Preface

The ubiquitin-dependent degradation of intracellular proteins is central to a remarkable assortment of physiological pathways, ranging from cell-cycle control and transcriptional regulation to DNA repair and signal transduction (Ciechanover, 2005; Varshavsky, 2017). Although well appreciated now, such intracellular proteolysis was originally regarded as a relatively uncommon feature of cellular regulation (Hochstrasser, 1996). However, it was also recognized early on that most such degradation requires energy despite the fact that proteolysis is an exergonic process (Simpson, 1953). This energy requirement suggested that the elimination of proteins from cells includes mechanistic processes that go beyond simple peptide-bond hydrolysis. For the ubiquitin-proteasome system, this is now well understood: ATP is expended in both the selective tagging of proteins with ubiquitin and the unfolding and translocation of the tagged proteins within the 26S proteasome. The proteasome is a complex molecular machine, and underlying the aforementioned energy expenditure is the execution of a specific cycle of conformational changes coupled to the movement of the substrate through narrow internal channels to the proteolytic core (de la Pena, Goodall, Gates, Lander, & Martin, 2018; Eisele et al., 2018).

The ability of the lysosome, a membrane-bounded organelle filled with hydrolytic enzymes, to degrade cellular proteins was recognized long ago (de Duve, 2005). The lysosome (called the vacuole in yeast) was thought to be fairly nonspecific in choosing its substrates. In a process known as macroautophagy, double-membrane structures envelope random segments of cytoplasm under starvation conditions, and the resulting autophagosomes fuse with the lysosome to disgorge and degrade their contents. This picture of indiscriminate destruction has turned out to be an oversimplification—macroautophagy is now known also to be able to target specific proteins, and specific organelles, to the lysosome.

Intriguingly, both ubiquitin and ubiquitin-like protein (Ubl) ligation have been found to be important for macroautophagy (Gomez-Diaz & Ikeda, 2018; Ohsumi, 2001). Two Ubls that are very distantly related to ubiquitin, but still display the classic β-grasp fold, play key roles. One (ATG12) is ligated to another autophagy factor, resulting in a protein complex that then promotes the attachment of the second Ubl, ATG8, to phosphatidylethanolamine; the membrane-remodeling activity of ATG8

helps drive autophagosome formation (Nakatogawa, Suzuki, Kamada, & Ohsumi, 2009). This mechanism is fundamental to all macroautophagy. On the other hand, selective autophagic elimination of particular cellular proteins or organelles often depends on their prior conjugation to ubiquitin. Adaptor proteins with binding sites for both ATG8 and ubiquitin target the ubiquitinated substrates to the developing autophagosome (Grumati & Dikic, 2018).

Other routes of substrate trafficking to the lysosome have also been found to depend on ubiquitin. Downregulation of cell-surface proteins usually involves endocytosis of the proteins from the plasma membrane; maturation of the endosomes by repeated rounds of invagination and vesiculation of the limiting membrane; and, finally, trafficking of the resulting multivesicular body (MVB) to the lysosome (MacGurn, Hsu, & Emr, 2012). Ubiquitination of target proteins at the cell surface signals the initiation of endocytosis, and their persistent ubiquitination—as well as that of membrane proteins in many other intracellular organelles—results in their being routed to the lysosome, where they are degraded. The primary exception to this mode of degrading ubiquitinated membrane proteins occurs at the endoplasmic reticulum (ER), where most ER-associated protein degradation occurs through extraction of the targeted ubiquitinated protein into the cytoplasm and degradation by the proteasome (Hampton & Sommer, 2012; Mehrtash & Hochstrasser, 2018).

In this volume, a wide range of in vitro and in vivo methodologies is described for the study of intracellular protein degradation in its many guises. As in the previous volume, the description of protocols in each chapter is preceded by a concise summary of the state of understanding in the field. An important aspect of intracellular proteolysis, which is mostly ubiquitin dependent, is its role in protein quality control. Methods for studying such processes for proteins residing in the cytoplasm, ER, and nuclear envelope are detailed. The proteasome is a large protein complex that serves as a platform for multiple reactions. In a series of chapters, protocols are presented for assaying these different features of the proteasome and how they are integrated into the proteolytic mechanism. Finally, techniques are described for the study of lysosomal/vacuolar protein degradation, initiated either by macroautophagy or ubiquitin-dependent membrane trafficking from the plasma membrane or internal membranes.

Altogether, it is hoped that researchers of all stripes will be able to dip into this two-volume collection for experimental insights and detailed technical

guidance for their own studies of the ubiquitin system. No doubt, many more new and exciting discoveries will be realized in these endeavors.

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