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Introduction to Intracellular Protein Degradation

Organisms must synthesize and fold an enormous variety of cellular proteins, and many of these proteins need to be incorporated into complex and often dynamic higher-order structures. The metabolic turnover of individual proteins varies over a wide range and is subject to precise regulation. For instance, many important regulatory proteins are rapidly degraded because this allows tight control of their concentrations in the cell. Moreover, any protein can suffer biosynthetic errors or post-translational mishaps such as improper folding or assembly into complexes. Accumulation of such defective proteins is potentially toxic and has been associated with a variety of degenerative disorders such as Alzheimer's and Parkinson's disease. In eukaryotes, much of this regulatory and quality-control proteolysis falls under the jurisdiction of the ubiquitin-proteasome system. This special issue of Chemical Reviews is focused on various aspects of cellular proteolysis with a particular emphasis on the ubiquitin-proteasome system.

In the first article, Grabbe and Dikic present a general discussion of the "β-grasp" or ubiquitin fold in the context of protein−protein interactions important for proteolysis and other physiological mechanisms. In some cases, the β-grasp or ubiquitin-like domain (ULD) is imbedded in a larger polypeptide, but the regulatory power of the β-grasp fold comes primarily from the ability of proteins with this fold to be covalently conjugated to other proteins or macromolecules. The first identified member of this modifier family was ubiquitin, but at least ten conjugation-competent ubiquitin-like proteins have been characterized at this point. Over the past decade, it has become clear that a wide range of ubiquitin-binding domains (UBDs) mediate the action of ubiquitin-modified proteins. In several important examples, a UBD is present in the same protein as a ULD. Such ULD−UBD proteins are subject to intramolecular associations and can act as adaptor molecules. For example, Grabbe and Dikic discuss how certain ULD−UBD proteins can shuttle ubiquitin-modified proteins to the proteasome for degradation.

When the 76-residue ubiquitin protein is used as a tag for targeting proteins to the proteasome, it is usually found in the form of ubiquitin polymers. Polyubiquitin modifications are highly dynamic structures subject to rapid disassembly by an array of specialized proteases called deubiquitinating enzymes (DUBs). In their contribution, Reyes-Turcu and Wilkinson focus on how polyubiquitin chains are recognized and how they are disassembled by DUBs. They concentrate on those DUBs for which high-resolution structural information is available. The amide linkages between the ubiquitin units in post-translationally synthesized polyubiquitin chains can be through different lysine side chains as well as the N^{α}-amino group of ubiquitin. These topologically distinct chains often have distinct functions. For example, chains linked through ubiquitin lysine-48 are most frequently responsible for proteasomal targeting, whereas those coupled at lysine-63 have functions in endocytosis, signal transduction, and DNA repair. Reyes-Turcu and Wilkinson describe how these different types of chains are deployed in specific physiological settings and how different DUBs can discriminate among them.

Marques, Palanimurugan, Matias, Ramos, and Dohmen provide a comprehensive summary of our current understanding of the proteasome itself. This ~2.5 MDa complex of over 33 different polypeptides rivals the ribosome in size and complexity. It consists of a cylindrical core particle (20S proteasome), with a central chamber housing the protease active sites, flanked on one or both sides by a 19S regulatory particle that includes six ATPase subunits for protein unfolding and translocation into the core as well as polyubiquitin receptors and DUBs. Marques et al. also summarize the still limited information available on the de novo assembly of these massive protein complexes within the cell. Several dedicated assembly chaperones for 20S

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proteasome assembly have recently been characterized. Analogous factors can be anticipated for 19S regulatory particle assembly.

The next two contributions describe the functions of ubiquitin-dependent signaling and proteolysis in several intricate physiological regulatory systems, namely, cell-cycle control and signal transduction by vertebrate "NF-κB signaling pathways". Wickliffe, Williamson, Jin, and Rape detail how the ubiquitin-dependent degradation of specific regulatory proteins controls various aspects of cell cycle progression, including regulation by "checkpoints" that delay the cycle in response to damage suffered by DNA or the mitotic spindle. Ubiquitin-mediated proteolysis ensures the irreversibility of key cell-cycle transitions, such as entry into S phase, when chromosomes are replicated, and entry into mitosis, when they are divided into two daughter cells. Exit from the cell cycle during terminal cellular differentiation is another setting in which ubiquitin-dependent degradation of specific proteins is required for proper regulation.

Chen describes the many ways in which ubiquitin-protein modification is deployed to regulate the multifaceted activities of the transcription factor NF-κB. NF-κB activity is primarily regulated by its association with an inhibitor, IκB, which normally keeps it in the cytoplasm away from its DNA targets. In response to signals at the cell surface, IκB is phosphorylated, triggering its modification by lysine-48-linked polyubiquitin chains and degradation by the proteasome, thereby allowing NF-κB to enter the nucleus and activate transcription. Surprisingly, attachment of polyubiquitin chains—assembled with lysine-63 linkages—on upstream signal-transducing proteins is also a crucial part of the NF-κB signaling pathway.

