F. The ubiquitin proteasome system structure and function

I. Introduction

Intracellular protein degradation is a fundamental process that occurs in all organisms, from bacteria to humans. This degradation is necessary to regulate the intracellular concentrations of enzymes that control all metabolic reactions, as well as the overall content of all other proteins, in response to physiological changes. In general, all intracellular proteins are degraded and their half-lives are variable (from a few minutes to more than 60 hours) depending on the proteins in question within the cell.

Today, three major proteolytic systems have been described:

- The degradation of extracellular proteins within a vacuolar system rich in proteases (endosome/lysosome). In this specific case, the proteins targeted for degradation enter the cell by endocytosis and are degraded in this vacuole after fusion with a lysosome, which is an acidic compartment containing numerous acid hydrolases (cathepsin-type proteases, lipases, etc.).
- Autophagy, which allows cytoplasmic components to be degraded via lysosomes.
- The ubiquitin-proteasome system (UPS), which is a selective proteolytic system in which the conjugation of ubiquitin molecules to the substrate allows the ubiquitinated protein to be directed to the proteasome for degradation. The ubiquitin-proteasome system accounts for approximately 75% of cellular proteolysis.

II. Ubiquitin and the ubiquitylation enzyme cascade

Ubiquitination is a post-translational modification that involves covalently binding ubiquitin (a protein consisting of 76 amino acids) to its substrate. This modification begins with the formation of an isopeptide bond between the C-terminal end of ubiquitin and (usually) a lysine residue of the substrate to be ubiquitinated. Ubiquitin has seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) that can themselves be ubiquitinated, thus forming a chain of ubiquitin chains linked together by isopeptide bonds. An eighth type of chain can be generated when ubiquitin is attached to the N-terminal end of another ubiquitin, thus creating Met1 chains.

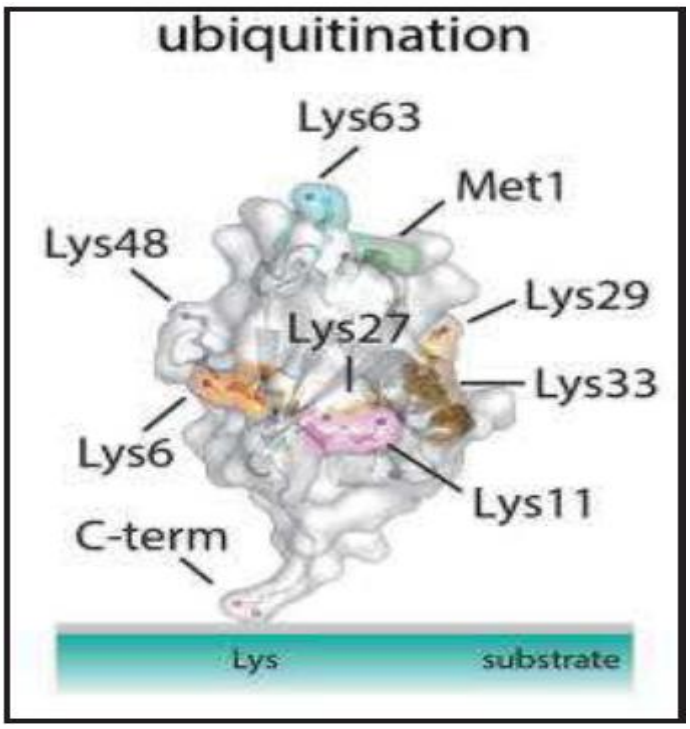

Figure III. 27. 3D representation of ubiquitin-tagged residues of ubiquitin **[37].**

The attachment of a ubiquitin molecule to a substrate requires the consecutive action of three enzymes:

- The first is the ubiquitin-activating enzyme, or E1, which performs ATP-dependent activation of ubiquitin by forming a high-energy thiol-ester bond between the C-terminal glycine of ubiquitin (via the COOH carried by its α carbon) and the thiol group (SH) group of a cysteine in E1;
- The second is a ubiquitin conjugating enzyme, or E2, to which activated ubiquitin is transferred at the thiol group of its active cysteine;
- Finally, the third is E3, called ubiquitin ligase. It promotes the transfer of ubiquitin from E2 to the substrate by forming an amide bond between the COOH group of the C-terminal glycine of ubiquitin and the ε-amine group of a lysine in the substrate, thus forming an isopeptide bond.

