Published in final edited form as:
Int Rev Cell Mol Biol. 2012 ; 293: 269–309. doi:10.1016/B978-0-12-394304-0.00013-0.

Membrane Trafficking and Phagosome Maturation During the Clearance of Apoptotic Cells

Nan Lu[*] and **Zheng Zhou**[*,†]

[*]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, USA

[†]Program in Developmental Biology, Baylor College of Medicine, Houston, Texas, USA

Abstract

Apoptosis is a cellular suicide process that quietly and efficiently eliminates unwanted or damaged cells. In metazoans, cells that undergo apoptosis are swiftly internalized by phagocytes and subsequently degraded inside phagosomes through phagosome maturation, a process that involves the fusion between phagosomes and multiple kinds of intracellular organelles and the gradual acidification of phagosomal lumen. In recent years, rapid progress has been made, in particular, through studies conducted in the model organism, the nematode *Caenorhabditis elegans*, in understanding the membrane trafficking events and molecular mechanisms that govern the degradation of apoptotic cells through phagosome maturation. These studies revealed the novel and essential functions of a large number of proteins, including the large GTPase dynamin, multiple Rab small GTPases and their regulatory proteins, the lipid second messenger PtdIns(3)P and its effectors, and unexpectedly, the phagosomal receptors for apoptotic cells, in promoting phagosome maturation. Further, novel signaling pathways essential for phagosome maturation have been delineated. Here, we discuss these exciting new findings, which have significantly deepened and broadened our understanding of the mechanisms that regulate the interaction between intracellular organelles and phagosomes.

Keywords

Apoptotic cell; Phagosome maturation; Apoptotic cell degradation; Membrane trafficking; Membrane fusion; PtdIns(3)P; PtdIns(3)P effectors; PI 3-kinase; Rab GTPase; V-type ATPase; Phagosomal tubules; Phagocytic receptor; CED-1; Dynamin; Endosomes; Lysosomes; *C. elegans*

1. Introduction

1.1. Importance of the removal of apoptotic cells for animal development and health

During animal development and adulthood, a large number of unwanted cells are eliminated via apoptotic cell death. Apoptotic cells display a number of morphological features, including cellular shrinkage, chromatin condensation, nuclear fragmentation, and plasma membrane blebbing yet maintain the integrity of the plasma membrane (Wyllie et al., 1980). Cells undergoing apoptosis are removed by phagocytosis, during which a phagocyte recognizes an apoptotic cell via a specific cell-surface receptor(s) and extends pseudopods along the surface of the dying cell; the extending pseudopods eventually fuse, leading to the scission of an apoptotic cell-containing membrane vacuole, known as a phagosome, from the plasma membrane (Fig. 8.1). The lumen of a nascent phagosome resembles the extracellular environment and lacks degradation capacity. Shortly after its formation, a nascent phagosome undergoes “phagosome maturation,” a process that results in the degradation of phagosomal contents. This process heavily relies on fusion events between phagosomes and organelles in the endocytic pathway, which lead to the phagosomal

acquisition of digestive enzymes as well as the gradual acidification of phagosomal lumen (Fig. 8.1) (Kinchen and Ravichandran, 2008; Vieira et al., 2002; Zhou and Yu, 2008).

Phagocytic removal of apoptotic cells eliminates excessive, damaged, or harmful cells from animal bodies and is important for organ sculpting, tissue homeostasis, immune tolerance, and tumorigenesis surveillance (Henson and Hume, 2006; Vaux and Korsmeyer, 1999). The efficient removal also prevents apoptotic cells from undergoing secondary necrosis and the releasing of potentially immunogenic contents (Savill and Fadok, 2000). Moreover, in mammals, the engulfment of apoptotic cells by macrophages induces the production of anti-inflammatory cytokines to suppress inflammatory responses (Erwig and Henson, 2007; Savill and Fadok, 2000). Defects in either engulfment or degradation of apoptotic cells contribute to autoimmune diseases, suggesting that eating and digesting apoptotic cells are both essential for proper immune responses (Elliott and Ravichandran, 2010; Nagata et al., 2010). The importance of the apoptotic cell degradation is best illustrated by DNase II knockout mice, which lack a lysosomal enzyme that degrades the nuclear DNA of apoptotic cells as well as nuclei expelled from erythroid precursor cells. The knockout mice suffer improper activation of innate immunity and die at birth due to severe anemia (Kawane et al., 2001; Yoshida et al., 2005a,b). The conditional knockout of DNase II in mice spleen and bone marrow after birth led to the production of TNF-α and various other cytokines, which resulted in the development of chronic polyarthritis (Kawane et al., 2006). Given that a large number of cells are eliminated via apoptosis every day in a human body as a result of physiological tissue turnover, understanding how apoptotic cells are degraded is of fundamental significance to human health.

