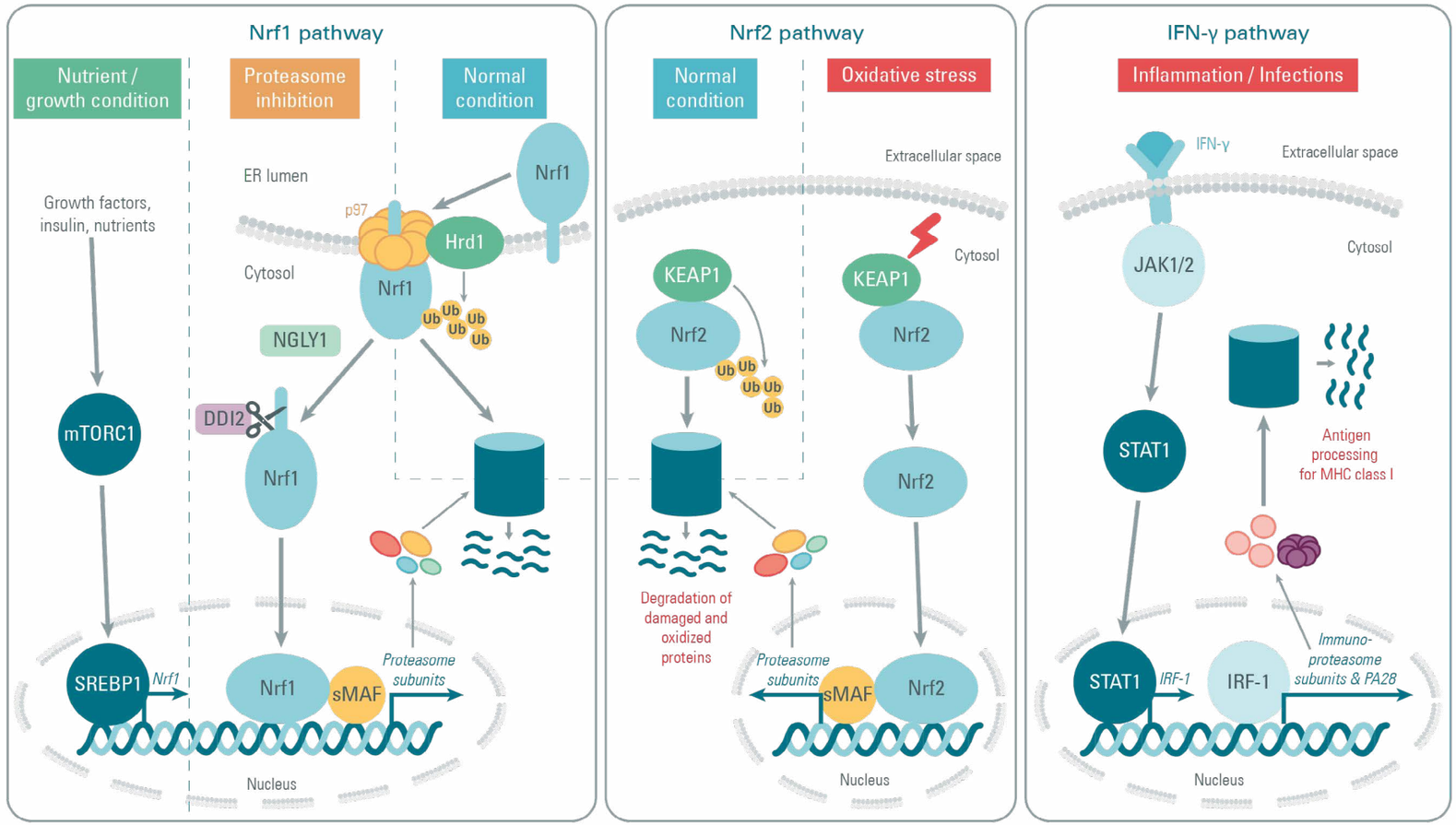


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

POST-TRANSLATIONAL MODIFICATIONS

Proteasome PTMs offer additional opportunities to regulate proteasome assembly, activity, localization, and abundance. To date, over 350 PTM sites have been identified on the 26S proteasome, including phosphorylation, acetylation, methylation, ubiquitination, O-GlcNAcylation, and ADP ribosylation. There seem to be differences in PTMs between cell types and tissues, adding another layer of complexity to this regulation process. For most PTMs, the specific proteasomal subunits/sites and associated effects on proteasome function are largely unknown. However, more and more modifications have been studied in recent decades, revealing their role in proteasome regulation. One common PTM that affects almost all proteasome subunits is phosphorylation, which is regulated by numerous proteasome-interacting kinases and phosphatases. As an example illustrating the key role of phosphorylation, treatment of purified mammalian proteasomes with alkaline phosphatase induces the dissociation of the CP and RP. The table presented here lists several examples of PTMs for which the target, the enzyme, and the effect on proteasomal function are known [8, 11].

Examples of known PTMs and their effects on proteasome regulation

PTM	TARGET	ENZYME(S)	EFFECT ON PROTEASOME
O-GlcNAcylation	19S / RPT2	OGT/OGA	↓ ATPase activity; ↓ chymotrypsin-like (ChT-L) activity; ↓ ubiquitinated protein degradation
Acetylation	20S / α6, β3, β6, β7	HDAC	↑ Trypsin-like (T-L) activity
Phosphorylation	19S / RPT6	PKA/PP1γ	↑ 26S proteasome assembly; ↑ ChT-L and T-L activities
Ubiquitination	19S / RPN13	UBE3C	↓ substrate binding; ↓ ubiquitinated protein degradation
Poly-ADP ribosylation	Nuclear 20S	PARP	↑ ChT-L activity; ↑ oxidized protein (e.g. histone) degradation

TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

PROTEASOME-DEPENDENT CELLULAR PROCESSES

Proteasome function is essential to cellular homeostasis. In addition to maintaining proteostasis, the proteasome plays a key role in regulating various cellular processes such as cell cycle control, cell proliferation and survival, and apoptosis, as well as immune and inflammatory responses [20].

Proteostasis maintenance: One of the main functions of the proteasome is to ensure the rapid degradation of abnormal proteins such as misfolded proteins (e.g. due to genetic mutations), oxidized proteins, and other types of damaged proteins (caused by other cellular stresses), in order to avoid the accumulation of toxic proteins (e.g. aggregated proteins) within the cell. The UPS is also at the center of the ERAD machinery which degrades newly synthetized proteins of the ER that fail in proper folding or assembly, in order to clear the ER of these harmful species [21].

Cell cycle control: The cell cycle is a strictly regulated process controlled by the oscillating activities of cyclin-dependent kinases (CDKs) which are activated by cyclins and inhibited by CDK inhibitors (CKIs). The cell cycle is regulated by diverse mechanisms, including the periodic UPS-mediated degradation of cyclins (such as cyclins A, B, and E), CKIs (such as p21 and p27), and other cell cycle regulators (such as Cdc6 and Cdc25A). This irreversible mechanism assures the strict unidirectionality of the cell cycle and mediates the precise spatial and temporal proteolysis of the main players in the cell cycle. The activity of the tumor suppressor p53 involved in cell cycle arrest and DNA repair is also regulated by the UPS [20, 22].

Cell proliferation and survival: A large number of proteins necessary for the control of cell proliferation and survival are regulated by the ubiquitin proteasome pathway. Among them, we can cite the Wnt signaling activator β-catenin, which is constantly degraded by the proteasome in the absence of the Wnt ligand [23]. Another example is HIF-1α, which constitutes the oxygen sensitive subunit of the transcription factor HIF-1. Its expression is induced under hypoxic conditions, whereas it undergoes quick proteasomal degradation under normoxic conditions [24]. In normal cells, the expression of the proto-oncogene c-Myc is also tightly controlled by the UPS [25].

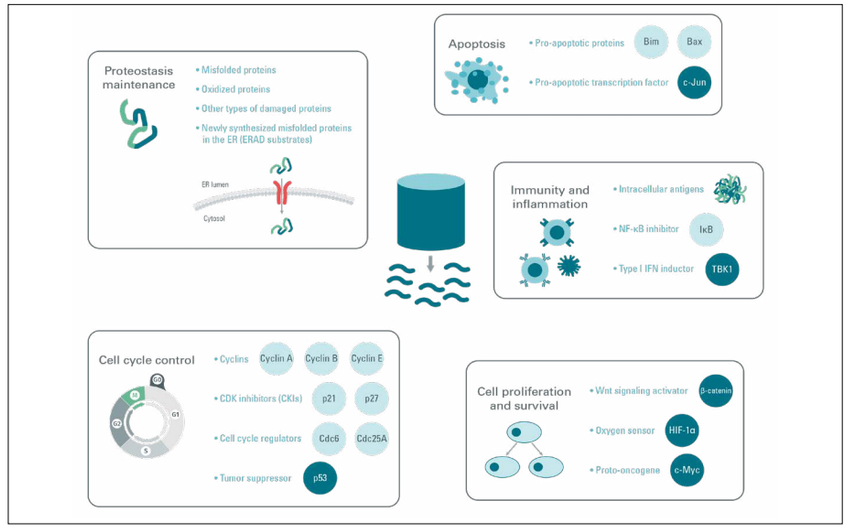
TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

Apoptosis: The UPS is required to ensure the normal regulation of cell apoptosis. Bax and Bim are well known examples of proteins whose activities are regulated by proteasomal degradation. These pro-apoptotic Bcl-2 family members control a critical step in commitment to apoptosis, by regulating the permeabilization of the mitochondrial outer membrane and the release of cytochrome c [26]. The pro-apoptotic transcription factor c-Jun, which is activated by JNK signaling and involved in death receptor-initiated extrinsic as well as mitochondrial intrinsic apoptotic pathways, is also regulated by the ubiquitin proteasome pathway [27].

Immunity and inflammation: As previously mentioned, the proteasome plays a key role in the processing of intracellular antigens, which are then used to release peptides for MHC class I antigen presentation in order to activate cytotoxic T cells. Proteasomal degradation of IκB is also essential for NF-κB transcription factor activation and the expression of genes involved in innate and adaptive immunity, inflammation, B-cell development, and lymphoid organogenesis [20]. TBK1-mediated induction of type I IFN plays a critical role in host antiviral responses and immune homeostasis. One mechanism of negative TBK1 activity regulation is via SOCS3, which triggers the polyubiquitination of TBK1 and promotes its proteasomal degradation [28].



Click to Enlarge

TABLE OF CONTENTS

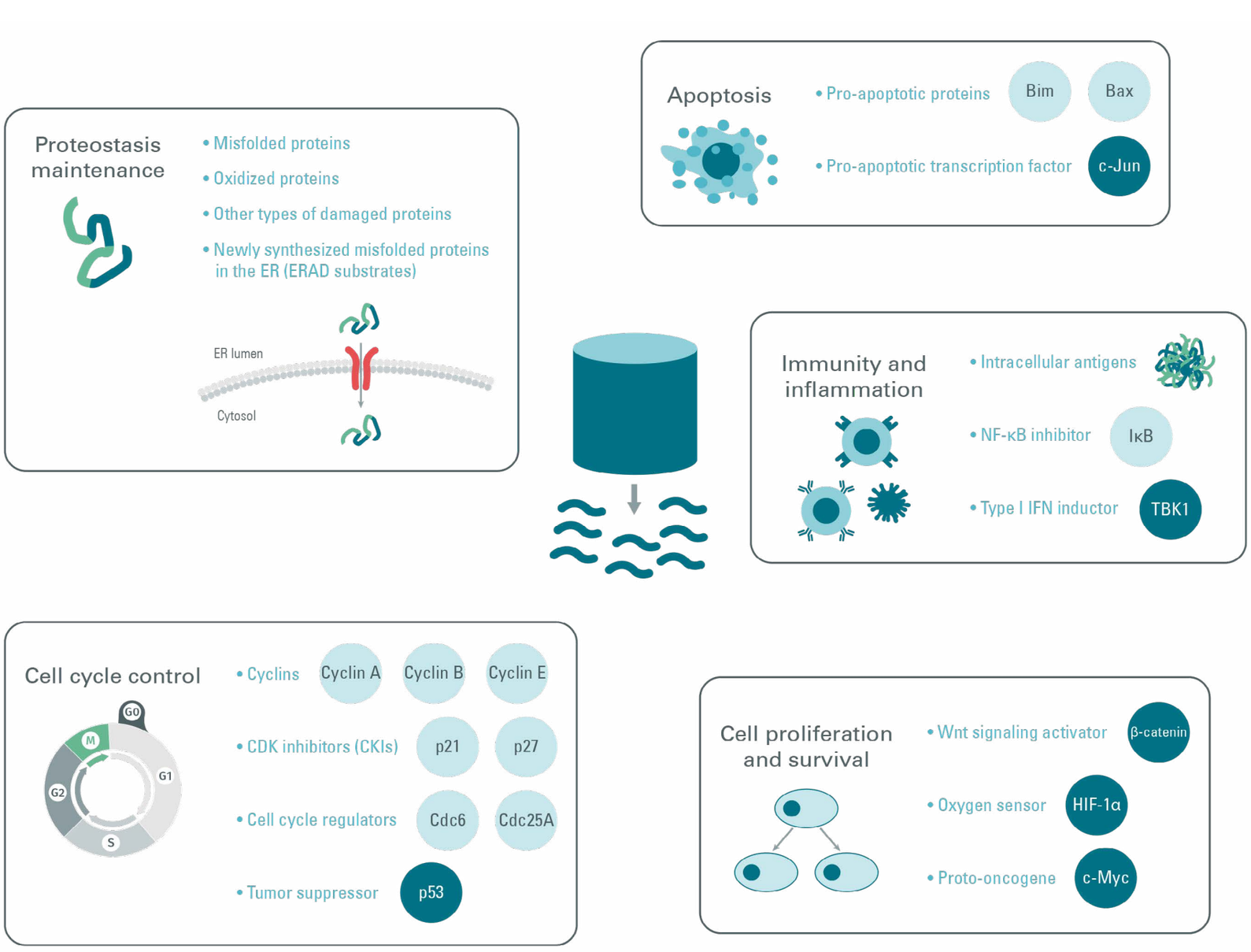


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The ubiquitin proteasome system

PROTEASOME DYSREGULATION IN PATHOLOGICAL DISORDERS

If an abnormality occurs in the regulation of protein degradation, normal proteins will be degraded and/or abnormal proteins will not be degraded, leading to proteasome-related diseases such as neurodegeneration, cancer, cardiac dysfunction, and autoimmune or metabolic disorders [29].

