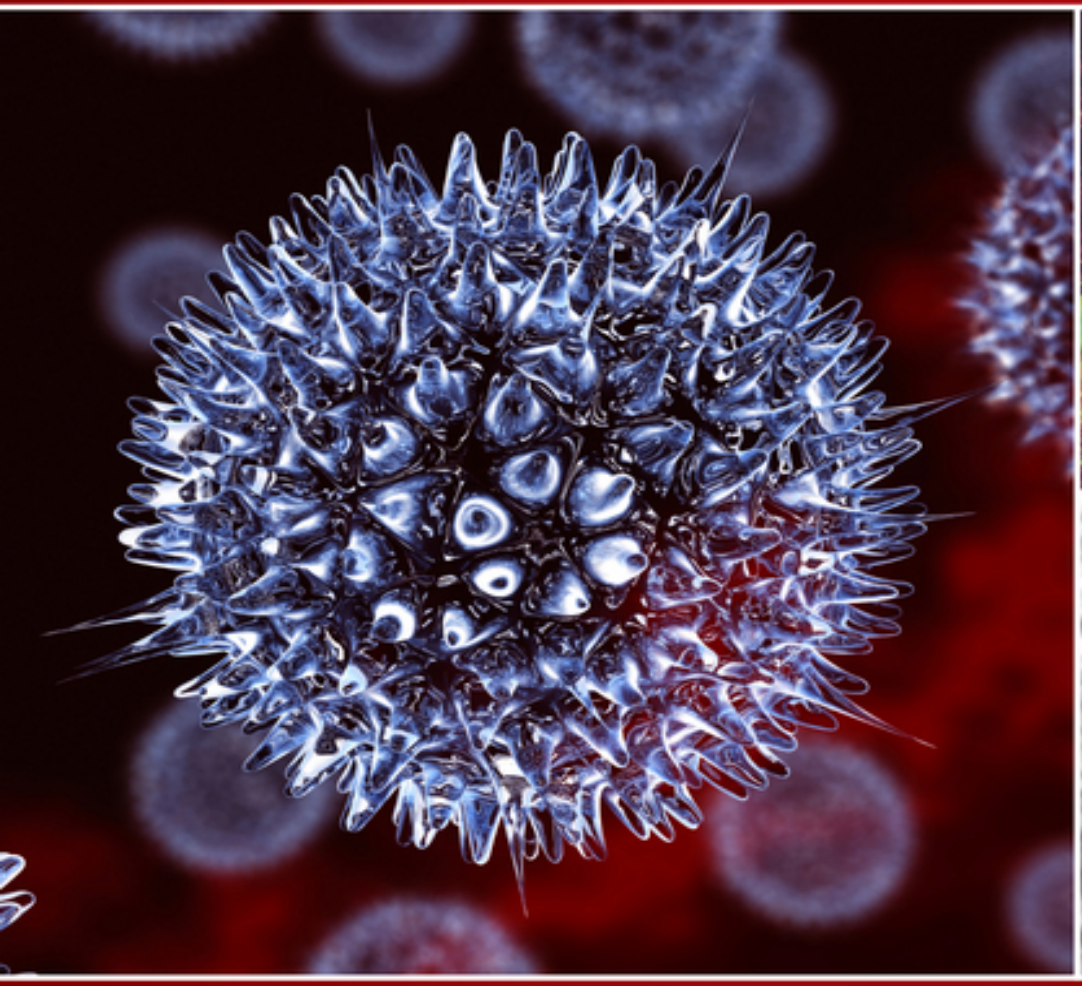
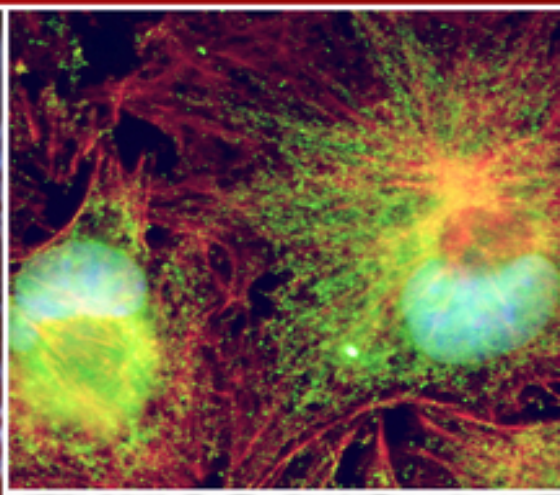
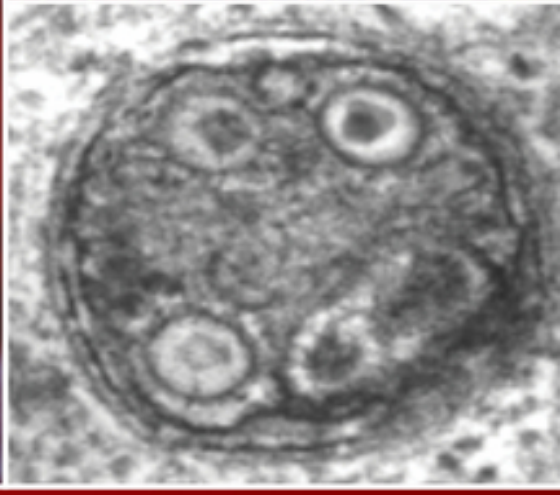
AUTOPHAGY, INFECTION, AND THE IMMUNE RESPONSE

