PostDoc Journal
Vol. 2, No. 1 January 2014

Journal of Postdoctoral Research
www.postdocjournal.com

Protein turnover: to self-eat or not to self-eat, that's the question.

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Abstract
The regulated balance between protein synthesis and protein degradation is crucial for tissue integrity, homeostasis, and maintenance. There are two principal routes for intracellular protein degradation, namely the ubiquitin proteasome system (UPS) and the autophagy-lysosome pathway (ALP) (Alfred 2003; Martinez-Vicente, Sovak et al. 2005). There are also emerging evidences for the interaction of the two pathways through specific molecular chaperones that can assist in recognizing, tethering, and degrading misfolded or aggregated protein substrates. Any misfolded or aggregated protein needs to be immediately cleared through either or both degradation pathways since the failure to do so can lead to cytotoxic stress due to accumulation of unwanted protein substrates. This stress can then lead to pathogenesis and tumorigenesis. In fact, most age-related diseases and cancers are characterized by a deregulation in either the UPS or ALP machineries. In this review, I intend to analyze the recent findings of cellular and molecular moieties in the autophagic degradation pathway, how this pathway is studied, and how it pertains to disease.

Keywords: ALP, pathogensis, proteasome, protein degradation, protein turn-over, tumorigenesis

Introduction

There are a number of parameters that determine the levels of protein expression, from de novo synthesis and post-translational modifications to degradation. The governing forces of synthesis and degradation for individual proteins at homeostatic cellular conditions are of great discovery values. Therefore, the study of protein turnover necessitates a thorough analysis of transcriptome and proteome, as well as the interaction between protein substrates and other macromolecules all of which dictate the overall cellular protein levels. There are a number of experimental approaches to determine the dynamics of protein turnover from stable isotope labeling to high throughput proteomic technologies (Hinkson and Elias 2011). In this review, the major protein degradation pathways will be discussed along with common techniques used in the field to detect such processes. In the end, protein degradation and its role in disease and more specifically in cancer and Huntington's disease will be discussed with major emphasis given to autophagy.

Protein degradation

Protein degradation is essential for various cellular processes from proliferation, growth, differentiation to cell death and aging. It can also act as a defensive mechanism against harmful pathogen invasion as well as an adaptive response to changing extracellular environments as well as to hazardous damaged and altered proteins (Martinez-Vicente, Sovak et al. 2005).
Therefore, proper protein degradation is essential for survival and one whose benefits outweigh the immense cellular energy expenditure. One way of covering the costs and maintaining cellular homeostasis is to reuse the amino acids for synthesis of new proteins (Alfred

2003).

Protein turnover rate can be categorized into two main groups: fast protein turnover for short-lived proteins with half lives of 10 to 20 minutes and slow protein turnover for long-lived proteins with half lives of up to a day (Eden, Geva-Zatorsky et al. 2011).

It is generally known that the majority of short-lived proteins are degraded through the UPS and the long-lived protein substrates incur ALP for their degradation. There's also evidence that he ALP machinery is important for bulk proteolysis whereas the UPS is more selective. However, these are not strict rules as short-lived proteins can undergo ALP degradation and some protein substrates are selectively recognition for ALP (Nedelsky, Todd et al. 2008).

Moreover, the amino acid constitution of protein substrates as well as their size, mass, and isoelectric point and surface area can affect their properties as well as stabilities. The question that still remains valid for many proteins is how they are degraded, through the UPS or ALP, and what molecular complexes and organelles are involved in the process of their degradation.

The aim of this review is to introduce the UPS and ALP in more detail and analyze our current thinking of how UPS and ALP are studied and involved in the various cellular processes and disease states.

The Ubiquitin Proteasome System (UPS)

The genes of the UPS and its regulatory molecules constitute ~5% of the genome and is quite conserved in eukaryotic cells as well as archaea and some bacteria. The UPS are the major pathway by which cells regulate their protein turnover. In fact, the majority of proteins are degraded through the proteasome system. This constant turnover ensures that aberrant proteins are quickly removed and other proteins are minimally exposed to hazardous environments that can consequently damage them (Martinez-Vicente, Sovak et al. 2005).

