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The ubiquitin-proteasome system and autophagy: Coordinated and independent activities

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ABSTRACT

The living cell is an ever changing, responsive, and adaptive environment where proteins play key roles in all processes and functions. While the scientific community focused for a long time on the decoding of the information required for protein synthesis, little attention was paid to the mechanisms by which proteins are removed from the cell. We now realize that the timely and proper activity of proteins is regulated to a large extent by their degradation; that cellular coping with different physiological cues and stress conditions depends on different catabolic pathways; and that many pathological states result from improper protein breakdown.

There are two major protein degradation systems in all eukaryotic cells—the ubiquitin- proteasome and the autophagy-lysosome. The two systems are highly regulated, and—via degradation of a broad array of proteins—are responsible for maintenance of protein homeostasis and adaptation to environmental changes. Each is comprised of numerous components responsible for its coordinated function, and together they encompass a considerable fraction of the entire genome.

In this review, we shall discuss the common and diverse characteristics of the ubiquitin-proteasome system (UPS) and autophagy—their substructure, mechanisms of action, function and concerted regulation under varying pathophysiological conditions.

1. Introduction

1.1. *The ubiquitin-proteasome and autophagosome–lysosome systems*

Although intracellular protein breakdown by lysosomes was described more than half a century ago, our understanding of the underlying molecular and cellular mechanisms, and mostly the regulation of the process, has been advanced significantly much later with the unraveling of the autophagosome-lysosome system. The UPS is recognized as a major highly specific and selective route for cellular protein degradation, whereas autophagy is involved mostly in bulk destruction in response to stress. It is now realized that both systems act simultaneously and in conjunction with one another, and play important roles in the maintenance of the proteome homeostasis and quality.

1.1.1. *The ubiquitin-proteasome system*

The UPS is a large machinery consisting of numerous components that act in a highly regulated manner, generating a chain of events which results in substrate ubiquitination and their subsequent degradation. Ubiquitination involves three types of enzymes: E1 (the ubiquitin-activating enzyme) encoded by two genes; E2 (a ubiquitin-carrier protein also called ubiquitin-conjugating enzyme) encoded by at least 37 genes; and E3 (a ubiquitin ligase) encoded by ~800 genes. Conjugation of ubiquitin (Ub) begins with an ATP-dependent activation of the C-terminal glycine residue (G76) of the molecule. Ub is then transferred as an activated moiety to an E2. The last step of substrate ubiquitination is carried out by the formation of a complex consisting of Ub-loaded E2 and a specific E3 to which the substrate protein is bound. It is then transferred from the E2 either directly to a lysine residue of the substrate when the E3 is of the RING-finger type, or to an internal cysteine residue in the E3 when the enzyme is of the HECT-domain type. Finally, it is conjugated to the substrate. Additional ubiquitins are then added by the same cascade, creating a Ub chain (Ciechanover, 1994; Finley, 2009). Furthermore, Ub chains can be also elongated by an

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additional type of ligase—E4, also termed the "Ub-chain elongation factor" (Koegl et al., 1999).

Polyubiquitinated substrates are delivered to the proteasome—the catalytic arm of the UPS. The 26S proteasome is composed of a 20S core particle (CP) that can be capped with one or two 19S regulatory particles (RP). The ubiquitinated substrates are recognized by 19S RP Ub receptors. Subsequent to recognition, the substrate undergoes deubiquitination by deubiquitinating enzymes (DUBs), following by unfolding and translocation to the proteolytic chamber of the 20S CP, the last two processes require metabolic energy (Gallastegui and Groll, 2010; Livneh et al., 2016).

In addition to the 'canonical' proteasomal degradation which depends on Ub and ATP, there are several alternative mechanisms, some of which do not require ubiquitination and/or ATP hydrolysis (see below).

1.1.2. Autophagosome-lysosome system

Autophagy (from Greek- "self-eating") is a process in which cytoplasmic material is degraded by the lysosome. There are three types of autophagy: (1) microautophgy, where the lysosome itself engulfs a small portion of the cytosol; (2) chaperone-mediated autophagy (CMA), in which chaperones target selective substrates to the lysosome; and (3) macroautophagy, usually termed autophagy, in which a phagophore is generated, expands, and forms an autophagosome, which can contain besides cytosol, also subcellular organelles. The autophagosome fuses with the lysosome and pours its contents into it to be degraded (Kroemer et al., 2010). Although macroautophagy was considered for many years as a non-selective process, recent studies reveal that autophagic substrates are also being selectively targeted for degradation, similar to the way substrates are being recognized by the UPS (Onodera and Ohsumi, 2004).

Autophagy consists of three conserved sequential steps: (1) nucleation; (2) elongation and substrate sequestration; and (3) fusion with the lysosome.

More than 35 autophagy related genes (ATGs) have been identified in yeast, many of which are well conserved in mammals. The core of the autophagic machinery is comprised of 18 ATGs (ATG1–10, ATG12–14, ATG16–18, ATG29, and ATG31) that are shared across different types of autophagy and are necessary for the formation of the autophagosome (Suzuki and Ohsumi, 2007).