NEURODEGENERATIVE DISEASES

Maintaining proteostasis in neurons is crucial to eliminate aggregation-prone proteins, especially as neuronal cells possess a complex architecture and a long lifespan, and are unable to dilute the aggregate load by cell division. Impaired proteasome function has been implicated as a primary cause or as a secondary consequence in the pathogenesis of many neurodegenerative disorders, including Alzheimer's Disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD). In these disorders, proteins that are normally degraded are not properly degraded after misfolding occurs, leading to the accumulation of toxic protein aggregates. This in turn results in the progressive death of neurons. In AD, microtubule-associated protein Tau becomes hyperphosphorylated, causing its misfolding and aggregation into neurofibrillary tangles (NFTs). Additionally, there is a pathological accumulation of amyloid-β peptides (Aβ) 1-40 and 1-42 that aggregate to form amyloid plaques. PD patients display a pathological accumulation of α-synuclein that further adopts oligomeric forms and then fibrils due to various causes such as oxidative stress and mutations. These fibrils then associate with other aggregated proteins (such as Tau or Aβ) to form bigger structures called Lewy bodies. HD is caused by a mutation in a gene of the protein huntingtin. As a result, the translated protein contains disease-causing expansions of glutamines (polyQ) that make it prone to misfold and aggregate. Several recent studies have provided evidence that these different types of aggregated proteins adopt a common 3D conformation that is capable of interacting and impairing ubiquitin-dependent and ubiquitin-independent proteasome function. This suggests that a common mechanism of proteotoxicity could contribute to the development and progression of these distinct neurodegenerative diseases [30].

TABLE OF CONTENTS

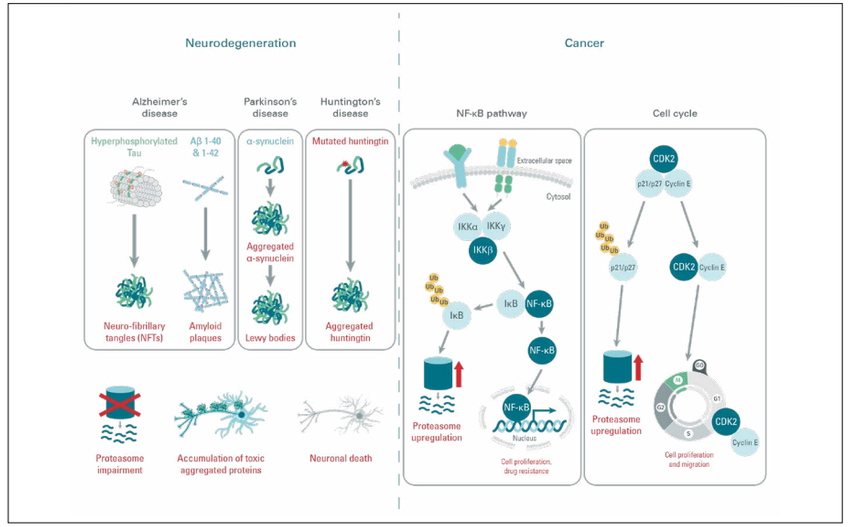
GENERAL KNOWLEDGE

The ubiquitin proteasome system

CANCER

Proteasomes play a critical role in regulation of cell growth and survival in both normal and cancer cells. In cancer cells, they are found to be highly expressed leading to their hyperactivation. Many proteins, such as IκB, p21, and p27, are degraded through proteasomes and are known to regulate tumorigenesis in a variety of cancers. The NF-κB pathway is constitutively activated in cancers. Extracellular signals activate the IKK complex composed of IKKα, IKKγ, and IKKβ. This complex is phosphorylated, and in turn induces the phosphorylation of IκB, which is further polyubiquitinated and degraded by the 26S proteasome. Degradation of IκB consequently releases the transcription factor NF-κB, which then translocates to the nucleus and activates the expression of genes involved in the proliferation and drug resistance of cancer cells [31].

As previously mentioned, cell cycle progression is governed by CDKs whose activity is inhibited by CKIs. In cancer cells, there is a loss of expression of the two G1-checkpoint CKIs p21 and p27 due to their upregulated ubiquitination and proteasomal degradation. Their degradation promotes G1/S phase transition via activation of cyclin E/Cdk2 and the subsequent proliferation and migration of cancer cells [32].



Click to Enlarge

TABLE OF CONTENTS

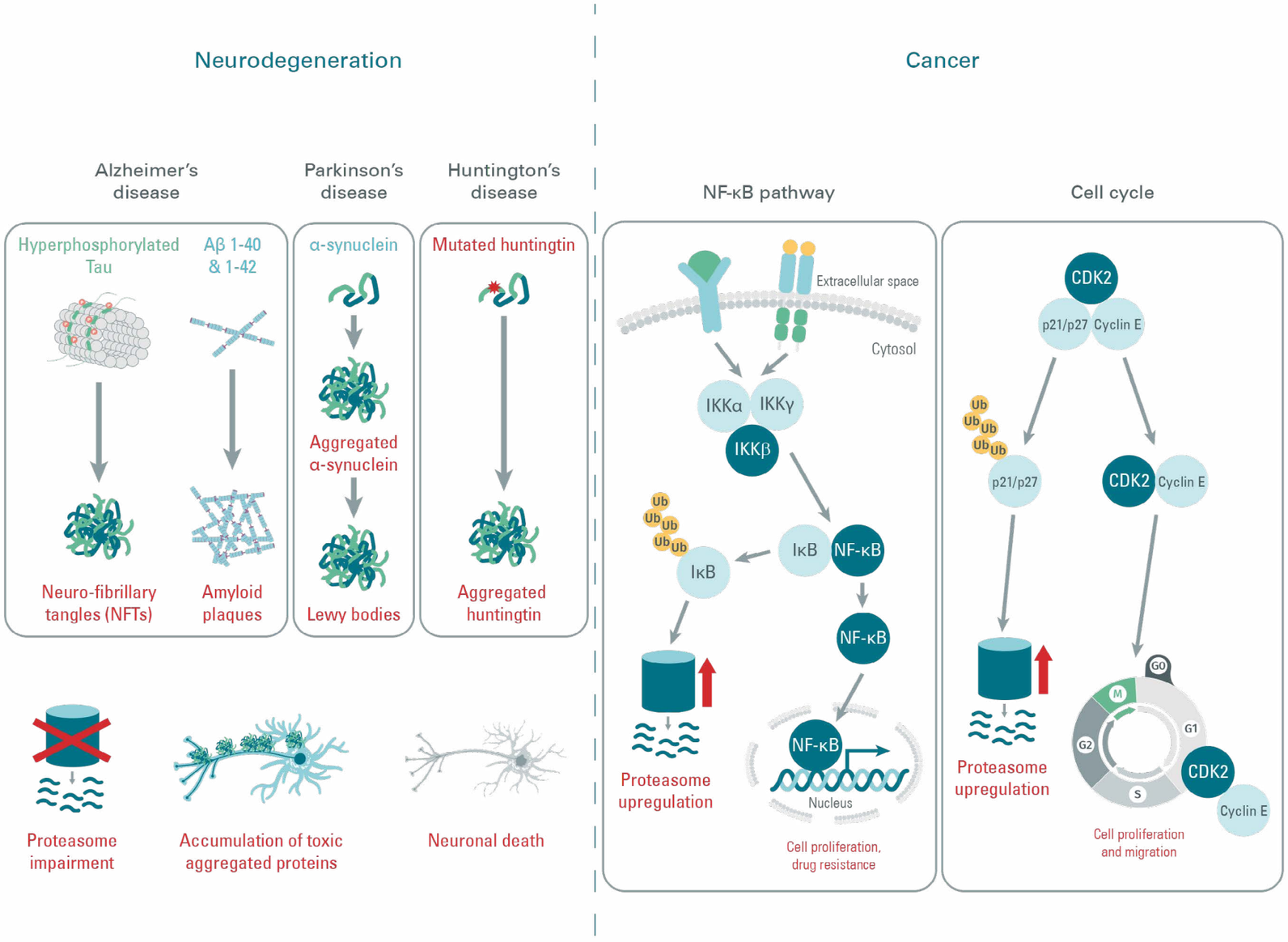


TABLE OF CONTENTS

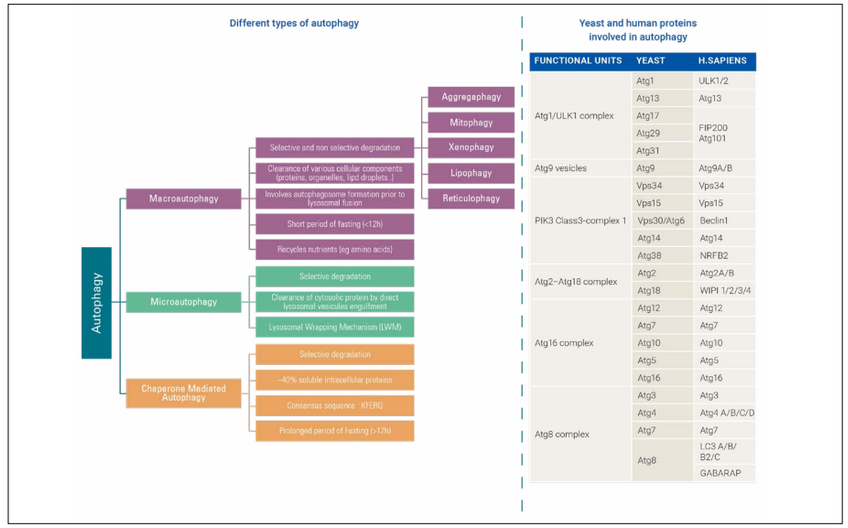
GENERAL KNOWLEDGE

The autophagy-lysosomal system

Autophagy is an evolutionary conserved cellular process that occurs in virtually all eukaryotic cells, ranging from yeast to mammals. Initially observed in 1957, it took nearly 30 years before Ohsumi's group paved the way for our current autophagy molecular mechanism understandings. Autophagy is crucial in the maintenance of homeostasis, being involved in numerous physiological processes including stress responses (e.g. starvation, hypoxia, high temperature), cell growth, and aging. Conversely, dysfunctions in autophagic mechanisms have been associated with diseases such as cancer, neurodegenerative diseases, infectious diseases, and cardiac and metabolic diseases.

From the Greek, auto means “self” and phagy means “eating”, and thus autophagy is a cellular process where a cell can eat and digest its own components. Autophagy is a catabolic process that can be compared to a cellular rubbish-disposal mechanism where cytoplasmic cargos are engulfed. These cargos can be proteins, where the process is called proteophagy, lipid droplets in lipophagy, organelles such as in mitophagy or reticulophagy, or pathogens in xenophagy. Unlike the ubiquitin-proteasome system, which is involved in the degradation of short-lived proteins, autophagy is not only involved in the clearance of long-lived proteins and organelles, but also in the recycling of building blocks such as amino acids.

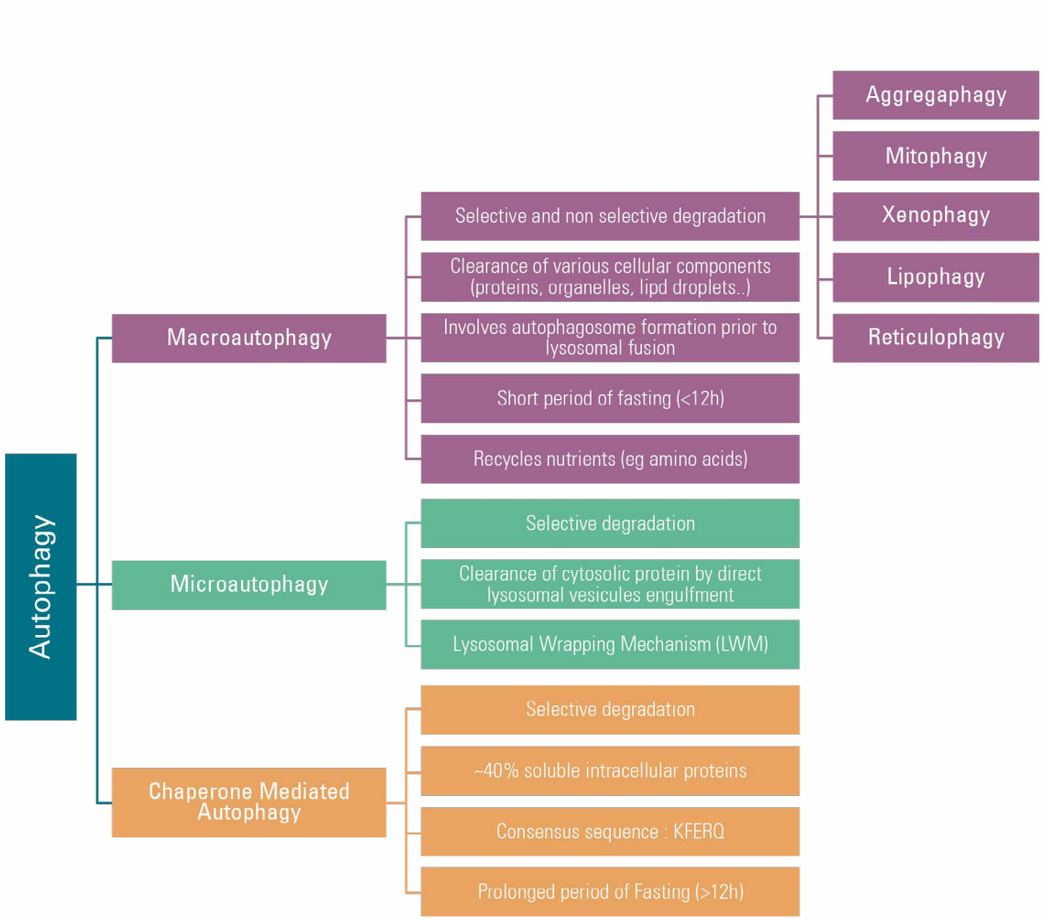
There are 3 types of Autophagy: 1) Macroautophagy, which is commonly referred to as autophagy, can be either selective or non-selective (“bulk”). Selective autophagy removes and recycles defective or unneeded cellular components, such as protein aggregates, damaged mitochondria, unneeded or excesses of peroxisomes, endosomes, or lipid droplets, as well as intracellular pathogens. Bulk autophagy is triggered by starvation and helps cell survival by providing lipids, amino acids, carbohydrates, and nucleotides. 2) Microautophagy and 3) Chaperone-Mediated Autophagy. They all direct cellular components for degradation within the lysosomes, the final destination where degradation and recycling occur.