Edited by **William T. Jackson and Michele Swanson**







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Preface

Since the discovery nearly 20 years ago that pathogenic bacteria and viruses intimately associate with autophagosomal membranes, scientists have determined that autophagy is a critical component of innate and acquired immunity. Of course, as with all aspects of the host immune response, some pathogens have turned autophagy to their advantage. For this volume, experts in the fields of bacteriology, virology, mycology, parasitology, immunology, and cell biology describe the cellular mechanisms of autophagosome formation and maturation, its contribution to host defenses, and the mechanisms pathogenic microbes have acquired to overcome and subvert this formidable barrier to infection. In addition, specialists discuss current efforts to exploit knowledge of the autophagy pathway to improve vaccine design. Accordingly, this thorough examination of an extraordinary cellular battleground between host and pathogen can stimulate ongoing research to understand and to manipulate autophagy to improve human health.

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Chapter 1
Autophagy and Immunity

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1.1 Introduction

Autophagy is a highly controlled process in which cellular components are self-degraded and subsequently recycled. This pathway in part plays a “house cleaning” role in the cell, directing numerous cargoes to the lysosome (or the vacuole in yeast and plants) for degradation. Depending on the specific conditions, the cargoes include random portions of cytoplasm, protein aggregates, and damaged or superfluous organelles such as mitochondria and peroxisomes. Dysfunction of autophagy is linked with many pathologies, including cancer, diabetes, myopathies, heart, liver and lung diseases, and certain types of neurodegenerative disease (Castets et al., 2013; Gonzalez et al., 2011; Klionsky and Codogno, 2013; Murrow and Debnath, 2013; Rubinsztein et al., 2012; Yang and Klionsky, 2010).

Emerging studies have revealed that autophagy plays important roles in immunity. In 2004, independent studies demonstrated for the first time that invading pathogens can be cargoes for autophagy (Gutierrez et al., 2004; Nakagawa et al., 2004). Today it is well accepted that autophagy can directly eliminate intracellular pathogens, including bacteria, fungal parasites, and viruses. Autophagy can also activate innate immune signaling cascades such as Toll-like receptor (TLR) signaling to

attack invading pathogens (Lee et al., 2007; Xu et al., 2007). However, microbes constantly undergo strong selective pressure to develop strategies to block host defense mechanisms. Indeed, studies indicate that some adaptations that confer pathogenicity involve microbial inactivation or subversion of autophagy through distinct mechanisms (Deretic and Levine, 2009; Kuballa et al., 2012; Levine et al., 2011; Yuk et al., 2012; Zhou and Zhang, 2012).

Autophagy's role in immunity is not limited to controlling infection by direct elimination of pathogens. For example, autophagy facilitates MHC (major histocompatibility complex) antigen presentation, indicating that autophagy is involved in adaptive as well as innate immunity (English et al., 2009; Paludan et al., 2005). Moreover, defects in autophagy are associated with autoimmune diseases such as Crohn disease (Levine et al., 2011; Schroder and Tschopp, 2010; Shi et al., 2012). Thus, autophagy is an integral part of our response to infection and plays a key role in immunity. A comprehensive understanding of autophagy as it pertains to microbial infection and the molecular mechanisms that underlie the interplay between autophagy and immune signaling pathways may enable us to unravel the pathogenesis of many infectious and immune diseases, and develop more effective therapeutic strategies for their treatment.

1.2 Autophagy

1.2.1 Types of Autophagy

There are three main types of autophagy: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (Figure 1.1). CMA is a process where a cytosolic chaperone protein, HSPA8/HSC70, specifically

recognizes its cargo proteins through a KFERQ-like motif and facilitates their translocation directly across the lysosomal membrane for degradation (Dice, 2007; Kaushik and Cuervo, 2012). Microautophagy involves the uptake of portions of cytoplasm by the direct invagination or protrusion of the lysosomal or vacuolar membrane (Mijaljica et al., 2011). The third process, macroautophagy, hereafter referred to as autophagy, is the best characterized and will be the focus of this chapter.