The UPS consists of a cylindrical multicatalytic 26S proteasomal complex containing alpha and beta subunits (20S proteasome and 19S regulatory subunit) that make a barrel with a central pore containing active sites so that the target proteins can bind and be degraded in an energy dependent manner (ATP hydrolysis) (Lecker, Goldberg et al. 2006). The discovery of the biochemistry of ubiquitin conjugation to protein substrates that prime them for degradation by Avram Hershko, Aaron Ciechanover, and Irwin Rose lead to Nobel Prize award of chemistry in 2004 (http://nobelprize.org/chemistry/laureates/2004/). There are three main ubiquitin activating enzyme ligases (E1, E2, and E3) that link single or polychain ubiquitin moieties to misfolded or mutated protein substrates and tag them for degradation (Aaron 2005). There are also deubiquitinase enzymes responsible for removal of ubiquitin moieties (Byung-Hoon, Min Jae et al. ; Mocciaro and Rape 2012). Both ubiquitin ligase and deubiquitinase enzymes play crucial roles in regulating protein degradation. Although we already know a lot about specificity of these enzymes and the amino acid moieties on which they act on, there are still current fields of study that focus on deciphering the specific roles of each. To complicate the matters, some post translational modifications such as phosphorylation prime proteins to be tagged for ubiquitination (Lecker, Goldberg et al. 2006). Also ubiquitination, although not in all protein cases (Zhang, Xu et al. 2013), can be a signal for degradation in the case of K48 ubiquitination or a regulatory signal transduction tag in the case of K63 ubiquitination (James, Hyoung Tae et al. 2013). Nevertheless, the UPS like the lysosomal autophagic pathway is essential for many cellular processes from cell cycle and gene expression to protection against environmental stress.

The Autophagic Lysosomal Pathway (ALP)

In the past decade and with the advanced technological tools, there has been an immense surge of interest in deciphering the molecular and cellular mechanisms of self-eating or autophagy. This attention demands due respect

as autophagy can be thought of as the chief culprit in various essential cellular processes including: cell cycle control, apoptosis, development, stress response, waste disposal, signal transduction, metabolism, transcription, DNA repair, antigen presentation, as well as inflammation, neurological disorders, and cancer (Figure 2).

Figure 1

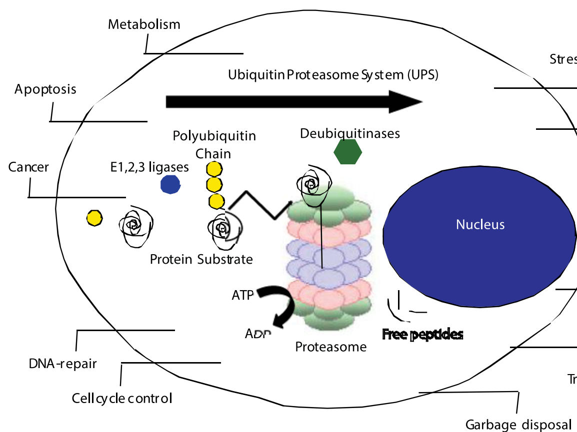

Figure 1. Proteasomal degradation system and its cellular functions. Protein substrates are conjugated to single ubiquitins or polyubiquitin chains with the aid of E1, E2, and E3 ligases which primes them to bind to the proteasome. The substrate engages the proteasome and the ubiquitin moieties get removed by deubiquitinases which initiates the degradation process. The protein subunit gets degraded sequentially along the proteasomal subunits in an energy dependent manner and the remaining peptides get recycled for incorporation into new proteins during de novo protein synthesis.

Autophagy (self-eating) was discovered after the visualization of electrodense vesicles in electromicrographs by de Duve, which he called lysosomes in mouse kidney cells in 1957 (de Duve and Wattiaux 1966). Ever since and with the advent of technology, scientists have been able to detect the sequestration of cytoplasmic components, proteins, and organelles (autophagosomes) and their delivery to lysosomes (autophagolysosomes) for degradation by lysosomal proteases, cathepsins, and hydrolases, a process known as autophagy (Noboru, Beth et al. 2008). This association of lysosomes with protein substrates has been well established (Strømhaug, Berg et al. 1998; Aaron 2005; Klionsky 2005; Cao, Cheong et al. 2008; Morvan, Köchl et al. 2009; Mizushima, Yoshimori et al. 2010).