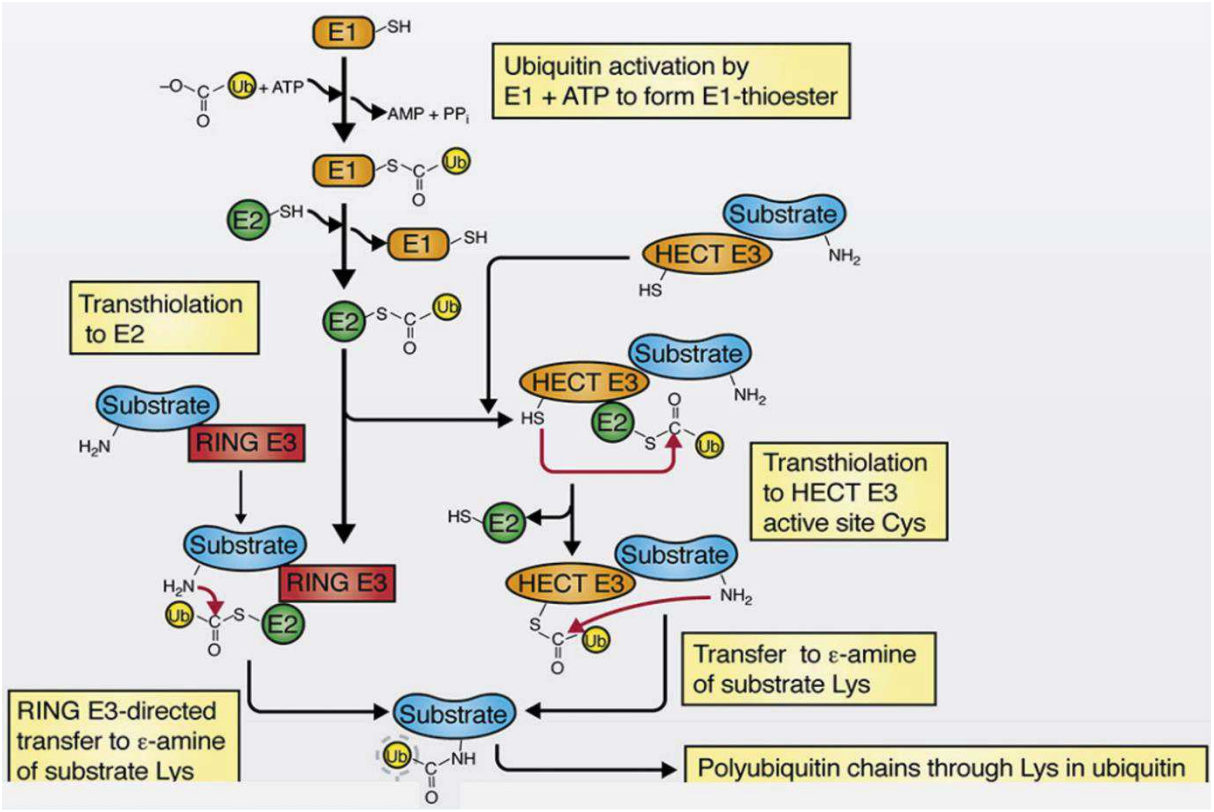

Figure III.28. The ubiquitylation cascade **[37].**

III. Proteasome and protein degradation

The 26S proteasome is the proteolytic component of the UPS involved in the degradation of target proteins, which are most often polyubiquitinated. The 26S proteasome is an enzyme complex with a very high molecular weight (2500 kDa) composed of around sixty proteins. It is a large cylindrical structure located in both the cytoplasm and the nucleus and has several proteolytic activities. It has three main parts: the 20S proteasome, which forms the core and catalytic subunit of the proteasome, and two caps, the 19S proteasome, which is considered the regulatory subunit.