In the final four articles, attention is turned to the degradation of transmembrane or membrane-enclosed proteins, where the mechanisms of proteolysis turn out to be remarkably diverse. Hampton and Garza describe the intriguing intersection at the endoplasmic reticulum (ER) between ubiquitin-dependent protein quality control and regulatory protein degradation. Because of the stringent demands placed on folding, maturation, and assembly of integral membrane and secreted proteins at the ER, extensive quality-control mechanisms are in place. If a protein fails to fold or assembly properly in the ER, it is retrotranslocated back into the cytoplasm for degradation by the proteasome, and this usually requires its polyubiquitination at the ER surface. Interestingly, the rate-limiting enzyme in the synthesis of cholesterol and related sterols, the ER-localized HMG-CoA reductase, is degraded in sterol-replete conditions. This degradation involves some of the same ubiquitin-conjugation machinery that targets misfolded proteins during protein quality control. Locally disordered regions of the target protein are thought to be the proximal signals for recognition by the ubiquitin system in both cases.

A very different type of ubiquitin-dependent protein degradation is described by Davies, Lee, Oestreich, and Katzmann. Transmembrane proteins at the cell surface, such as plasma membrane receptors, enter internal membrane-bound organelles through the process of endocytosis. Elimination of such proteins requires their subsequent trafficking to the lysosome, an organelle filled with proteases and other hydrolytic enzymes. These transmembrane proteins present a topological dilemma for complete breakdown into amino acids because they generally have segments not only within the membrane but also facing the cytoplasm and the cell exterior. This problem is overcome by the invagination and vesiculation of the endosomal membrane to form multivesicular bodies (MVBs). Fusion of the remaining outer membrane of the MVB to the lysosomal membrane releases the internal vesicles into the lysosome interior where lipases break down the membranes and proteases digest the proteins in their entirety. In an unexpected twist, ubiquitin conjugation to the cytoplasmic segments of membrane proteins provides an endocytosis signal at the cell surface as well as a sorting signal for proteins to enter the invaginations that will go on to form the internal vesicles in the MVB. Thus, ubiquitin again functions as a protein degradation signal but in a more indirect way than for proteasomal targeting. Katzmann et al. discuss the mechanistic features of MVB formation and ubiquitin-dependent sorting of proteins from both the plasma membrane and from the trans-Golgi network.

Besides the degradation of membrane proteins, the lysosome is also able to degrade cytoplasmic proteins, primarily through a process called autophagy. In macroautophagy, a double membrane structure forms around a volume of cytoplasm, creating an autophagosome, and the autophagosome then fuses with the lysosome in a process at least superficially similar to MVB−lysosome fusion. Surprisingly, the formation of autophagosomes is strictly dependent on a pair of linked ubiquitin-like protein-conjugation systems, as described by Noda, Ohsumi, and Inagaki. The proteins required for autophagy, called ATG (autophagy) factors, were identified first in budding yeast but turn out to be broadly conserved. Two ATG proteins, ATG8 and ATG12, are β-grasp proteins distantly related to ubiquitin. Both are activated and conjugated to their targets by enzymatic mechanisms similar to ubiquitin-protein conjugation. ATG12 is attached to a single target protein, ATG5, and remarkably, ATG8 is reversibly linked not to a protein but to the headgroup of a phospholipid, phosphatidylethanolamine. Noda et al. describe these two conjugation systems, emphasizing the recent structural studies that have clarified their mechanisms and substrate specificity.

In the final contribution to the issue, Wolfe discusses a fascinating group of transmembrane proteolytic enzymes that are able to cleave integral membrane proteins within the plane of the membrane. How hydrolysis reactions can occur in the hydrophobic interior of lipid bilayers has been a long-standing puzzle. Among the intramembrane cleaving proteases (I-CliPs) are the Site-2 proteases (S2Ps), metalloproteases involved in the regulation of mammalian cholesterol synthesis; the presenilin-containing γ-secretase complex, an aspartyl protease which has been associated with the pathogenesis of Alzheimer's disease; a related aspartyl protease called signal peptide peptidase; and the rhomboid serine proteases, which are important in various growth and developmental regulatory mechanisms. Biochemical and structural data on the rhomboid proteases and an archaeal S2P have now shed considerable light on how intramembrane proteolysis can occur, as detailed by Wolfe.

This collection of reviews provides an in-depth look at the current state of understanding for many central problems of intracellular protein degradation, primarily in eukaryotes. Although by no means comprehensive, this volume will serve as a valuable resource for anyone seeking to learn the background and current status of some of the most fascinating issues confronting researchers in this ever-expanding field.

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