Click to Enlarge

TABLE OF CONTENTS

Different types of autophagy



Yeast and human proteins involved in autophagy

FUNCTIONAL UNITS	YEAST	H.SAPIENS
Atg1/ULK1 complex	Atg1	ULK1/2
	Atg13	Atg13
	Atg17	FIP200 Atg101
	Atg29	
	Atg31	
Atg9 vesicles	Atg9	Atg9A/B
PIK3 Class3-complex 1	Vps34	Vps34
	Vps15	Vps15
	Vps30/Atg6	Beclin1
	Atg14	Atg14
	Atg38	NRFB2
Atg2–Atg18 complex	Atg2	Atg2A/B
	Atg18	WIPI 1/2/3/4
Atg16 complex	Atg12	Atg12
	Atg7	Atg7
	Atg10	Atg10
	Atg5	Atg5
	Atg16	Atg16
Atg8 complex	Atg3	Atg3
	Atg4	Atg4 A/B/C/D
	Atg7	Atg7
	Atg8	LC3 A/B/ B2/C
		GABARAP

TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

MACROAUTOPHAGY, MICROAUTOPHAGY AND CHAPERONE MEDIATED AUTOPHAGY

Even though selective and bulk autophagy or macroautophagy are triggered by different signals, they both converge towards a single pathway that initiates the formation of pre-autophagosome, also known as phagophore, which matures into autophagosome prior to its fusion with lysosomes. Different autophagy related proteins, or Atg proteins, are sequentially involved in the formation of autophagosome and fall into 6 functional groups: (i) the ULK1/Atg1 protein kinase complex; ii) Atg2–Atg18/WIPI proteins (iii) Atg9 vesicles; (iv) phosphatidylinositol (PI) 3-kinase (PI3K) complex I; two ubiquitin-like protein conjugation systems; v) the Atg16 complex, and vi) LC3/Atg8 conjugation systems [33] [34]. For a better understanding of protein names found in yeast and human, please refer to the table.

Autophagy starts with the formation of a bud from lipid phosphatidylinositol 3-phosphate rich membranes such as RE, Golgi, or plasma membranes and known as omegasome or pre-autophagosomal structure (PAS). This a cup-shaped structure requires the ULK1/Atg1 complex formed by Atg13, Atg101, FIP200, and ULK1 proteins. A transmembrane protein Atg9 contained in vesicles is involved in the recruitment of Atg2-Atg18 to the PAS. With its phospholipid transfer activity, the Atg2 protein supplies phospholipids necessary for membrane elongation. ULK1 phosphorylates and activates Beclin1, which partners with Vsp15, Vsp34, Atg14, and NRFB2/Atg38 forming a class III Phosphatidylinositol 3-kinase (PI3KC3-Complex 1). This complex leads to the production of phospho-inositol triphosphate (PIP3) which further directs the recruitment of PI3P binding Atg18/WIPI 1–4 proteins and the Atg12-Atg5-Atg16 complex. The latter complex, along with Atg4 and Atg7, is implicated in the conjugation of Phosphatidyl Ethanolamine to LC3-GABARAP/Atg8, a process also known as LC3-GABARAP protein family lipidation. LC3-GABARAP/Atg8 -PE conjugates serve as receptors involved in the recognition of ubiquitinated cargos through adapter proteins such as p62SQSTM1, NBR1, Optineurin (OPTN), or NDP52. After the overall structure is completed, the newly formed autophagosome fuses with lysosomes, where trapped cellular components are eventually degraded and building blocks recycled. SNARE proteins such as VAMP8 and Vti1b, and tether factors such as PLEKHM1 and HOPS are required for the fusion process, as well as lysosomal small GTPases, Arl8b, and RAB7. The lysosome contains approximately 60 different soluble acid hydrolases, such as sulfatases, glycosidases, peptidases, phosphatases, lipases, and nucleases. One important lysosomal complex is the LYNUS machinery, which is involved in lysosome nutrient sensing. LYNUS is a multiprotein complex including mTORC1, Rag GTPases, the small GTPase Rheb, Ragulator, and the proton pump V-ATPase, and is positively regulated by the transcription factor TFEB [35] [36] [37] [38] [39] [40].

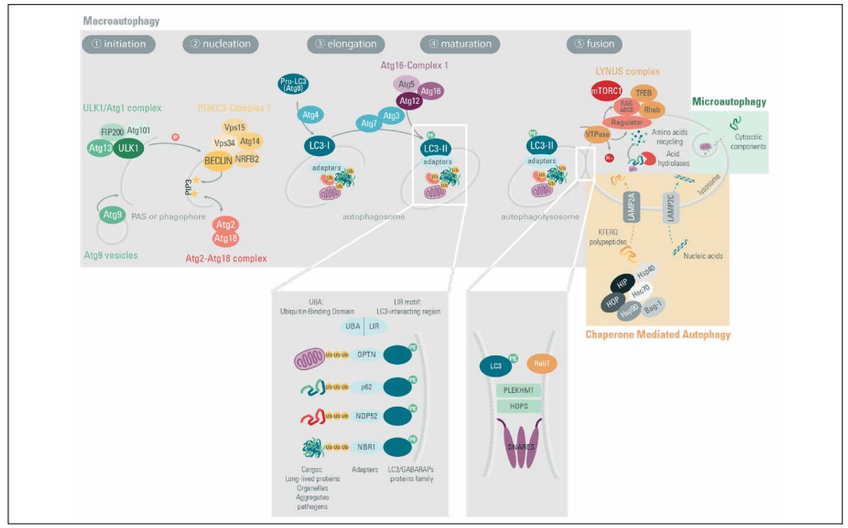
TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

Microautophagy refers to a process where cytosolic components are directly engulfed by lysosomes through membrane invaginations. Endosomal microautophagy (eMI) has recently been reported, but the molecular basis still remains elusive [41].

Chaperone-mediated autophagy (CMA) is the direct translocation of cytosolic protein substrates bearing a KFERQ motif into the lysosome through LAMP2A. This process involves different proteins, such as HSP90, 40, or HCS70. Nucleic acids are translocated via LAMP2C. CMA can be activated in response to multiple stress conditions such as starvation, hypoxia, and oxidative stress [42].



Click to Enlarge

TABLE OF CONTENTS

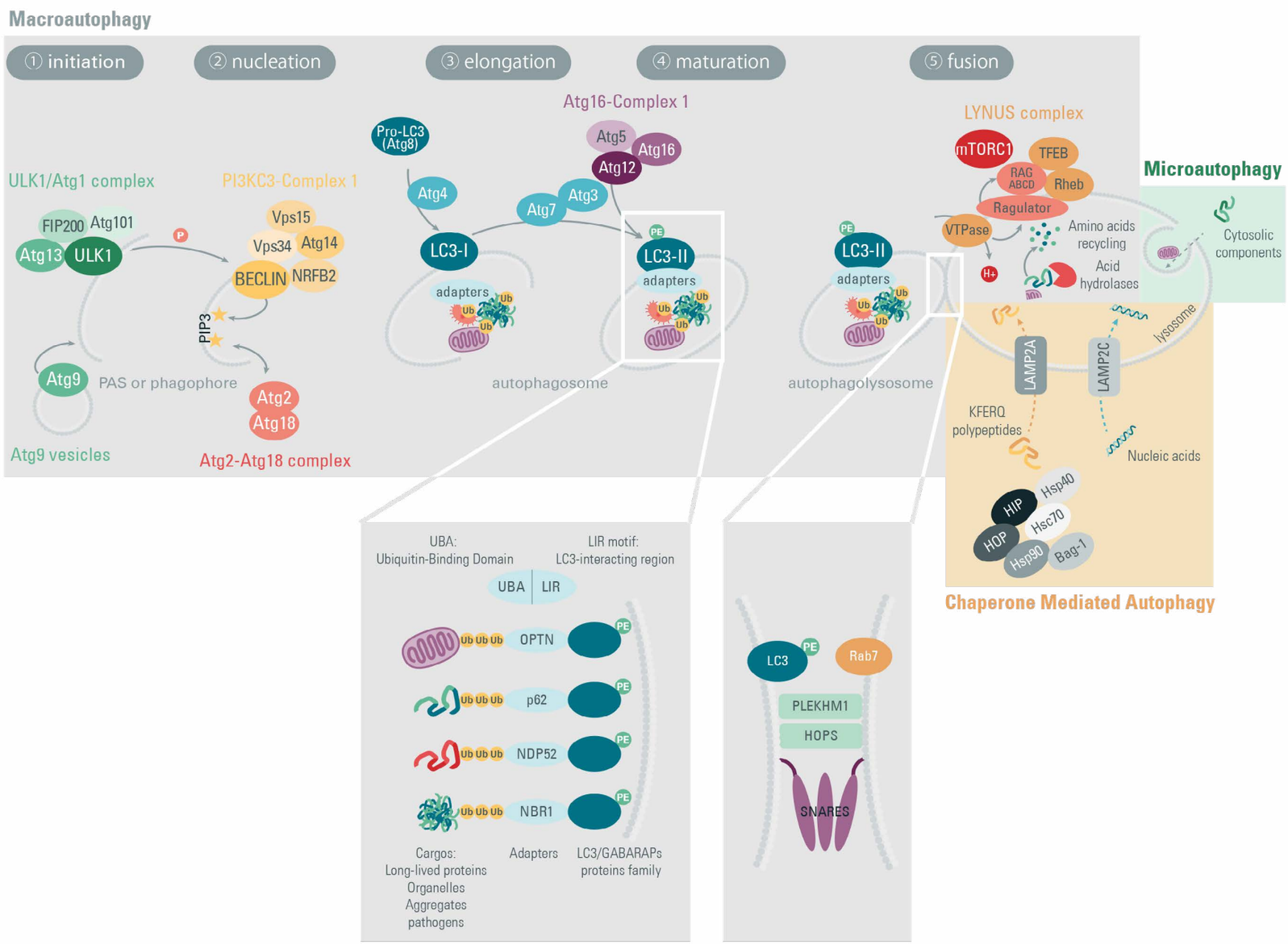


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

REGULATIONS OF AUTOPHAGY

Under normal conditions, a constitutive basal autophagy ensures intracellular quality control. But in stress conditions, such as nutrient deprivation, the autophagy process is rapidly induced to maintain the pool of amino acids and ensure cell survival. Multiple regulations in the autophagic pathways occur at both post-translational and transcriptional levels.

POST-TRANSLATIONAL REGULATIONS

The main post-translational modifications involved in autophagy regulation are phosphorylation, ubiquitination, and acetylation.

When nutrients are present, the upstream AKT kinase phosphorylates and activates mTORC1, which in turn inhibits autophagy by phosphorylating ULK1 at Ser638 and Ser758, as well as its associated partners ATG13 at Ser389 and AMBRA1 at Ser52.

In nutrient deprivation conditions, mTORC1 is downregulated and AMBRA1 is dephosphorylated. This results in ULK1 autophosphorylation at Ser180 and the subsequent phosphorylation of ATG13 (Ser389), ATG101 (Ser11 and Ser203), FIP200 (Ser943, Ser986, Ser1323), and AMBRA1 (Ser465, Ser635). AMP-activated protein kinase (AMPK), which is a nutrient sensor, is a positive regulator of autophagy involved in the activation of ULK1 by phosphorylation at Ser317 and Ser777. In fact, the ULK complex is considered to be an upstream hub which integrates and relays the activities of mTORC1 and AMPK.

A complex network of autophagic regulators modulates autophagy either positively (green arrows on the scheme) or negatively (red arrows on the scheme). For example, dephosphorylated AMBRA interacting with ULK1 leads to TRAF6 mediated-ULK1 ubiquitination and stabilization; phosphorylation of BCL2 by JNK releases Beclin-1 and promotes autophagy, whereas EGFR and Cdk5 phosphorylate and deactivate Beclin-1 functions. While MAPK15/ERK8 positively regulates autophagy through pro-LC3 phosphorylation, PKA plays the opposite role. Autophagy is inhibited by the acetyltransferase p300, which acetylates ATG7, ATG5, LC3, and ATG12, whereas the deacetylase SIRT1 is a positive autophagy regulator [43] [44] [45] [46].

