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Review

Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems

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ABSTRACT

The ubiquitin proteasome system (UPS) and macroautophagy (hereafter called autophagy) were, for a long time, regarded as independent degradative pathways with few or no points of interaction. This view started to change recently, in the light of findings that have suggested that ubiquitylation can target substrates for degradation via both pathways. Moreover, perturbations in the flux through either pathway have been reported to affect the activity of the other system, and a number of mechanisms have been proposed to rationalise the link between the UPS and autophagy. Here we critically review these findings and outline some outstanding issues that still await clarification.

1. Introduction

The UPS and autophagy are two cornerstones of cellular catabolism that are involved in most aspects of normal physiology and development, and are also implicated in a broad array of pathological states, including cancer, neurodegeneration and aging. Protein degradation controls processes like the cell cycle, signaling, DNA transcription, repair and translation, by downregulating their critical regulatory elements. Additionally, the UPS and/or autophagy are involved in the degradation of virtually every type of surplus, dysfunctional or damaged cellular component, ranging from soluble proteins to whole organelles. This allows recycling of both matter and energy and therefore serves to save valuable resources. Thus, autophagy and the UPS are critical in the maintenance of cellular homeostasis, suggesting that their activities need to be carefully orchestrated. Yet, the two pathways differ so significantly with respect to their mechanistic details (autophagy is a vesicular trafficking pathway, while the enzymatic reactions of the UPS occur directly in the cytosol), substrates (the activity of UPS is restricted to soluble proteins, while autophagy is practically omnivorous), machinery, specificity, kinetics, elements of control, etc., that this leaves very little room to suspect any cross-talk. Indeed, for a long time these processes were viewed as independent of each other [1,2]. Here, we review the evidence generated during recent years that challenge this view and offer a glance into a complex and often an unexpected interplay between these two cellular waste conveyors.

2. Basic mechanics of the UPS and autophagy

Proteins are targeted for destruction by the UPS via a series of enzymatic reactions that tag them with homopolymers of a small, 76-amino acid residue, protein called ubiquitin [3,4]. Polyubiquitylation marks the UPS clients for transportation by a poorly understood shuttling machinery to a specialized organelle called the proteasome, where proteins are degraded to oligopeptides, which are released into the cytoplasm or nucleoplasm, where they can be digested into amino acids by soluble peptidases. The specificity and selectivity of the ubiquitylation process is achieved by a combination of three types of enzymes [5]. E1 enzymes, two of which are known in mammals, initiate the reaction by activating ubiquitin and transferring it onto E2 ubiquitin-conjugating molecules, of which around 40 are thought to be encoded in the mammalian genome. A substrate is selected in our cells by one of several hundred E3 ligases, which bind the

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ubiquitin-carrying E2 enzyme, resulting in the transfer of the ubiquitin onto lysine residues of the target substrate [6,7]. As a result of such a reaction, the substrate becomes monoubiquitylated in one or more places. These initial modifications are not yet sufficient for proteasomal targeting. Since ubiquitin itself contains lysine residues in positions 6, 11, 27, 31, 33, 48 and 63, all of these sites could become acceptors of another ubiquitin moiety in a subsequent round of ubiquitylation, which would lead to the generation of different types of polyubiquitin chains. It is thought that chains of at least four ubiquitins [8] interconnected via K48 residues, which are characterized by a closed conformation [9], are optimal for delivery to the proteasome. The proteasome is a barrel-shaped proteolytic organelle found throughout the cell that consists of a 20S central complex and two 19S lid complexes. The 19S complexes bind cargo-loaded shuttling proteins, deubiquitylate the substrates and control access to the six proteolytic sites of the inner core of 20S subunit [10,11]. The catalytic activities of the proteasome have different specificities, and are considered trypsin-, chymotrypsin- and peptidyl-glutamyl peptide-hydrolyzing-like [12]. The narrow size of the proteasomal catalytic pore suggests that protein substrates need to be partially-unfolded prior to entry into the 20S subunit. Thus, protein complexes and aggregates can only be digested if disassembled, which makes them poor proteasome substrates [13].

In contrast to the UPS, autophagy is restricted to the cytoplasm, but is capable of degrading a much wider spectrum of substrates, which, on average, tend to be longer-lived and bulkier. These include functional or misfolded soluble proteins, protein complexes, oligomers and aggregates. Although limited, there appears to be a certain overlap in function between the two degradative pathways, as both seem to be capable of degrading soluble misfolded polypeptide chains [14]. Additionally, autophagy can degrade whole cellular organelles. Terms like pexophagy, mitophagy or ribophagy have been coined to describe autophagosomal degradation of peroxisomes, mitochondria or ribosomes, respectively. Interestingly, also, proteasomal subunits were found to be degraded by lysosomes [15]. This provides a possibility that the autophagy-lysosome system could affect the activity of the UPS by controlling the numbers of proteasomes, a hypothesis that, to our knowledge, has not yet been investigated.