Upon induction, proteins of the *UNC51-like kinase* (ULK) complex (ULK1 or ULK2 and ATG13, *FAK family kinase-interacting protein of 200 kDa* (FIP200) and ATG101) assemble and initiate phagophore nucleation. Additional nucleation requires the ULK complex to activate a class III PI3K complex (BECLIN1 (or Atg6 in yeast), *vacuolar protein sorting 15* (VPS15), VPS34 and ATG14). This activation promotes the generation of a specific autophagosome phosphatidylinositol 3-phosphate (Wang and Klionsky, 2003). The origin of the phagophore membrane is still elusive. Historically, it was considered to be a *de novo* synthesis product, but current evidence suggests it originates from pre-existing organelles (such as the endoplasmic reticulum (ER) and Golgi apparatus) (Geng et al., 2010; Yamamoto et al., 1990). Regardless, the autophagosome stems from an expansion of this membranal sheet—the phagophore. As it grows in size, the phagophore curves and is sealed to form the autophagosome (Knævelsrud et al., 2013). As in the case of the phagophore origin, the source of the membrane required for its expansion is still under investigation.

The elongation and expansion step which includes also the recruitment of the substrate receptor LC3, is mediated by two ubiquitin-like conjugation systems. In the first one, the ubiquitin-like protein ATG12 is attached to ATG5 by the E1-like ATG7 and the E2-like ATG10 proteins. Then, ATG16L1 binds ATG5-ATG12 to form the active ligase-like complex (Geng and Klionsky, 2008; Kuma et al., 2002). In the second conjugation process, LC3, which is synthesized as a pro-LC3 form, is cleaved by the cysteine protease ATG4B to expose its C-terminal glycine residue, forming the LC3-I form. LC3-I is then conjugated with a phosphatidylethanolamine (PE) by the combined activity of the E1-like ATG7, E2-like ATG3, and the E3-like ATG16L1-ATG5-ATG12 complex (Hanada et al., 2007; Kabeya et al., 2000; Tanida et al., 2004). LC3-PE (also called LC3-II), incorporates into the expanding autophagosomal membrane, and then binds different adaptor proteins, which facilitate selective sequestration of substrates (e.g. p62/Sequestosome 1 and *neighbor of BRCA1* (NBR1)) (Bjørkøy et al., 2005)

Certain stimuli, such as apoptotic signaling, have been shown to induce 'non-canonical' autophagy in two manners: the first bypasses proteins that are important for initiation (ULK1) and nucleation (BECLIN1) (Cheong et al., 2011). The second bypasses the proteins involved in autophagosome elongation and maturation (ATG7, ATG5 and LC3), and is mediated by fusion events of vesicles positive for the GTPase RAB9, which is known to be involved in vesicular trafficking between the *trans*-Golgi network and late endosomes (Codogno et al., 2011; Moreau et al., 2011; Nair et al., 2011).

2. Mechanism of action of the UPS and autophagy

Specific degradation of proteins by both the UPS and the autophagic pathway involve substrate ubiquitination, its targeting to the proteolytic machinery by shuttling proteins, and the recognition of the degradation signal by the catalytic arm of each system.

2.1. Degradation signals

2.1.1. Ubiquitin-dependent degradation in the UPS and autophagy

First discovered as a degradation signal used by the UPS (Hershko and Ciechanover, 1998), Ub was later found to mediate also proteolysis of substrates through endocytosis (Galan and Haguenauer-Tsapis, 1997; Terrell et al., 1998) and autophagy (Kim et al., 2008; Pankiv et al., 2007). Ub can be attached to a substrate either as a single moiety (monoubiquitination), several single moieties on different sites (multiple monoubiquitinations) or as a chain (polyubiquitination). After the first Ub has been conjugated to the substrate, a chain is polymerized by continuous attachment of additional Ub moieties to a lysine (K) residue (K6, K11, K27, K29, K33, K48, or K63 (Ikeda and Dikic, 2008)) or to the N-terminal methionine residue (Kirisako et al., 2006) of the previously attached Ub (Hershko and Ciechanover, 1998). The fate of a ubiquitinated substrate depends on the length of the Ub chain attached, as well as on the K residue through which it is linked (Pickart, 2000).

While it was first suggested that short-lived proteins which are degraded by the proteasome are marked selectively by K48-linked Ub chains (Thrower et al., 2000), it was later shown that all chain types are probably involved in proteasomal degradation (Hofmann and Pickart, 2001; Saeki et al., 2009; Xu et al., 2009).

It is now known that K63 Ub chains serve as a degradation signal also for selective autophagy (Olzmann et al., 2007; Tan et al., 2008; Wooten et al., 2008). Interestingly, deletion of the autophagy genes *ATG5* or *ATG7*, resulted in accumulation of all types of Ub chains (Riley et al., 2010), suggesting the involvement of other chain types in autophagy.

Monoubiquitination was also shown to be sufficient for targeting substrates to both the UPS (Boutet et al., 2007; Braten et al., 2016; Carvallo et al., 2010; Kravtsova-Ivantsiv et al., 2009) and to autophagy (Kim et al., 2008).

Besides common degradation signals, the two machineries also share several substrates and enzymes. The *inhibitor of transcrip-*

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