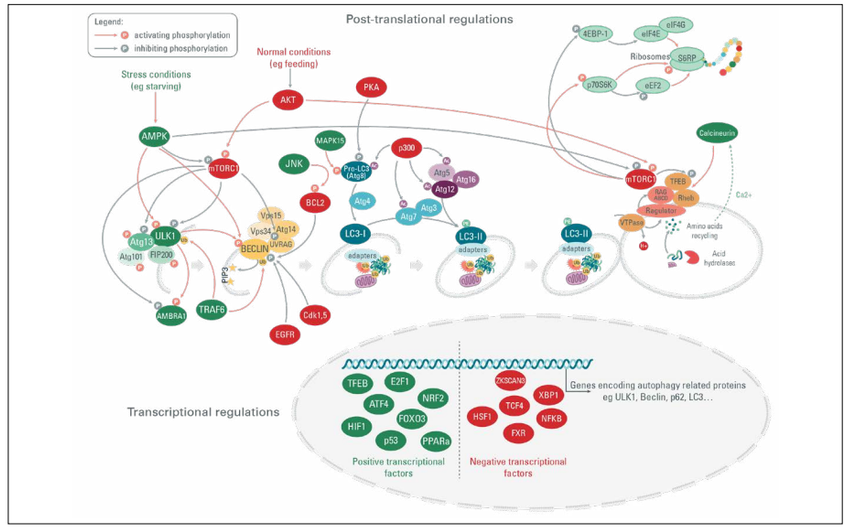
TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

TRANSCRIPTIONAL REGULATIONS

At the transcriptional level, TFEB and FOXO3 are key transcriptional factors which positively regulate both autophagy and lysosomal biogenesis. Transcription Factor EB (TFEB) is considered to be the master regulator of lysosomal and autophagic function. Inactive TFEB is phosphorylated by mTOR and sequestered in the cytoplasm. Upon dephosphorylation by phosphatases such as Calcineurin, TFEB translocates into the nucleus, where it binds to specific CLEAR DNA sequences and induces the upregulation of proteins involved in lysosome biogenesis and in the autophagy pathway. Whereas the transcription factors HIF1, ATF4, PPARa, or NRF2 also positively regulate autophagy, ZSCAN3, FXR, TCF4, or NFKB down regulate it [43] [47].



Click to Enlarge

TABLE OF CONTENTS

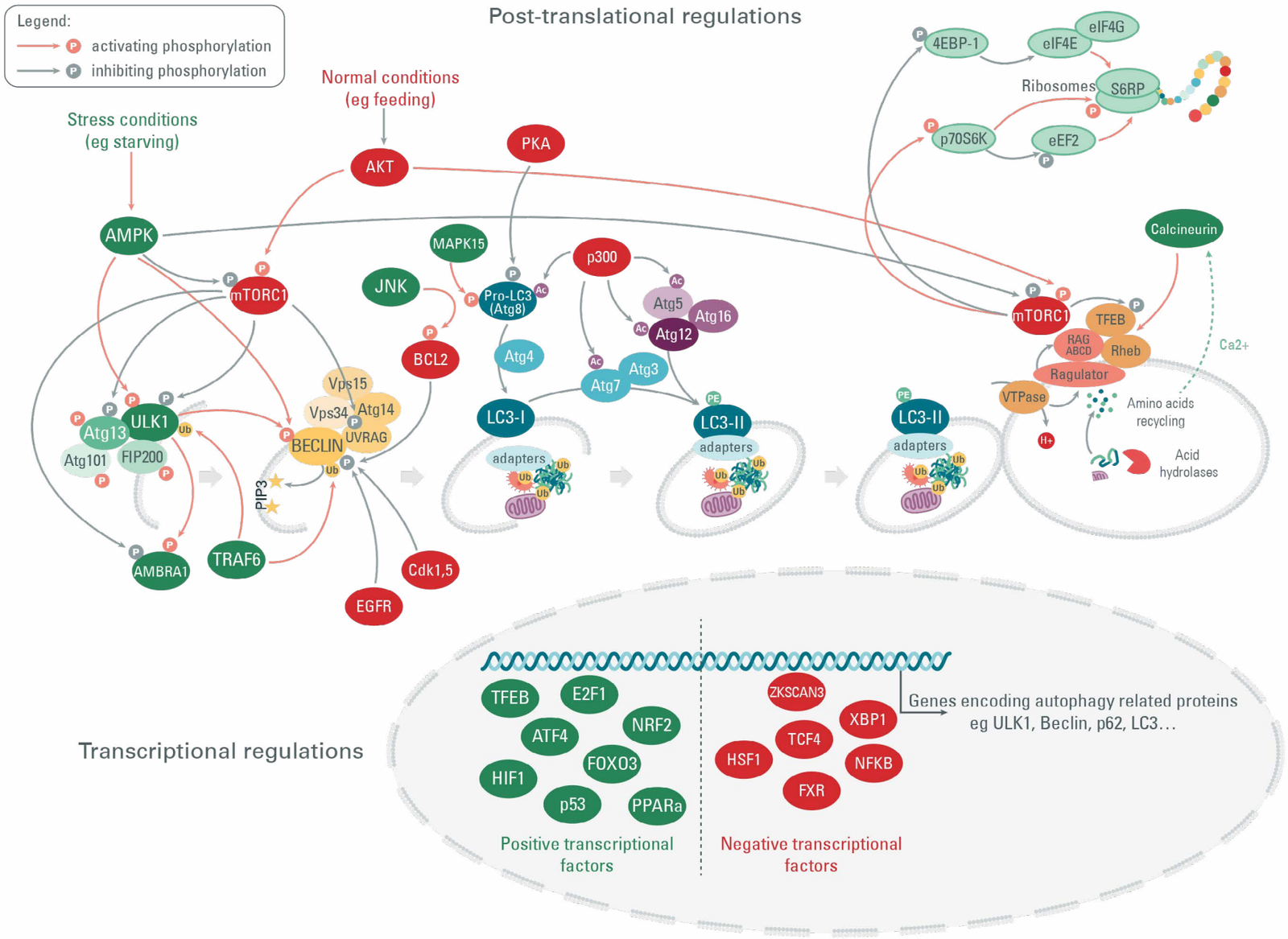


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

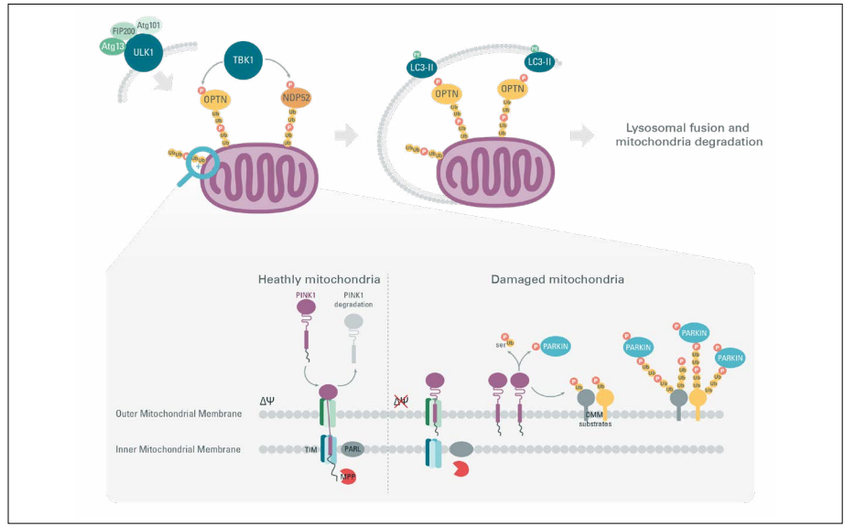
SPOTLIGHT ON MITOPHAGY

Mitochondria are essential organelles that provide cellular energy and contribute to cell death. This organelle is continuously exposed to intra and extra mitochondrial threats. For instance, mitochondrial oxidative phosphorylation produces ATP and byproducts such as reactive oxygen species (ROS) that can cause mitochondrial DNA damage. The removal of damaged mitochondria is critical for maintaining cellular homeostasis, and here mitophagy plays a key role in ensuring a selective control process to maintain mitochondria quality and quantity.

Mitophagy is controlled by two major proteins: PINK1 (PTEN-induced putative kinase 1) which is a serine/threonine-protein kinase, and PARKIN which is an E3-ubiquitin ligase.

PINK1 is addressed to heathy polarized mitochondria through a mitochondrial targeting sequence, and is processed by matrix processing peptidases (MPP) and the PARL protease in the mitochondrial inner membrane. The 52kD mature form of PINK is then released into the cytosol, where it is ubiquitinated and degraded by the proteasome.

In damaged depolarized mitochondria, PINK1 accumulates on the mitochondrial outer membrane at the TOM complex (Translocase of the Outer Membrane). Following its autophosphorylation, activated PINK1 in turn phosphorylates ubiquitin on serine 65 (Ser65) which promotes Parkin stabilization. In an active conformation, PINK1 directly phosphorylates and fully activates Parkin. Once activated, Parkin ubiquitinates many targets at the Mitochondrial Outer Membrane (MOM), such as mitofusin (MNF), VDAC, or the pro-apoptotic factor BAK, as well as cytosolic proteins. Polyubiquitinated mitochondrial substrates bind to LC3 adapters, such as OPTN or NDP52, which are phosphorylated by TBK1. Finally, damaged mitochondria are trapped in autophagosomes and further degraded by lysosomal enzymes [48] [49] [50].



Click to Enlarge

TABLE OF CONTENTS

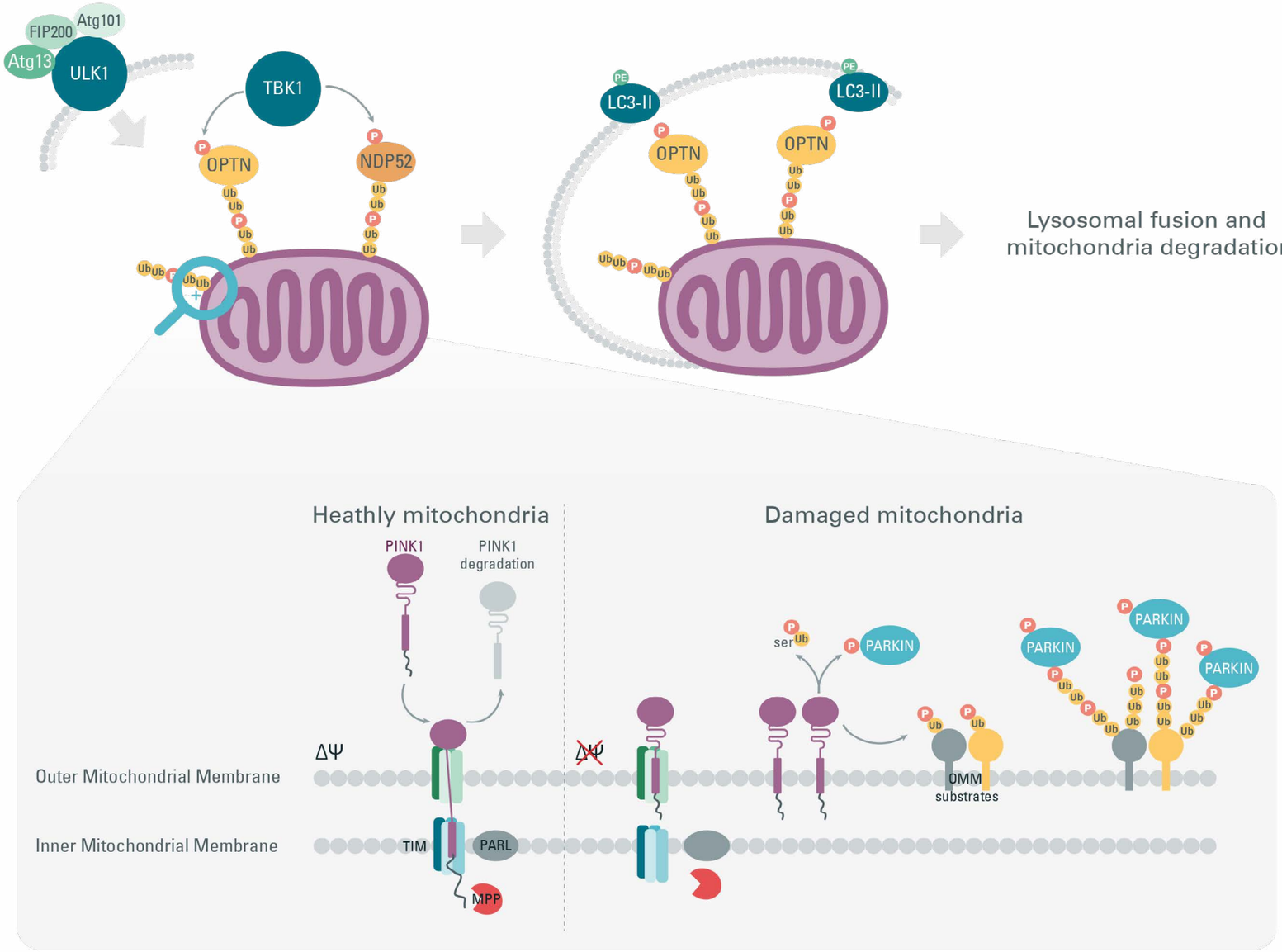


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

AUTOPHAGY DYSREGULATION IN PATHOLOGICAL DISORDERS

Not surprisingly, defective autophagy mechanisms have been associated with human diseases.

Mutations in ATG5 genes have been associated with autoimmune disease susceptibility, for example systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and multiple myeloma. ATG5 is involved in the activation of innate and adaptive immune responses by regulating antigen presentation, NF-κB signaling, and cytokine production. ATG5 mutants are likely to contribute to SLE onset by perturbing antigen presentation and cytokine over-production [51] [53].

Deletion of the Beclin encoding gene is associated with breast, ovarian, prostate, and colorectal cancers. It is likely that the role of autophagy varies along with cancer progression, being protective at early stages but harmful in advanced cancer stages [52] [54] [55] [56].

Dysregulated autophagy is also involved in metabolic syndromes, obesity, and diabetes, as well as vascular diseases [53] [54] [55] [56].

Lysosomal storage disorders or LSDs are a family of about 50 diseases including Gaucher or Niemann Pick diseases, caused by gene mutations that impair lysosomal functions. In most LSDs the fusion of autophagosome with lysosome is defective, leading to an accumulation of ubiquitinated proteins, damaged organelles such as mitochondria, and autophagy proteins such as SQSTM1/p62 [53] [54].