Briefly, classical autophagy begins with the formation of autophagosomes and autophagolysosomes whose contents are to be degraded. The organelles that contribute to the double membrane formation are ER, Golgi, mitochondria, plasma membrane and various intracellular vesicles (Yen, Shintani et al. 2010). Autophagy is a conserved process from yeast to mammals (Lee, Giordano et al. 2012). Autophagy is characterized by these double membrane vesicles and detected through microscopic techniques (Hailey, Rambold et al. 2010; Hayashi-Nishino, Fujita et al. 2010).

There are three types of autophagy identified in mammalian cells: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (Mizushima 2007). Other specific types include aggrephagy, endoplasmic reticulum (ER)-phagy, mitophagy, pexophagy, and xenophagy (van der Vaart and Reggiori 2010). Macroautophagy and microautophagy can engulf and degrade cytoplasmic materials in bulk (e.g., protein aggregates and organelles) in a selective or nonselective manner whereas other types of autophagy are strictly selective (Mizushima 2007).

Techniques for studying protein turnover

There are many techniques that are utilized by researchers to study protein turnoverand to assess proteasomal and autophagic degradation. Among the classical tests to decipher proteasomal versus autophagic degradation of protein substrates is utilizing inhibitors of each

pathway and measuring the proteins of interest by immunoblotting, microscopy, autoradiography, and spectroscopy. An exhaustive list of genes, inducers, and inhibitors of proteasomal and autophagic pathways can be found on the Invivogen website (http://www.invivogen.com/autophagy).

Figure 2

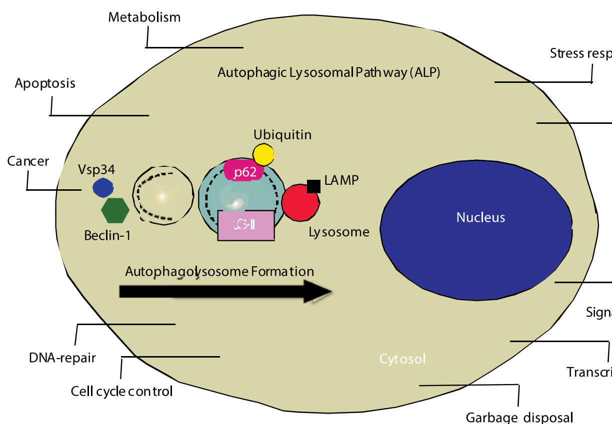

Figure 2. Autophagic machinery and its cellular functions. This is a simplified schematic of the different levels of autophagic mechanism and regulation. There are first signaling pathways that induce autophagy. Second step is the autophagosome formation in which many different molecules play important roles. P62 binds ubiquitin and LC3, Vsp34 and Beclin-1 also form a complex with the help of ULK, and FIP200 and Atg16 interact and recruit other Atg proteins to the site of autophagosome. The third step is the maturation of autophagosome by fusing with lysosome to degrade the transported cargo through the acidic lysosomal hydrolases. The various cellular pathways that are regulated by autophagy are also added to the diagram.

The study of autophagy may prove to be more complicated as there is a surge of signaling and non-signaling molecules that need to come together to assemble and form the autophagosomal double membrane. Some of the date and techniques used to decipher them are presented below:

There is already a massive amount of data through quantitative proteomic characterization of autophagosomes that have identified a set of autophagosome-associated proteins and regulators (Dengjel, Høyer-Hansen et al. 2012). The list starts with the identification of AuTophaGy specific Atg genes and their roles in various cellular processes (Legakis, Yen et al. 2007). Various proteins including Vsp15, Vsp34, Beclin-1-class III PI3K, FIP200, uncoordinated family member-51-like kinase (ULK1,2), VRAG, Atg14, Ambra1, and Bif1 (Hara and Mizushima 2009; Kang, Zeh et al. 2011) selectively come together in order to form and elongate a macromolecular complex with protein substrates and initiate autophagy (Blommaart, Luiken et al. 1997; Cheong, Lindsten et al. 2011). The list of interacting partners of autophagy are growing as even more molecules previously not known to be involved in the initiation of autophagy are being discovered, for instance Rab GTPase Rab5 and Rab7, lysosomal transmembrane protein (LAMP), ubiquitin, and acetyl-lysine (Lee, Giordano et al. 2012).