Autophagy is initiated by the formation and elongation of a double-layered isolation membrane (the origin of which remains an intensely debated topic) also called a phagophore, that enwraps and sequesters portions of cytoplasm containing autophagic substrates, to form autophagosomes. The formation of autophagosomes is regulated by a set of Atg genes, where Atg stands for autophagy-related, the nomenclature taken from yeast where they were originally identified [16]. These can be grouped, according to their function, into the Atg1 complex (Atg1, Atg13 and Atg17 controlling autophagosomal induction), the PI3K complex III (including phosphatidyl inositol 3-phosphate kinase vps34, Beclin 1 (Atg6 orthologue) and UVRAG (UV radiation resistance associated gene)) regulating vesicle nucleation, and two interconnected ubiquitin-like conjugation systems that mediate vesicle elongation and sealing. The first of these conjugation systems involves the formation of Atg5-12 conjugate, mediated by the E1-like enzyme, Atg7, and the E2-like enzyme, Atg10. The second involves conjugation of Atg8 (in mammalian cells also known as microtubule-associated protein 1 light chain 3, LC3) to the lipid, phosphatidylethanolamine, regulated by Atg7, along with Atg3, as the E2-like enzyme [17]. Following the formation of autophagosome, Atg5-12 conjugate is removed from the vesicle, while LC3 remains attached. Thus, LC3 serves as a reliable autophagosomal marker that can be used to estimate both the rates of autophagosome formation and degradation [18]. Autophagosomes are transported along microtubules in a dynein-dependent manner and fuse with endosomes or directly with lysosomes where autophagosomal contents are degraded by lysosomal hydrolases [19].

3. Ubiquitin as a unifying factor linking the UPS and selective autophagy

Autophagy is often thought of as a non-specific process that degrades cytoplasmic proteins and organelles in bulk, a situation likely to occur when cell survival depends on autophagy during periods of starvation [20]. However, as early as the 1970s, the first evidence of selective autophagy was suggested for organelles such as the endoplasmic reticulum or mitochondria, although further understanding of such selectivity was impossible until more recent insights into the molecular mechanisms of selective autophagy [21]. While this process is still poorly understood, it is postulated that during selective autophagy, certain autophagic substrates may be specifically targeted for destruction, rather than being randomly taken up along with bulk cytoplasm. The relevance of this issue to the topic of our discussion becomes evident when we learn that it is ubiquitylation, just like in the ubiquitin proteasome pathway, that serves as the signal for selective autophagy. Thus, it might be tempting to speculate that ubiquitin coordinates the catabolism of cellular targets by both the UPS and autophagy. Indeed, many proteins are known to be substrates of both degradative systems, and in certain conditions ubiquitylated proteasomal substrates, which are normally degraded by the UPS, can also be digested by autophagy, and vice versa [22–24]. Moreover, impairment of proteasome activity was found to activate autophagy, which was thought to be a compensatory mechanism allowing the cell to reduce the levels of UPS substrates (see below) [25–28]. However, the overall contribution of autophagy to the degradation of the total pool of cellular ubiquitylated proteins remains unknown, and so it is unclear whether ubiquitylation is an important mechanism for autophagic targeting of many proteins.

In addition, although ubiquitylation may appear to be a universal tag targeting substrates for destruction via both catabolic systems, the exact type of modification recognised by each pathway appears to be different. While K48-linked polyubiquitin chains are employed by the UPS, substrates recognised by autophagosome-lysosome pathway are thought to be modified either by K63-linked chains (adopting a more open conformation than K48 chains), or may just be monoubiquitylated [29]. Thus, despite the use of ubiquitin in both catabolic pathways, the structural complexity of different polyubiquitin chains may be sufficient to maintain selectivity and specificity of the UPS and autophagy towards their substrates. However, some potential overlap may result from incomplete specificity of the different adaptor molecules that have been proposed to retrieve ubiquitylated substrates for each degradative pathway. In this category, there are several proteins that appear to serve as linkers between ubiquitylated cargo and the phagophore, including p62 (also called SQSTM1/A170), NBR1 (neighbour of BRCA1 gene 1), HDAC6 (histone deacetylase 6) and Alfy [30]. These proteins have the capacity to interact directly or indirectly with both ubiquitin and components of autophagic machinery, thus providing the type of link that would be required from an adaptor molecule. The most established of these adaptors, p62, is itself an autophagy substrate that forms homo-oligomers to which ubiquitylated proteins are recruited via its UBA (ubiquitin-associated) domain [30–33]. It was proposed that these complexes serve to sequester ubiquitylated substrates that are recognised by the autophagic machinery (p62 interacts directly with LC3 via a dedicated LIR motif [33]), and then engulfed and degraded [30,31]. The UBA domain of p62 appears to have a slightly higher affinity for monoubiquitin or polyubiquitin chains with open conformations (K63-linked), compared to those with a closed