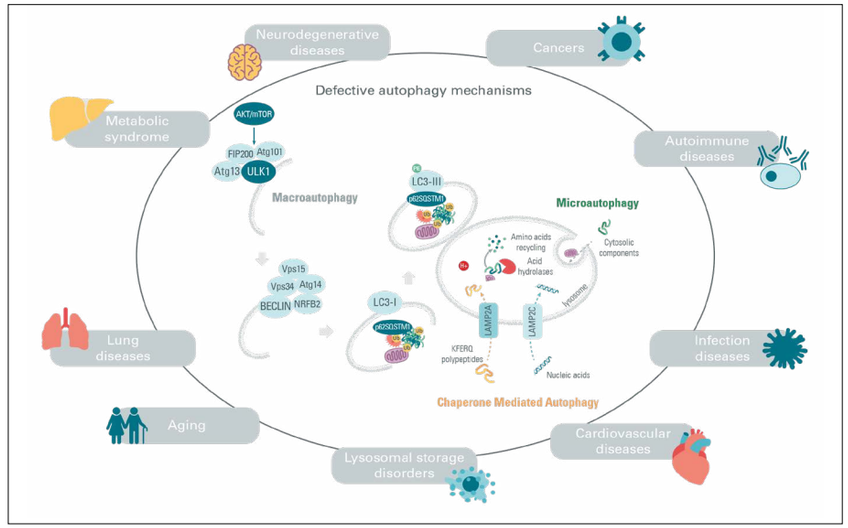
Dysfunctions in autophagy mechanisms are a hallmark of neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's Diseases, and results in the accumulation of abnormal proteins and damaged organelles. For instance, mutations in a-synuclein, a protein involved in PD pathogenesis, impair its degradation by inhibiting the Chaperone Mediated Autophagy (through high affinity binding to LAMP2A) and autophagy pathways, resulting in the accumulation of toxic a-synuclein aggregates in the cytoplasm [55] [56] [57] [58].

TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

Mitochondrial dysfunctions which encompass impaired mitochondrial biogenesis, dynamics, and trafficking, Ca2+ imbalance, and oxidative stress, as well as mitophagy, are also associated with neurodegenerative disorders. More particularly, losses of function mutations in genes encoding PINK1 or Parkin are associated with an autosomal recessive form of PD, where mitochondrial biology is compromised due to impaired mitophagy and subsequent mitochondria degradation, and impaired mitochondria morphology and trafficking. Besides their implication in defective autophagy, a-synuclein mutants contribute mitochondrial defects by disturbing the Ca2+ balance, decreasing energy production, and downregulating mitochondria biogenesis. In PD, the LRRK2 G2019S mutant also contributes to defective mitophagy by interfering with mitochondrial dynamics and trafficking [57] [59].



Click to Enlarge

TABLE OF CONTENTS

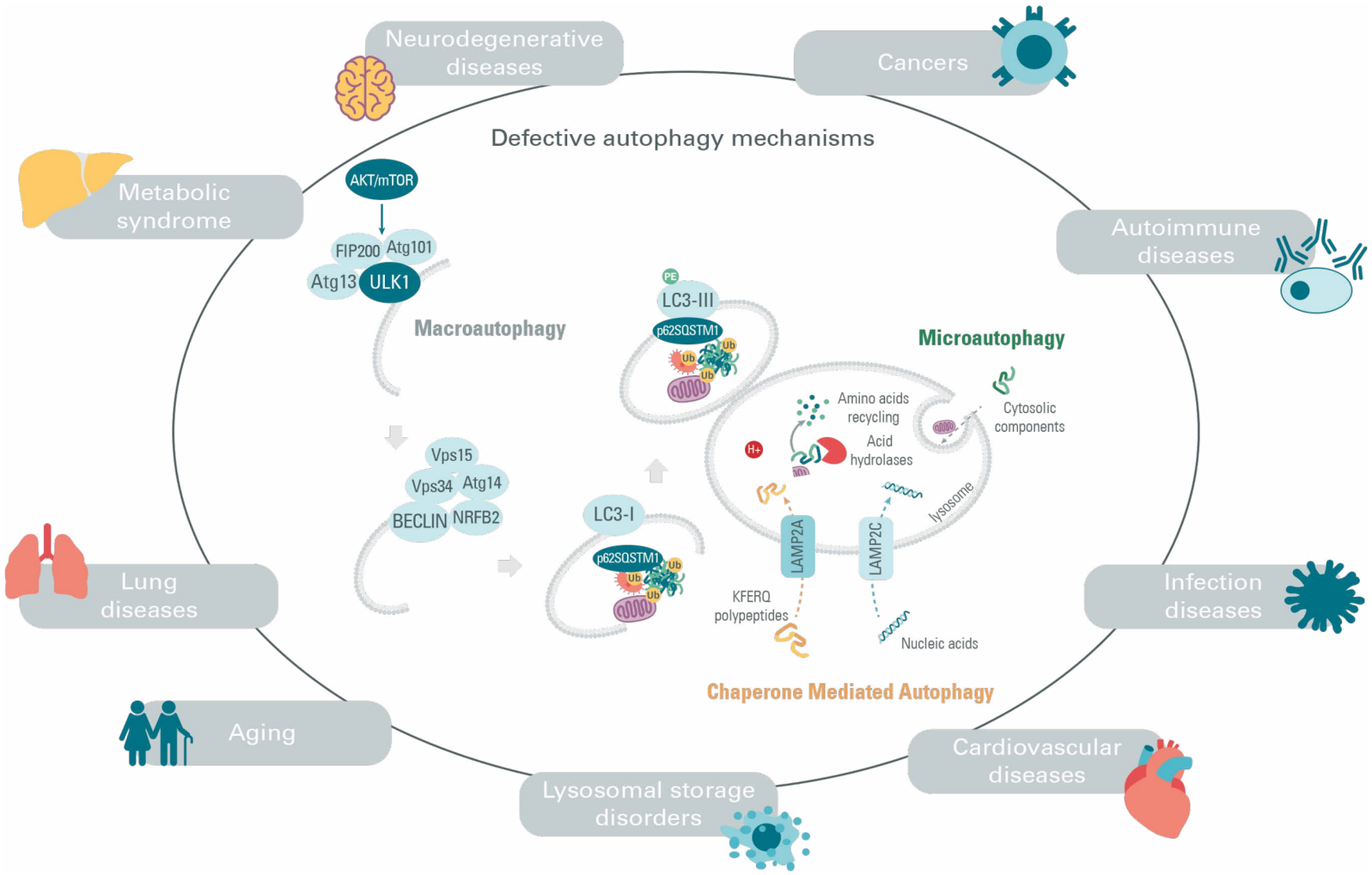


TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

THE UPS-ALS CONNECTION

Cellular protein homeostasis is maintained by two major degradation pathways: UPS and autophagy. Even if both systems recognize their targets through their ubiquitin tags, they had been viewed as two independent machineries with different components, action mechanisms, and substrate selectivity. However, recent studies have indicated the presence of overlaps and interconnections between the UPS and autophagy, suggesting that cells operate in a single coordinated proteolytic network to maintain proteostasis under fluctuating environments [60].

MUTUAL REGULATION THROUGH PROTEOLYSIS

Components of autophagy are regulated through degradation by the UPS and vice versa. The UPS modulates the half-life of various autophagy proteins, such as LC3 and Beclin1, to control cellular autophagic activity. On the other hand, the UPS is regulated via a specific form of selective autophagy called proteaphagy, corresponding to the lysosomal degradation of whole proteasomes. Nutrient starvation or an accumulation of proteins activates this process which is mediated by “proteaphagy receptors”, such as p62SQSTM1 [60, 61].

COMPENSATORY MECHANISMS

In order to maintain homeostasis, compensation mechanisms exist between the UPS and autophagy. Inhibition of one system leads to a compensatory upregulation of the other. This means that proteins which accumulate following inhibition of one degradation pathway are cleared by the other.

For example, inhibition of the UPS by the proteasome inhibitors MG132 and Bortezomib results in an increase in the autophagy proteins Beclin1/LC3 and ATG5/ATG7 respectively, leading to autophagy upregulation. It has been shown that proteasomal inhibition is sensed by AMPK and mTORC1, two key regulators of autophagy. Conversely, several studies based on the chemical inhibition of autophagy or the knock down of ATG genes have demonstrated that impaired autophagy correlates with the upregulation of the UPS. Nevertheless, in some cases compensation does not always function, since autophagy impairment can also correlate with UPS defects and vice versa. The success of this compensatory mechanism largely depends on the cell types, cellular and environmental conditions, and target protein load [60, 62].

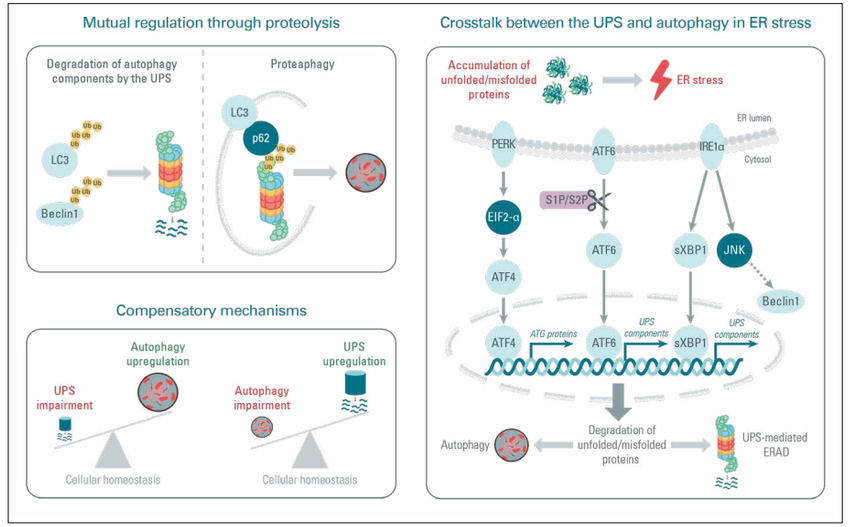
TABLE OF CONTENTS

GENERAL KNOWLEDGE

The autophagy-lysosomal system

CROSSTALK DURING ER STRESS

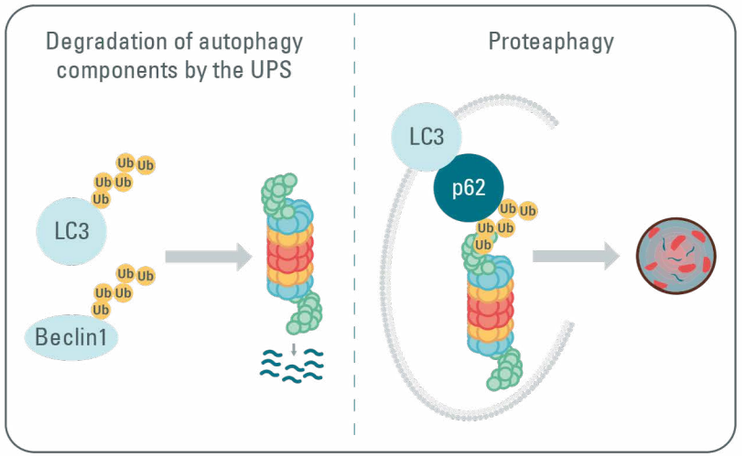
ER stress is a well-known example of a condition under which both the UPS and autophagy are activated by the unfolded protein response (UPR) and the ERAD process. Upon ER stress caused by an accumulation of unfolded/misfolded proteins, the ER transmembrane proteins PERK, ATF6, and IRE1α are activated. PERK phosphorylates EIF2α which in turn activates the transcription factor ATF4, leading to the expression of ATG proteins. ATF6 is processed in the Golgi by the proteases S1P and S2P, leading to its translocation into the nucleus and the transcription of UPS components involved in ERAD. IRE1α induces the expression of the active spliced form of XBP1 (sXBP1) which also triggers the expression of UPS proteins for ERAD. In parallel, IRE1α activates JNK, leading to the activation of the autophagy protein Beclin1. These pathways induce the activation of the UPS and autophagy, which act in a complementary manner to remove improperly folded proteins and restore ER function [60, 63].