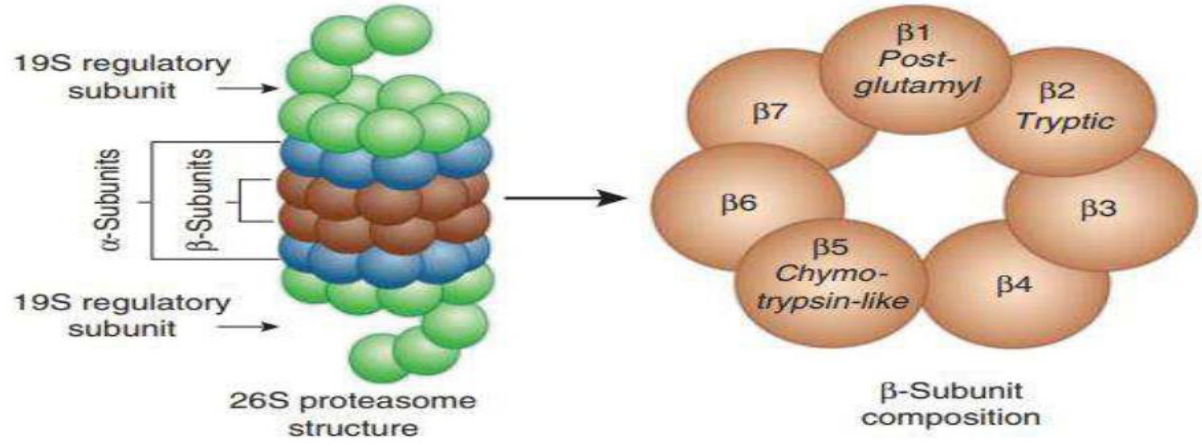

Figure III.29. Structure of the 26S proteasome and the β ring **[37].**

III.1. Structure and function of the 20S proteasome

The 20S proteasome structure is conserved from bacteria to mammals, with only its subunit composition varying. It is composed of four rings, each consisting of seven protein subunits forming a cavity 5 nm in diameter. The two central rings are each composed of seven different β subunits of approximately 35 kDa (from β1 to β7) that contain proteolytic activity. Two α rings composed of 7 different α subunits of approximately 25 kDa (α1 to α7) are located on either side of the β rings to control access to the catalytic cavity. Access to the catalytic chamber has determined the formation of a pore within the α rings, the opening of which is controlled by the 19S proteasome.

The catalytic chamber, formed by the two β rings, has three distinct proteolytic activities carried out by specific β subunits:

- A caspase-like activity (cleavage after acidic residues) via the β1 subunit,
- A trypsin-like activity (cleavage after basic residues) via the β2 subunit,
- A chymotrypsin-like activity (cleavage after hydrophobic residues) via the β5 subunit.

Like the α subunits, the four other β subunits have no catalytic activity.

III.2. Structure and function of the 19S proteasome

The 19S proteasome, also known as the cap, is a 900 kDa complex composed of around twenty proteins. It consists of a base of 10 subunits, six of which have ATPase activity directly linked to the α ring of the 20S proteasome, and a cap composed of nine non-ATPase subunits. The 19S proteasome can be located at one or both ends of the 20S proteasome. It is the regulatory part of the proteasome and performs four main functions, some of which require energy.

- Activation of the 20S proteasome (opening of the pore in the α ring),
- Recognition of polyubiquitinated substrates,
- Deubiquitination of substrates (ensures recycling of ubiquitin),
- Unfolding and translocation of the substrate to the 20S proteasome.