Normally, there is an even distribution of microtubule associated protein 1 light chain 3 (LC3), a mammalian homologue of yeast Atg8, in normal cell conditions. However, under starvation or induction of autophagy through chemicals, there's an accumulation of LC3 proteins and recruitment to autophagosome membranes. The fluorescent LC3 puncta can be quantified in micrographs of cells under normal and autophagic conditions as a signifier of autophagy (Kimura, Noda et al. 2007; Geng, Baba et al. 2008). Therefore, the second (and possibly the most reliable) method for detecting autophagy is to measure LC3-II protein (Mizushima, Yamamoto et al. 2004). During autophagy, the cytosolic form of LC3 (LC3-I, 18 kDa) is lipidated and translocates to autophagosome membranes (Yukiko, Noboru et

al. 2000). Since lipidated LC3-II (16 kDa) can be separated from LC3-I on an SDS–PAGE gel (Kabeya et al. 2000), autophagosome formation can be detected by measuring the conversion of LC3-I to LC3-II by Western blot (Chen, Azad et al. 2010).

There has been several cargo receptors that associate with autophagosomal marker LC3 including p62 and NRB1 to entrap certain proteins for degradation (Pankiv, Clausen et al. 2007).
Insights into the signaling networks that regulate the autophagic process is very important as regulating the mechanisms by which specific protein degradation occurs will have therapeutic function in many diseases.

Protein degradation and disease

The proteasomal and autophagic degradation systems deteriorate and decrease in efficiency with age and in many disease states (Lecker, Goldberg et al. 2006). Some of the common pathologies resulting from defective or inefficient proteolysis are obesity (Argilés, Busquets et al. 1999), muscle wasting disorders (Zhang, Rajan et al. 2011), cancer (Soyeon, Xueming et al. 2013), neurodegenerative disorders (Keller 2006; Wang, Wang et al. 2008), and diabetes (Huiling, Dajun et al. 2010). Below, the role of autophagic proteolysis in two of these pahtologies, cancer and a neurodegenerative disease will be discussed.

Firstly, the autophagic process has been shown to be associated with the inhibition of tumor development as the loss of several autophagic genes (e.g. Beclin, Atg, FoxO family of genes) have been directly or indirectly linked to many human cancers (Ying, Jing et al. 2010). For instance, Insulin/IGF-1 signaling can activate a cascade of kinases that inhibit FoxO activity and hence decrease autophagic rate of protein degradation. Or, SIRT1, an NAD-deacetylase, can deacetylate FoxO, increase its activity and therefore autophagy (Salminen and Kaarniranta 2009). In contrast, SIRT2, another subfamily of the sirtuin genes, has been shown to inhibit lysosome-mediated autophagic turnover by interfering with aggresome formation and sensitizing the cells to accumulated protein mediated cytotoxicity (Gal, Bang et al. 2012). Recent evidence has also implicated the role of autophagy in life span extension and slowing the aging process by degrading the misfolded or damaged proteins and inhibiting their deleterious accumulation (Vellai, Takács-Vellai et al. 2009).

Huntington disease is characterized by the accumulation of mutant Huntingtin (mHtt) in inclusion bodies (Proenca, Stoehr et al. 2013), however although inducing autophagy can rescue mHtt accumulation, a disturbance in the autophagic flux through Atg4b overexpression enhanced aberrant accumulation and disease progression (Tsvetkov, Miller et al. 2010).

This is by far not an exhaustive list for the role of protein degradation and autophagy in disease but aims to emphasize that the closer examination of macromolecular complexes that can regulate autophagy deems necessary for development of therapeutic targets for a range of diseases.

Future perspectives

The degradation of protein substrates is a way to rid the cell of mutated, damaged, unstable, non-functional and functional molecules and prevent their accumulation and caused cell stress at precise times and cell types. Aside from the binding partners and molecular chaperones that assist with the degradation process, more studies are needed to analyze whether regulated protein degradation applies to the already discovered specialized proteins or to all proteins. Also how and through which degradation pathways are the not so studied proteins degraded? Other relevant questions would be to determine at what cellular conditions and times do the cells decide to eliminate these proteins and what determines which degradation pathway is incurred for each specific cell type.

Acknowledgments

I would like to thank Dr. Maria Almira Correia for supporting my research work in the present. I also thank the anonymous reviewers for their thoughtful suggestions for improving this article.

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