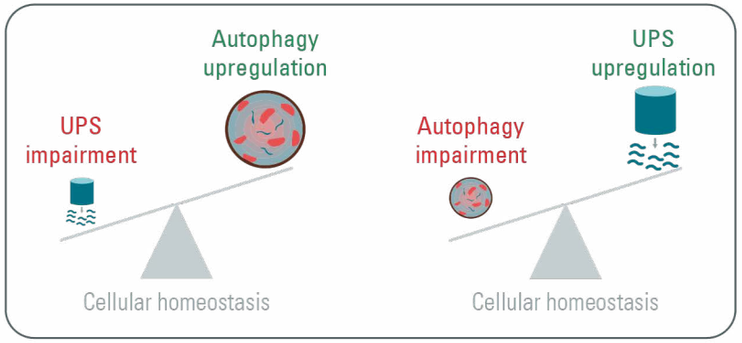
Click to Enlarge

TABLE OF CONTENTS

Mutual regulation through proteolysis



Compensatory mechanisms



Crosstalk between the UPS and autophagy in ER stress

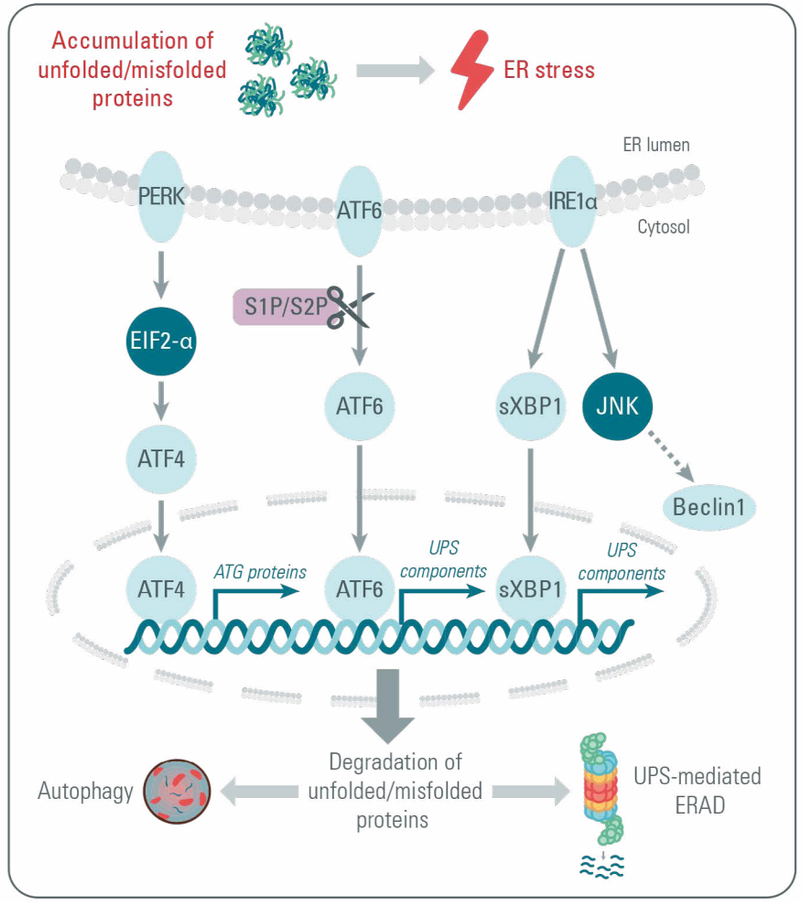


TABLE OF CONTENTS

THERAPEUTIC STRATEGIES

Targeting the ubiquitin proteasome system

TARGETING THE PROTEASOME COMPONENTS

For decades the well-known proteasome inhibitor MG-132 has been used as a research tool to unravel the molecular basics underlying proteasome mediated protein degradation, and nowadays drugs targeting proteasome components are part of the medicinal arsenal to fight against diseases. Defects in the Ubiquitin Proteasome System have been associated with neurodegenerative disorders, autoimmune diseases, and cancers. Given this new knowledge, bortezomid (BTZ) was the first proteasome inhibitor approved by the FDA in 2003 to treat Multiple Myeloma and other hematological malignancies. By binding to the 26S proteasome β5-subunit, BTZ blocks the chymotrypsin-like activity of the proteasome. BTZ exerts a cytotoxic effect on cancer cells, mainly through p53 induced apoptosis. However, the emergence of BTZ resistance led to the development of second-generation proteasome inhibitors such as carfilzomib or ixazomib. Since the immunoproteasome is abundantly expressed in lymphoid and hematopoietic cells, drugs that specifically target the immunoproteasome are also being investigated, especially for the treatment of hematological cancers and autoimmune diseases. Whereas inhibiting proteasomes is effective in the treatment of some diseases, enhancing their activity represents a strategy for other pathologies in which proteins accumulate and / or aggregate, like in aging associated diseases, neurodegenerative diseases, or cancers. For example, agonists of the 20S proteasome such as chlorpromazine or MK-886 have been shown to induce the degradation of α-synuclein, a protein involved in Parkinson's Disease [64] [65] [66].

TARGETING E1 AND E2 UBIQUITIN CONJUGATING ENZYMES, E3 LIGASES, AND DEUBIQUITINATING ENZYMES (DUBS)

UBA1 is the main E1 ubiquitin activating enzyme which binds ATP and forms a covalent bond between E1 and ubiquitin. Inhibition of ubiquitin activation can be achieved by inhibitors such as Pyr-41, NSC624206, JS-K, and PPZD-4409. Other inhibitors such as Bay 11-7821, CC 0651, or NSC 697923 have been shown to inhibit E2 ubiquitin conjugating enzymes, for example hCdc34 or UBE2N [67] [68].

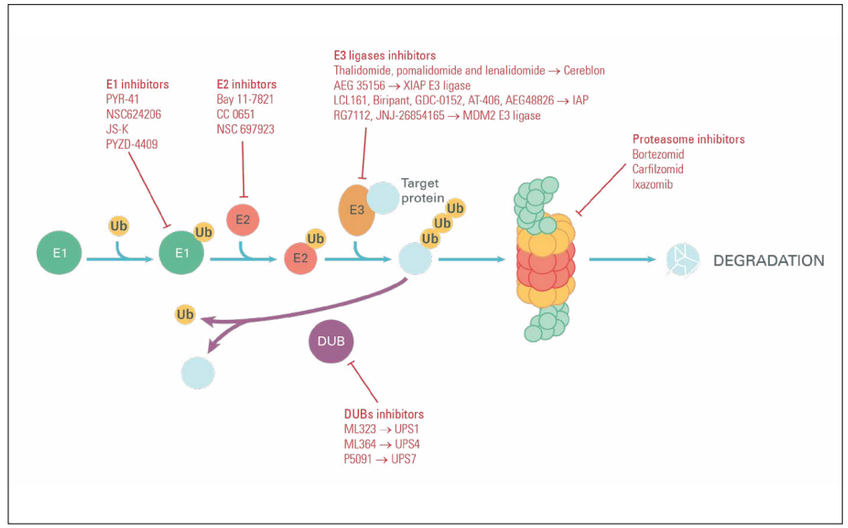
TABLE OF CONTENTS

THERAPEUTIC STRATEGIES

Targeting the ubiquitin proteasome system

Unlike proteasome inhibitors or activators which lack specificity, E3 ubiquitin ligases are substrate-specific and therefore represent attractive targets enabling gains in specificity and reduced side effects. The E3 ligase Cereblon activity is inhibited by Immunomodulatory drugs (IMIDs) such as thalidomide, pomalidomide, and lenalidomide, all approved for the treatment of multiple myeloma. Other small molecules modulating the XIAP E3 ligase (AEG 35156), IAP (LCL161, Biripant, GDC-0152, AT-406, AEG48826) and the MDM2 E3 ligase (RG7112, JNJ-26854165) are in clinical trials, whereas the development of compounds against βTrCP, VHL, or Parkin E3 ligases are less advanced in drug discovery [67] [68].

Deubiquitinating enzymes (DUBs) play the opposite role towards E3 ligases and remove ubiquitin moieties from proteins, thereby maintaining the equilibrium between ubiquitination and deubiquitination. Around 100 DUBs have been identified so far. They are involved in various biological processes, such as DNA damage response, and cell proliferation or apoptosis. Since DUBs can be overexpressed or mutated in some cancers, this class of proteases is being considered for cancer treatment. Different more or less specific inhibitors have been reported, such as ML323 for UPS1, ML364 for UPS4, or P5091 for UPS7 [67] [68].



Click to Enlarge

TABLE OF CONTENTS

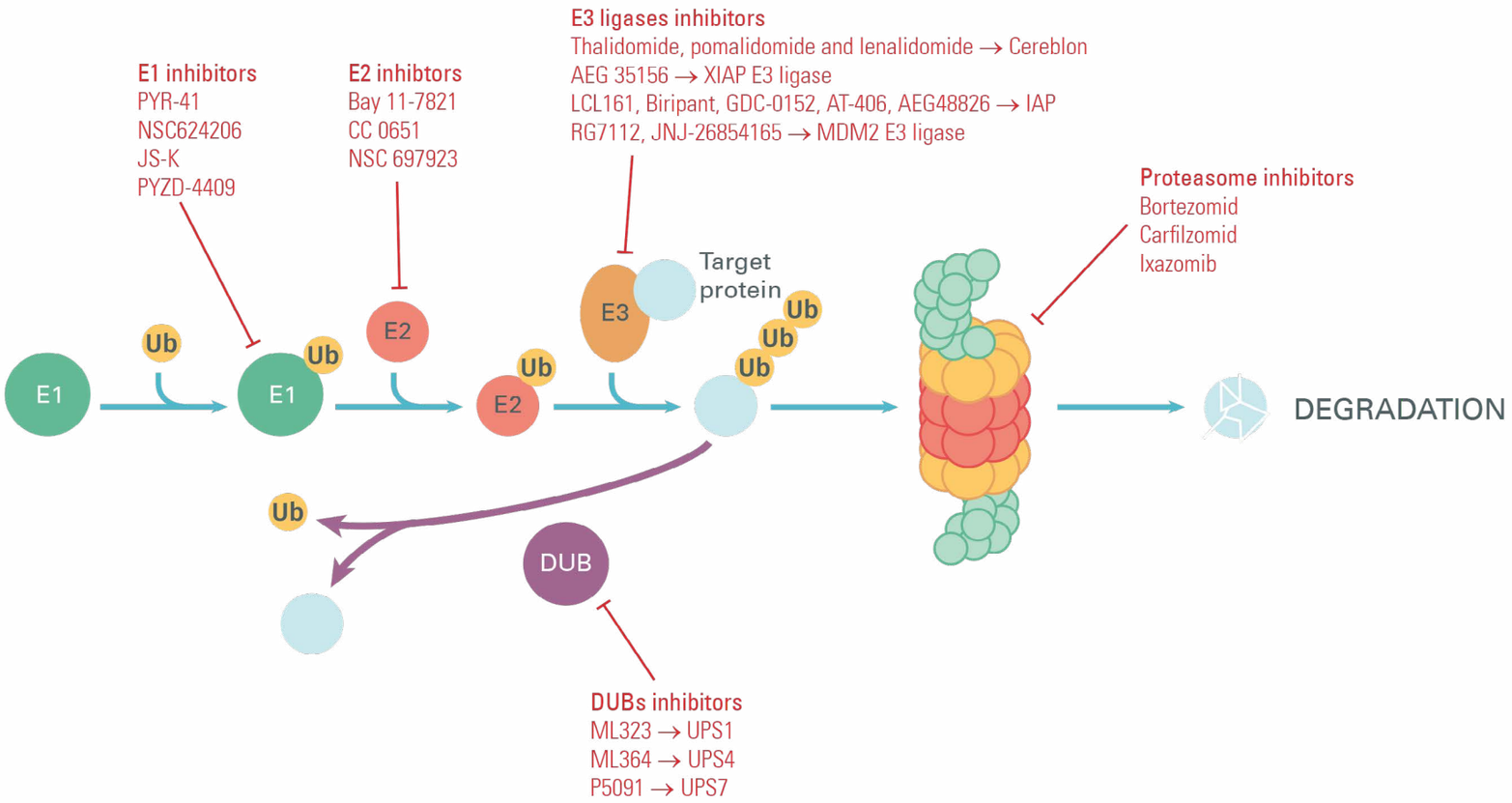


TABLE OF CONTENTS

THERAPEUTIC STRATEGIES

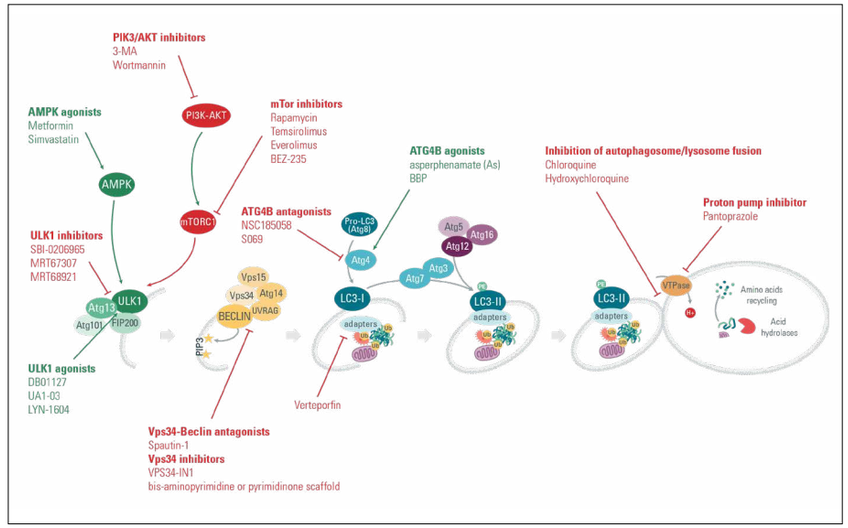
Targeting the ubiquitin proteasome system

TARGETING THE AUTOPHAGY LYSOSOMAL SYSTEM MACROPHAGES

Autophagy impairment is associated with several disorders such as autoimmune diseases, infection, neurodegeneration, and cardiovascular disorders. In cancer, autophagy is likely to play a protective or harmful role depending on the type and stage of the cancer. Thus, enhancing or inhibiting autophagy mechanisms are currently under investigation. Since autophagy is tightly regulated through the interplay of several molecular pathways such as MTORC1, AMPK, or MAPK, their modulation represents various interesting approaches.

Among autophagy inducer drugs, mTOR Inhibitors such as Rapamycin (Sirolimus) and its analogues (Temsirolimus, Everolimus) have shown positive effects on multiple sclerosis, and on breast and gastric cancers. BEZ-235 (Dactolisib) is a selective and reversible dual inhibitor of PI3K/mTOR which has shown anti-tumor activity. AMPK activators such as Metformin and Simvastatin are known to have anti-cancer properties. Carbamazepine is a MAPK activator enhancing autophagy by increasing inositol-triphosphate level [69] [70].

Among autophagy inhibitors, chloroquine and its derivative hydroxychloroquine block the autophagic flux and prevent autophagosome and lysosome fusion. Their anticancer effect in cancer patients has been demonstrated. The PI3K inhibitors 3-MA and Wortmannin, the ULK1/2 inhibitors SBI-0206965, MRT67307, or MRT68921, the Vsp14/Beclin interaction inhibitor Spautin-1, the catalytic VSP14 inhibitors Vps34, VPS34-IN1 and the bis-aminopyrimidine or pyrimidinone scaffold, as well as the ATG4B inhibitor NSC185058, are all used as autophagy inhibitors. Finally, a p62/SQSTM1 inhibitor called Verteporfin is FDA-approved for the treatment of macular degeneration [69] [70].