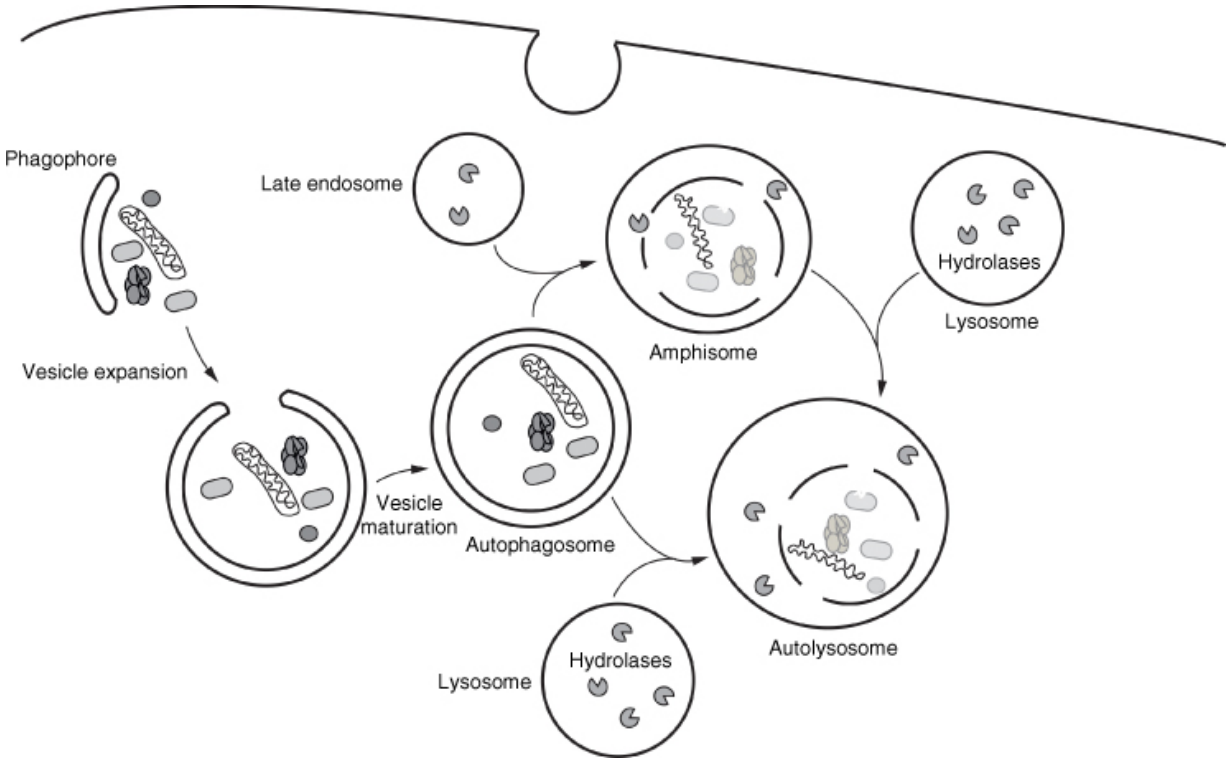

Figure 1.1 Schematic model of mammalian autophagy. Cargoes including cytosolic proteins, protein aggregates, and damaged organelles are sequestered by a phagophore, which will expand and mature to form a complete autophagosome. The outer membrane of the autophagosome fuses with either a late endosome (forming an amphisome, which then fuses with a lysosome) or lysosome, forming an autolysosome. Finally, the cargoes together with the inner membrane are degraded and the breakdown products are released back into the cytosol for reuse.

1.2.2 Morphology

The morphological hallmark of autophagy involves the *de novo* formation of a double-membrane organelle named the autophagosome; however, this structure is essentially an end product of the sequestration process and as such is not really the primary functional unit of autophagy. Rather, the precursor to the autophagosome, the phagophore, is the

dynamic membrane structure that is responsible for sequestering the cargos such as damaged organelles and invading pathogens (Figure 1.1). The phagophore expands with the addition of membrane, the sources of which are suggested to include almost every intracellular organelle. Upon completion, the phagophore seals and becomes a completed autophagosome. The autophagosome may fuse directly with a lysosome or, first, with a late endosome to form an intermediate amphisome. The subsequent fusion of the outer membrane of the autophagosome or the amphisome limiting membrane with a lysosome generates an autolysosome and exposes the cargoes to the degradative lysosomal enzymes. The degradation products, especially amino acids, are subsequently released back into the cytosol and are used in generating energy or as substrates for biosynthetic pathways.