IV. Functioning of the ubiquitin proteasome system

In general, the ubiquitin proteasome system breaks down target substrates (proteins) in two main steps:

- Substrate ubiquitination: thanks to the concerted action of three enzymes (E1, E2, and E3), a polyubiquitin chain is covalently attached to one or more lysine residues of the substrate.
- Once ubiquitinated, the substrate is directed to and degraded by the 26S proteasome. Degradation of ubiquitinylated proteins by the 26S proteasome can occur in the cytoplasm and nucleus and is ATP-dependent.

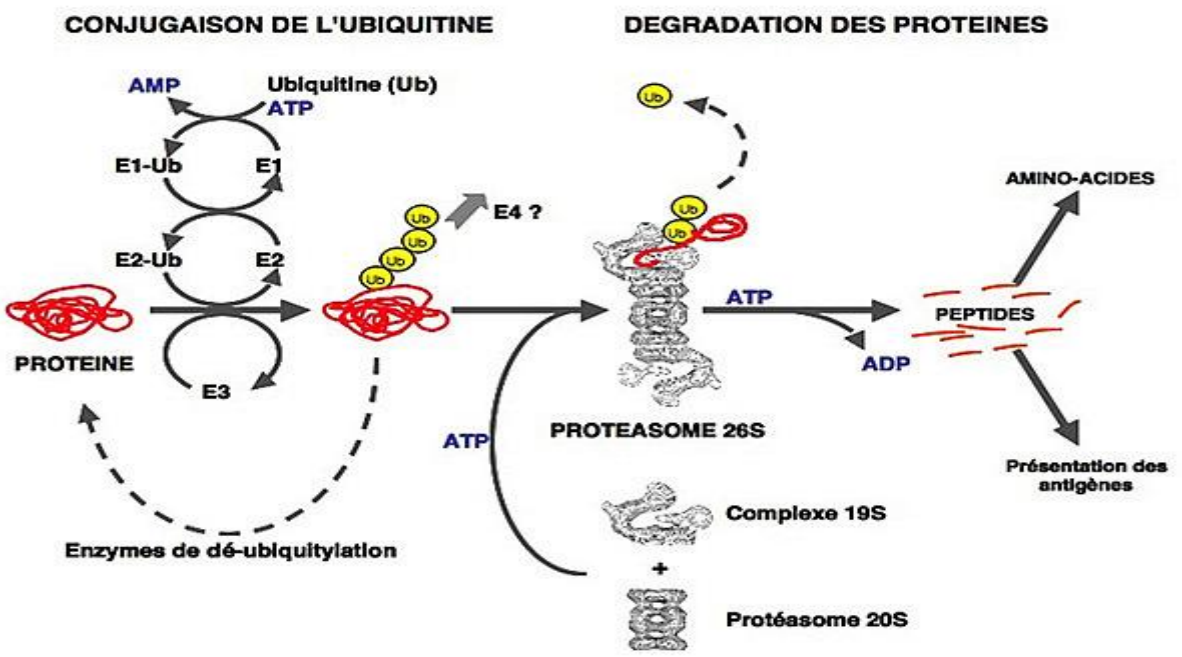

Figure III.30. General mechanism of action of the ubiquitin proteasome system **[38].**

The degradation process of a polyubiquitinated substrate can be simplified into a few key steps:

- Activation of the 20S proteasome (opening of the α ring),
- Recognition of the substrate or ubiquitinated protein by ubiquitin receptors (cap subunits: Rpn10 and Rpn13),
- Partial ATP-dependent substrate binding,
- Substrate deubiquitination,
- Unfolding and translocation of the substrate to the catalytic chamber,
- Proteolysis via the catalytic β subunits,
- Release of cleavage fragments (3 to 25 residues) which will be hydrolyzed by intracellular peptidases,
- Recycling of amino acids for the biosynthesis of new proteins.