Click to Enlarge

TABLE OF CONTENTS

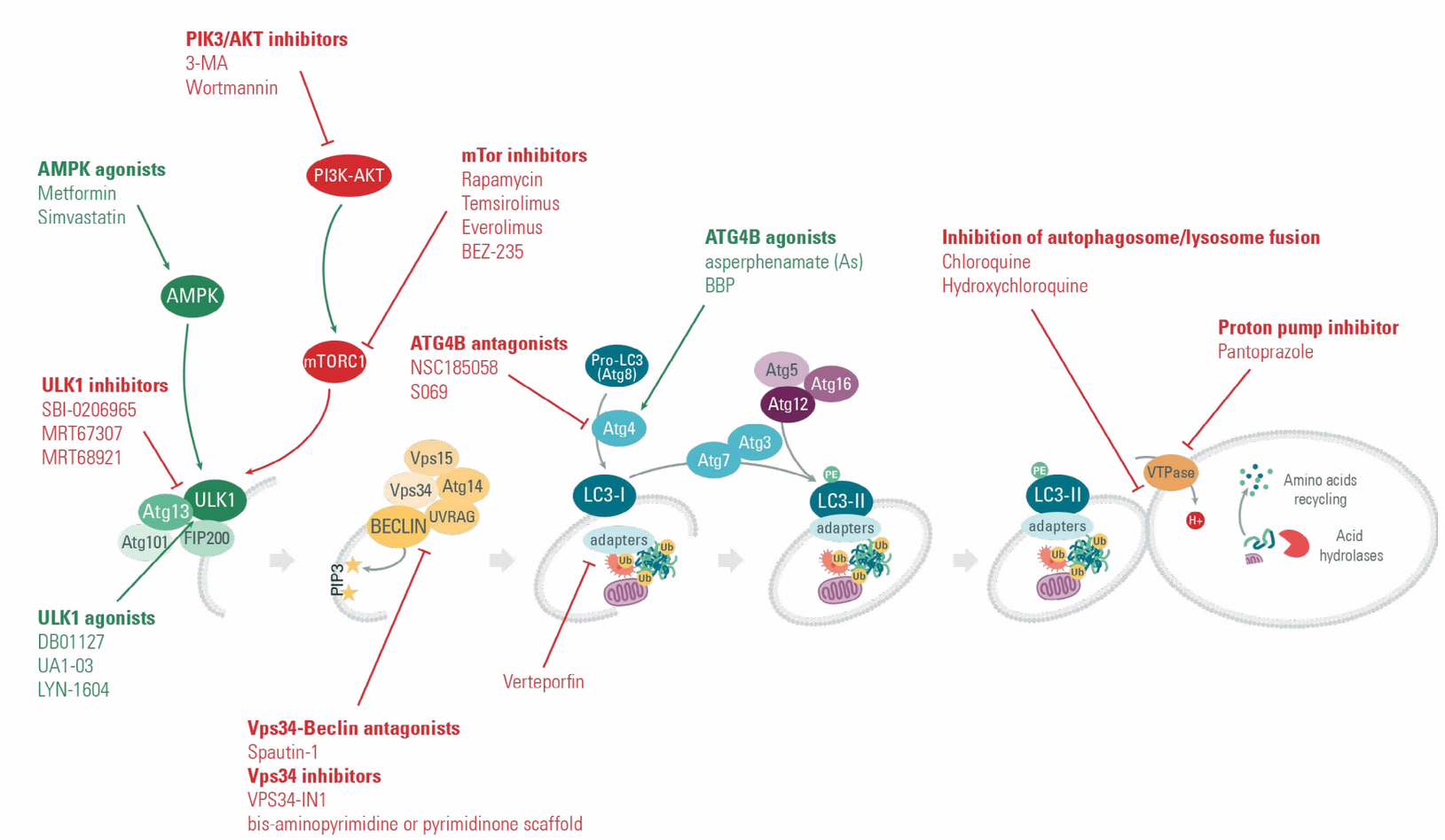


TABLE OF CONTENTS

THERAPEUTIC STRATEGIES

Targeted protein degradation: The PROTAC adventure

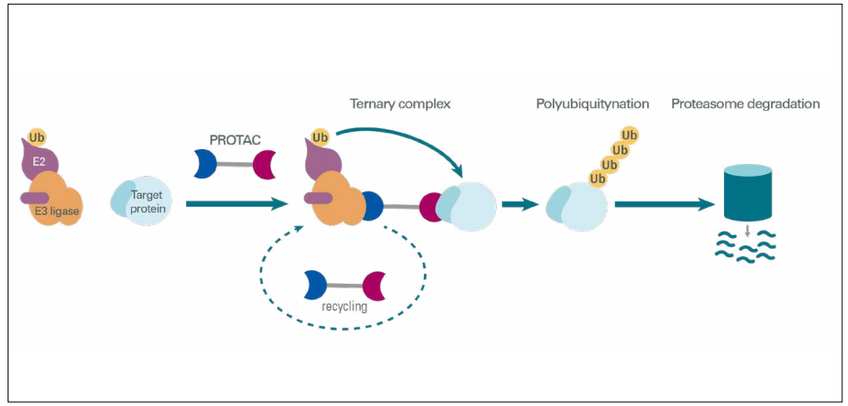
TARGETED PROTEIN DEGRADATION : THE PROTAC ADVENTURE

Taking advantage of proteasome capabilities in promoting protein degradation, Craig Crews and co-workers opened up the way to targeted protein degradation through the PROteolysis TArgeting Chimeras (PROTAC) approach. Described first in the early 2000s, this approach is gaining much more attention as it is expected to overcome mutations and drug resistance, to work at low concentrations due to the catalytic turnover, and to have enhanced target selectivity, thus creating fewer side effects and having better bioavailability compared to conventional monoclonal antibodies.

PROTACs are hetero bifunctional molecules comprising one moiety or ligand that binds to the Protein of Interest (POI), also called a Warhead, and a second ligand that binds to an E3 ubiquitin ligase (E3), plus a linker that bridges the two ligands. Once the POI, the PROTAC compound, and the E3 ligase are in a complex (also called the ternary complex), the POI becomes ubiquitinated via the E3 ligase activity and degraded through the proteasome. Although the first generation of peptide-derived PROTAC compounds were shown to induce efficient degradation of proteins such as FKBP12, their high molecular weight, low potency, and poor cell permeability led to the development of second-generation small molecule-based PROTACs.

The most studied E3 ligases in a PROTAC context are MDM2, XIAP (in this case the degraders are called SNIPER), VHL, and Cereblon (CRBN). For example, MDM2-PROTAC was used to induce androgen receptor degradation, and XIAP-PROTAC was shown to target Estrogen Receptor, while VHL-PROTAC was reported to be effective on RIPK2, BRD4, FLT-3, or ALK. Finally CRBN-PROTACs, which rely on IMIDs (Thalidomide, lenalidomide, or pomalidomide) as CRBN ligands, have been shown to induce the degradation of BRD4, CDK9, BTK, HDAC6, ALK, BCR-ABL, Sirt2, and PI3K [71] [72].

With more than 700 E3 ligases encoded in vertebrate genomes, it can be expected that other E3 ligases will enter the PROTAC field, further expanding PROTAC applicability to virtually any class of proteins, even the undruggable ones, and especially proteins that lack a catalytic site such as transcription factors or scaffolding proteins. Although PROTACs were initially applied to cancer, other diseases such as neurodegenerative, infectious, or cardiovascular diseases and many more may benefit from this approach.



Click to Enlarge

TABLE OF CONTENTS

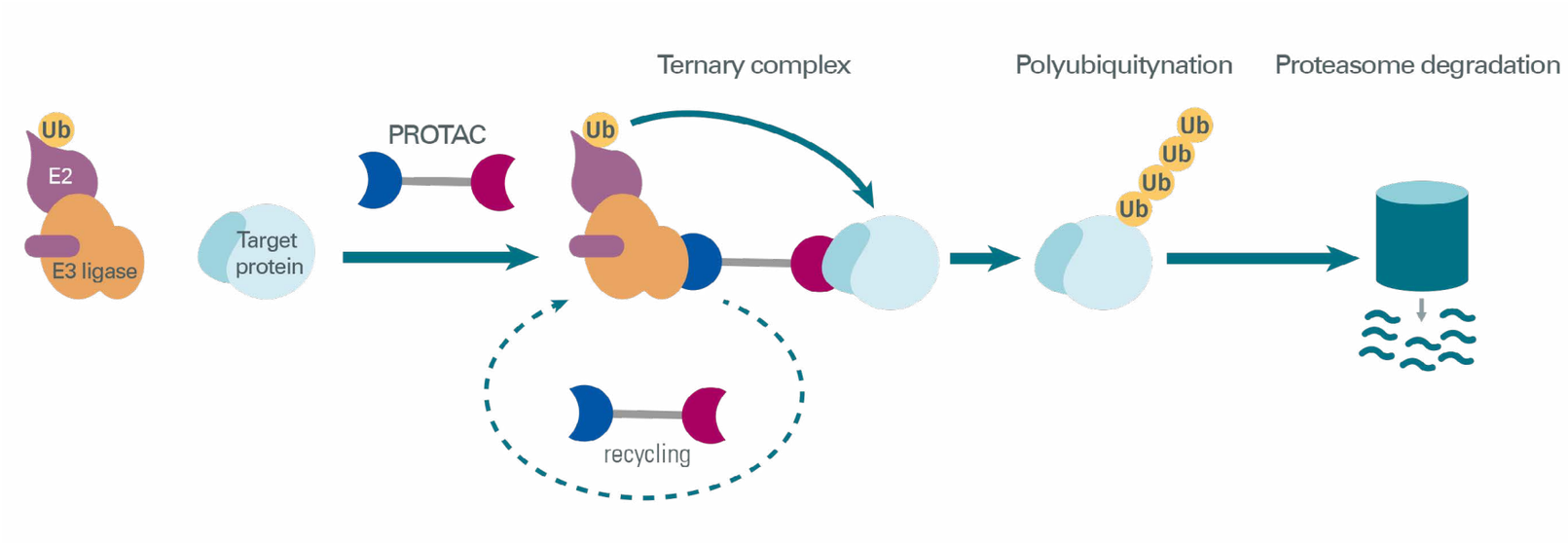


TABLE OF CONTENTS

BIBLIOGRAPHY

1. Swatek, Kirby N., Komander, David. Ubiquitin modifications. Cell Research 2016; 26:399-422.
2. Yau, Richard, Rape, Michael. The increasing complexity of the ubiquitin code. Nature Cell Biology 2016; 18: 579-586.
3. Yao, Tingting; Ndoja, Ada. Regulation of gene expression by the ubiquitin-proteasome system. Semin. Cell Dev Biol. 2012; 23:523-529.
4. Chen, Ruey-Hwa, Chen, Yu-Hsuan, Huang, Tzu-Yu. Ubiquitin-mediated regulation of autophagy. Journal of Biomedical Science. 2019, 26 (80).
5. Zheng, Ning, Shabek, Nitzan. Ubiquitin Ligases: Structure, Function and Regulation. Annu. Rev. Biochem. 2017; 86:129-57.
6. Wilkinson, Keith D. DUBs at a glance. Journal of Cell Science. 2019; 122:2325-2329.
7. Xie Y. Structure, Assembly and Homeostatic Regulation of the 26S Proteasome. Journal of Molecular Cell Biology 2010; 2:308-317
8. Marshall R.S., Vierstra R.D. Dynamic Regulation of the 26S Proteasome: From Synthesis to Degradation. Front. Mol. Biosci. 2019; 6(40)
9. Livneh I., Cohen-Kaplan V., Cohen-Rosenzweig C., Avni N., Ciechanover A. The life cycle of the 26S proteasome: from birth, through regulation and function, and on to its death. Cell Research 2016; 26:869-885
10. Tanaka K., Mizushima T., Saeki Y. The proteasome: molecular machinery and pathophysiological roles. Biol. Chem. 2012; 393:217-234
11. Kors S., Geijtenbeek K., Reits E., Schipper-Krom S. Regulation of Proteasome Activity by (Post-)transcriptional Mechanisms. Front. Mol. Biosci. 2019; 6(48).
12. Morozov A.V., Karpov V.L. Biological consequences of structural and functional proteasome diversity. Heliyon 4 2018; e00894
13. Morozov A.V., Karpov V.L. Proteasomes and Several Aspects of Their Heterogeneity Relevant to Cancer. Front. Oncol. 2019; 9(761)
14. Deshmukh F.K., Yaffe D., Olshina M.A., Ben-Nissan G., Sharon M. The Contribution of the 20S Proteasome to Proteostasis. Biomolecules 2019; 9(190)
15. Motosugi R., Murata S. Dynamic Regulation of Proteasome Expression. Front. Mol. Biosci. 2019; 6(30)
16. Hamazaki J., Murata S. ER-Resident Transcription Factor Nrf1 Regulates Proteasome Expression and Beyond. Int. J. Mol. Sci. 2020; 21(3683)
17. Zhang Y., Manning B.D. mTORC1 signaling activates NRF1 to increase cellular proteasome levels. Cell Cycle 2015; 14(13):2011-2017
18. Tonelli C., Chio I.I.C., Tuveson D.A. Transcriptional Regulation by Nrf2. Antioxid. Redox Signal. 2018; 29:1727-1745
19. Kapuy O., Papp D., Vellai T., Bánhegyi G., Korcsmáros T. Systems-Level Feedbacks of NRF2 Controlling Autophagy upon Oxidative Stress Response. Antioxidants 2018; 7(39)
20. Thibaudeau T.A., Smith D.M. A Practical Review of Proteasome Pharmacology. Pharmacol Rev. 2019; 71:170-197
21. Amm I., Sommer T., Wolf D.H. Protein quality control and elimination of protein waste: The role of the ubiquitin–proteasome system. Biochim. Biophys. Acta. 2014; 1843:182-196
22. Bassermann F., Eichner R., Pagano M. The ubiquitin proteasome system - Implications for cell cycle control and the targeted treatment of cancer. Biochim. Biophys. Acta. 2014; 1843(1)
23. Tauriello D.V.F., Maurice M.M. The various roles of ubiquitin in Wnt pathway regulation. Cell Cycle 2010; 9(18):3700-3709
24. Masoud G.N., Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. Acta Pharmaceutica Sinica B 2015; 5(5):378-389