1.2.3 Molecular Machinery

Even though autophagosomes have been observed by electron microscopy as early as the 1950s, the molecular mechanisms of autophagy have been poorly studied until the past two decades (Stromhaug and Klionsky, 2001). The molecular machinery was first identified through studies in budding yeast, *Saccharomyces cerevisiae*, and to date more than 30 autophagy-related (*ATG*) genes have been identified as being involved in this process (Harding et al., 1995; Klionsky et al., 2003; Thumm et al., 1994; Tsukada and Ohsumi, 1993). Subsequent work with mammalian cells has revealed homologs of the core autophagy machinery (Xie and Klionsky, 2007), supporting the notion that autophagy is evolutionarily conserved. At the same time, there are also increasing numbers of ATG proteins being identified in mammals and other model systems such as *Caenorhabditis elegans* that lack yeast homologs, suggesting an increased complexity and diversity of

function in higher eukaryotes (Klionsky and Codogno, 2013). For ease of discussion, the protein machinery of autophagy is subdivided into four major complexes in the following sections, and we focus on the mammalian autophagy machinery.

ULK1/ULK2 complex

Autophagy occurs at a basal level in cells under normal conditions. Upon stress or other stimuli, autophagy can be induced, and defects in regulation that prevent proper induction can lead to aberrant cell physiology; however, too much autophagy activity can also be detrimental to the cell. Thus, the level of autophagy must be tightly controlled. Accordingly, there are various factors that regulate autophagy induction, and studies have shown that the ULK1/ULK2 (unc-51 like autophagy activating kinase 1/2) complex functions in part in an early stage of autophagy regulation.

ULK1 and ULK2 are kinases and the other components of the complex include ATG13, RB1CC1/FIP200 (RB1-inducible coiled-coil 1), and ATG101. ATG13 directly interacts with ULK1/ULK2 and RB1CC1 regardless of the nutrient availability (Hosokawa et al., 2009; Jung et al., 2009); however, the phosphorylation status of these proteins changes under different conditions. In nutrient-rich conditions, a key upstream negative regulator of autophagy, the mechanistic target of rapamycin complex 1 (MTORC1) interacts with the complex and phosphorylates ULK1/ULK2 and ATG13, inhibiting ULK1/ULK2 kinase activity. Upon starvation, MTORC1 is released from the complex. ULK1/ULK2 and ATG13 are then partially dephosphorylated, leading to activation of ULK1/ULK2 kinase activity, which in turn leads to phosphorylation of ATG13 (presumably on distinct sites from those used by MTORC1) and RB1CC1 to induce autophagy (Chan, 2009;

Hara et al., 2008; Hosokawa et al., 2009). AMPK (AMP-activated protein kinase) also binds ULK1/ULK2 and positively regulates autophagy through phosphorylation upon glucose starvation; as expected, AMPK and MTORC1 phosphorylate ULK1 at different sites (Kim et al., 2011; Zhao and Klionsky, 2011).

Class III phosphatidylinositol 3-kinase complexes

The class III phosphatidylinositol 3-kinase (PtdIns3K) is generally thought to act downstream of the ULK1/ULK2 complex, mediating formation of phosphatidylinositol-3-phosphate (PtdIns3P) on the phagophore membrane, an event essential for autophagy. PtdIns3P serves to recruit downstream factors such as WIPI1 (WD repeat domain, phosphoinositide interacting 1) and WIPI2, which are involved in the trafficking of ATG9 and promote autophagosome maturation (Polson et al., 2010). In mammals, there are multiple class III PtdIns3K complexes with the core components being PIK3C3/VPS34 (phosphatidylinositol 3-kinase, catalytic subunit type 3), BECN1/Beclin 1 (beclin 1, autophagy related), and PIK3R4/VPS15/p150 (phosphoinositide-3-kinase, regulatory subunit 4). BECN1 can interact with several proteins, including AMBRA1 (autophagy/beclin-1 regulator 1), ATG14/ATG14L/Barkor, UVRAG (UV radiation resistance associated), KIAA0226/Rubicon and BCL2 (B-cell CLL/lymphoma 2) to form distinct complexes (Furuya et al., 2005; Itakura et al., 2008; Matsunaga et al., 2009; Petiot, 2000). BECN1 was first identified as a BCL2 binding protein. The interaction between BECN1 and BCL2 inhibits the binding of the former with PIK3C3, thus inhibiting autophagy. The ATG14–BECN1–PIK3C3–PIK3R4–AMBRA1 complex is specific for autophagy; ATG14 may direct this complex to the phagophore to promote autophagosome biogenesis (Itakura et al., 2008; Matsunaga et al., 2009),