TABLE OF CONTENTS

BIBLIOGRAPHY

25. Gregory M.A., Hann S.R. c-Myc Proteolysis by the Ubiquitin-Proteasome Pathway: Stabilization of c-Myc in Burkitt's Lymphoma Cells. Mol. Cell. Biol. 2000; 20(7):2423-2435
26. Fennell D.A., Chacko A., Mutti L. BCL-2 family regulation by the 20S proteasome inhibitor bortezomib. Oncogene 2008; 27:1189-1197
27. Xia Y., Wang J., Xu S., Johnson G.L., Hunter T., Lu Z. MEKK1 Mediates the Ubiquitination and Degradation of c-Jun in Response to Osmotic Stress. Mol. Cell. Biol. 2007; 27(2):510-517
28. Liu D., Sheng C., Gao S., Yao C., Li J., Jiang W., Chen H., Wu J., Pan C., Chen S., Huang W. SOCS3 drives proteasomal degradation of TBK1 and negatively regulates antiviral innate immunity. Mol. Cell. Biol. 2015; 35:2400-2413.
29. Dahlmann B. Role of proteasomes in disease. BMC Biochemistry 2007; 8(Suppl 1):S3
30. Thibaudeau T.A., Anderson R.T., Smith D.M. A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. Nature Communications 2018; 9(1097)
31. Jang H.H. Regulation of Protein Degradation by Proteasomes in Cancer. J. Cancer Prev. 2018; 23(4):153-161
32. Abukhdeir A.M., Park B.H. p21 and p27: roles in carcinogenesis and drug resistance. Expert Rev. Mol. Med. 2009; 10(19)
33. Mizushima N, Yoshimori T, Ohsumi Y: The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 2011,27:107-132.
34. Hironori Suzuki, Takuo Osawa, Yuko Fujioka and Nobuo N Noda: Structural biology of the core autophagy machinery Current Opinion in Structural Biology 2017, 43:10–17
35. James H. Hurley and Lindsey N. Young Mechanisms of Autophagy Initiation Annu Rev Biochem. 2017 June 20; 86: 225–244
36. Kevin Moreau, Maurizio Renna and David C. Rubinsztein. Connections between SNAREs and autophagy. Trends in Biochemical Sciences, February 2013, Vol. 38, No. 2
37. Bento CF, Renna M, Ghislat G, Puri C, Ashkenazi A, Vicinanza M, Menzies FM, Rubinsztein DC. 2016. Mammalian autophagy: how does it work? Annual Review of Biochemistry 85:685–713.
38. Ohsumi Y. 2014. Historical landmarks of autophagy research. Cell Research 24:9–23.
39. Settembre C, Fraldi A, Medina D, and Ballabio. A Signals for the lysosome: a control center for cellular clearance and energy metabolism. Nat Rev Mol Cell Biol. 2013 May; 14(5): 283–296.
40. Zhao and Zhang Autophagosome maturation: Machinery and regulation J. Cell Biol. 2019 Vol. 218 No. 3 757–770
41. Wen-wen Li 1 , Jian Li, Jin-ku Bao Microautophagy: lesser-known self-eating Cell Mol Life Sci . 2012 Apr;69(7):1125-36.
42. Susmita Kaushik , Ana Maria Cuervo. The coming of age of chaperone-mediated autophagy Nat Rev Mol Cell Biol 2018 Jun;19(6):365-381.
43. Botti-Millet Anna, Chiara Nascimbeni, Nicolas Dupont, Etienne Morel and Patrice Codogno. Fine-tuning autophagy: from transcriptional to posttranslational regulation. Am J Physiol Cell Physiol 311: C351–C362, 2016.
44. Bach M, Larance M, James DE, Ramm G. The serine/threonine kinase ULK1 is a target of multiple phosphorylation events. Biochem J 440: 283–291, 2011.
45. Chauhan S, Goodwin JG, Chauhan S, Manyam G, Wang J, Kamat AM, Boyd DD. ZKSCAN3 is a master transcriptional repressor of autophagy. Mol 50 :l 50: 16–28, 2013.

TABLE OF CONTENTS

BIBLIOGRAPHY

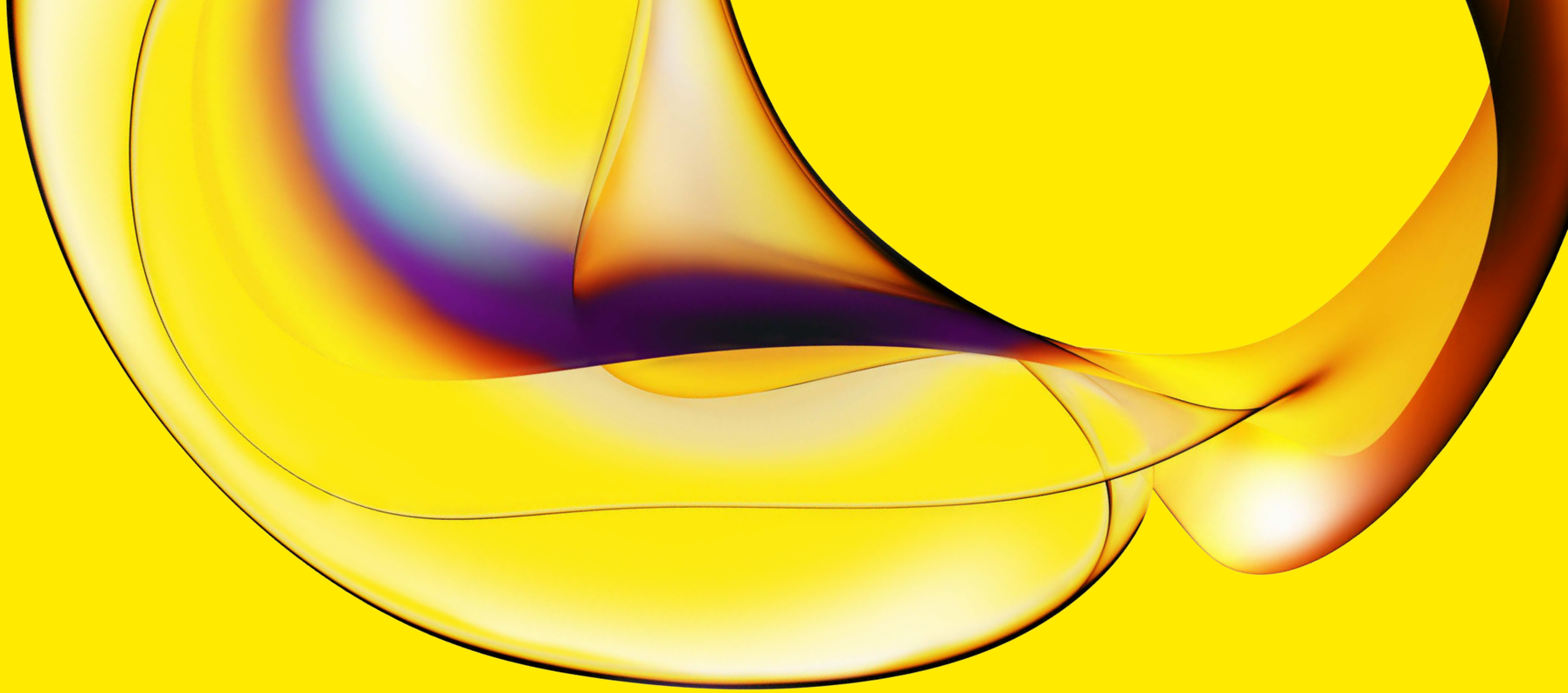
46. Kim YM, Jung CH, Seo M, Kim EK, Park JM, Bae SS, Kim DH. mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. Mol Cell 57: 207–218, 2015.
47. Jens Füllgrabe , Ghita Ghislat , Dong-Hyung Cho , David C Rubinsztein. Transcriptional regulation of mammalian autophagy at a glance. J Cell Sci. 2016 Aug 15; 129(16):3059-66
48. Seung-Min Yoo, and Yong-Keun Jung. A Molecular Approach to Mitophagy and Mitochondrial Dynamics Mol. Cells 2018; 41(1): 18-26
49. Seok Min Jin and Richard J Youle. PINK1- and Parkin mediated mitophagy at a glance
50. The pathways of mitophagy for quality control and clearance of mitochondria. G Ashrafi 1, T L Schwarz Cell Death Differ . 2013 Jan;20(1):31-42
51. Xin Ye, Xu-Jie Zhou and Hong Zhang Exploring the Role of Autophagy-Related Gene 5 (ATG5) Yields Important Insights into Autophagy in Autoimmune/Autoinflammatory Diseases. Front. Immunol. 9:2334.
52. Ravi Amaravadi, Alec C. Kimmelman, and Eileen White. Recent insights into the function of autophagy in cancer. Genes & Development 30:1913–1930
53. Peidu Jiang, Noboru Mizushima. Autophagy and human diseases. Cell Research (2014) 24:69-79.
54. Mizushima N. 2018. A brief history of autophagy from cell biology to physiology and disease. Nature Cell Biology 20:521–527.
55. Dikic I, Elazar Z. 2018. Mechanism and medical implications of mammalian autophagy. Nature Reviews Molecular Cell Biology 19:349–364.
56. Palaniyandi Ravanan, Ida Florance Srikumar, Priti Talwar. Autophagy: The spotlight for cellular stress responses Life Sci . 2017 Nov 1; 188:53-67
57. Yan Wang, Na Liu, Bingwei Lu. Mechanisms and roles of mitophagy in neurodegenerative diseases CNS Neurosci Ther . 2019 Jul;25(7):859-875.
58. Melinda A. Lynch-Day, Kai Mao, Ke Wang, Mantong Zhao, and Daniel J. Klionsky The Role of Autophagy in Parkinson's Disease. Cold Spring Harb Perspect Med 2012 ;2:a009357
59. Jin-Sung Park, Ryan L. Davis, Carolyn M. Sue. Mitochondrial Dysfunction in Parkinson's Disease: New Mechanistic Insights and Therapeutic Perspectives Current Neurology and Neuroscience Reports (2018) 18: 21
60. Kocaturk N.M., Gozuacik D. Crosstalk Between Mammalian Autophagy and the Ubiquitin-Proteasome System. Front. Cell Dev. Biol. 2018; 6(128)
61. Quinet G., Gonzalez-Santamarta M., Louche C., Rodriguez M.S. Mechanisms Regulating the UPS-ALS Crosstalk: The Role of Proteaphagy. Molecules 2020; 25(2352)
62. Nam T., Han J.H, Devkota S., Lee H.W. Emerging Paradigm of Crosstalk between Autophagy and the Ubiquitin-Proteasome System. Mol. Cells 2017; 40(12):897-905
63. Song J.Y., Wang X.G., Zhang Z.Y., Che L., Fan B., Li G.Y. Endoplasmic reticulum stress and the protein degradation system in ophthalmic diseases. PeerJ 2020; 8: e8638
64. Philipp M. Cromm, and Craig M. Crews. The Proteasome in Modern Drug Discovery: Second Life of a Highly Valuable Drug Target ACS Cent. Sci. 2017, 3, 830–838
65. Q. Ping Dou, and Jeffrey A. Zonder. Overview of Proteasome Inhibitor-Based Anti-cancer Therapies: Perspective on Bortezomib and Second-Generation Proteasome Inhibitors versus Future Generation Inhibitors of Ubiquitin-Proteasome System. Curr Cancer Drug Targets. 2014; 14(6): 517–536.
66. Evert Njomen, Jetze J. Tepe*Proteasome Activation as a New Therapeutic Approach to Target Proteotoxic Disorders. Med Chem. 2019 July 25; 62(14): 6469–6481
67. Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin-proteasome system. Nat Rev Drug Discov. 2006; 5:596-613.

TABLE OF CONTENTS

BIBLIOGRAPHY

68. Susanne Lub, Ken Maes, Eline Menu, Elke De Bruyne, Karin Vanderkerken and Els Van Valckenborgh. Novel strategies to target the ubiquitin proteasome system in multiple myeloma. Oncotarget, Vol. 7, No. 6
69. Siyu He, Qi Li, Xueyang Jiang, Xin Lu, Feng Feng, Wei Qu, Yao Chen,and Haopeng Sun. Design of Small Molecule Autophagy Modulators: A Promising Druggable Strategy. J. Med. Chem. 2018, 61, 4656−4687
70. Allison S. Limpert, Lester J. Lambert, [...], and Nicholas D. P. CosfordAutophagy in Cancer: Regulation by Small Molecules. Trends Pharmacol Sci. 2018 December; 39(12): 1021–1032
71. Xiuyun Sun, Hongying Gao, Yiqing Yang, Ming He, Yue Wu, Yugang Song, Yan Tong and Yu Rao. PROTACs: great opportunities for academia and industry (pas de date, de journal?)
72. Haixiang Pei, Yangrui Peng, Qiuhua Zhao and Yihua Chen. Small molecule PROTACs: an emerging technology for targeted therapy in drug discovery. RSC Adv., 2019, 9, 16967

TABLE OF CONTENTS



www.revvity.com

Revvity, Inc.
940 Winter Street, Waltham, MA 02451 USA
(800) 762-4000 | www.revvity.com

For a complete listing of our global offices, visit www.revvity.com

322467 (214977)