1.2. Membrane trafficking events as critical driving forces for phagosome maturation

The studies of phagosome maturation conducted in cultured mammalian phagocytes that internalize latex beads or microbes have revealed a basic maturation route likely adopted by all phagosomes, regardless of luminal contents, to progress into lytic and bactericidal membrane compartments. After scission from the host cell membrane, a nascent phagosome sequentially fuses with early (sorting) endosomes, late endosomes, and eventually lysosomes (Fig. 8.2) (Desjardins et al., 1994; Vieira et al., 2002). This process enables nascent phagosomes to gradually acquire the properties of the donor organelles, including the distinct membrane markers and the progressively acidic pH value (Fig. 8.2). As a consequence, a phagosome ultimately evolves into a hybrid intracellular compartment called phagolysosome. Similar to lysosomes, phagolysosomes contain various digestive enzymes, including proteases, nucleases, lipases, and glycosidases, which display optimal activities in the highly acidic lumens of phagolysosome ($pH \leq 5.0$) and efficiently degrade luminal contents (Vieira et al., 2002). Phagosomes also actively recruit NADPH-oxidase complexes, which mediate the killing of pathogens inside phagosomes (Underhill and Ozinsky, 2002).

During phagosome maturation, phagosomes actively remodel lipid composition and membrane-associated proteins on their membranes. Several proteomic studies of isolated phagosomes revealed the identities of hundreds of proteins that are associated with phagosomes (Boulais et al., 2010; Garin et al., 2001; Shui et al., 2008; Stuart and Ezekowitz, 2008; Stuart et al., 2007). The continuous alteration of phagosomal-associating molecules is believed to enable phagosomes to preferentially interact with distinct endocytic organelles and in this manner drives the stepwise progression of phagosome maturation (Fig. 8.2). The Rab small GTPases and the lipid second messenger phosphatidylinositol 3-phosphate (PtdIns(3)P) play important roles in the maturation of phagosomes containing latex beads and microbes (Fig. 8.2). Rab5, localized on early endosomes and early phagosomes, is critical for endosome–phagosome tethering and fusion (Alvarez-Dominguez et al., 1996; Duclos et al., 2000; Jahraus et al., 1998). Rab7, which is recruited to phagosomes at a later stage than Rab5, is required for tethering and fusion between lysosomes and phagosomes

(Harrison et al., 2003; Vieira et al., 2003). PtdIns(3)P, which is produced by class III PI 3-kinase Vps34 on nascent phagosomes (Ellson et al., 2001a; Vieira et al., 2001), recruits effector proteins that contain PtdIns(3)P-binding modules such as FYVE (Fab1p, YOTB, Vac1p, and EEA1) or PX (Phox) domain (Birkeland and Stenmark, 2004). Two FYVE domain-containing proteins, EEA-1 and Hrs, were reported to participate in the maturation of phagosomes-containing pathogens (Fratti et al., 2001; Vieira et al., 2004). $p40^{phox}$, a PX domain-containing protein and another PtdIns (3)P-effector, is part of the phagocyte oxidase (Phox) complex that generates reactive oxygen species, which facilitates pathogen killings (Ellson et al., 2001b; Kanai et al., 2001).

The acidification of phagosomes is established by the activity of vacuolar-type proton transporting ATPase (V-ATPase), a multi-subunit transmembrane enzyme that uses the energy from ATP hydrolysis to pump protons from the cytosol of the host cell to phagosomal lumen (Fig. 8.2) (Beyenbach and Wieczorek, 2006). Both endosomes and lysosomes have been reported to be the source for phagosomal V-ATPase (Beyenbach and Wieczorek, 2006; Clarke et al., 2002; Sun-Wada et al., 2009).

1.3. Special features of the maturation of phagosomes containing apoptotic cells

Until recently, not much study has been devoted to understanding how apoptotic cells are degraded inside phagosomes. Unlike macrophages that ingest bacteria, which elicit a proinflammatory response, macrophages that engulf apoptotic cells secrete anti-inflammatory cytokines and actively suppress the secretion of the proinflammatory cytokines (Fadok et al., 1998; Freire-de-Lima et al., 2006; Serhan and Savill, 2005; Voll et al., 1997). Further, recent studies revealed that phagosomes containing apoptotic cells or opsonized living cells matured at different rates (Erwig et al., 2006). Study of apoptotic cell degradation will help uncover the unique features of the maturation of phagosomes containing apoptotic cells and reveal how phagocytes processing different phagosomal cargos elicit different immunological responses.

Recently, genetic and cell biological studies in model animals, including the nematode, the fruit fly, and the mice, as well as the biochemical studies in cultured mammalian cells, have shed a great amount of light on how phagocytes engulf and degrade apoptotic cells, through identifying new components of the clearance machinery, building up signaling transduction pathways, and revealing the consequential immunological responses. These studies deepened our understanding of phagosome maturation in general as well as unique mechanisms utilized for degrading apoptotic cells inside phagosomes.

Here, we review the molecular mechanisms that regulate phagosome maturation and the consequential degradation of apoptotic cells inside phagocytes by highlighting the critical functions of Rab GTPases, PtdIns (3)P, the large GTPase dynamin, and phagocytic receptors in promoting the interaction between intracellular organelles and phagosomes. We will further explain the organization of the phagosome maturation pathways, which are established by integrating the knowledge of the function and regulation of each phagosome maturation factor.

2. Novel Players and Mechanisms That Drive Phagosome Maturation

2.1. *Caenorhabditis elegans* as a model organism for studying the engulfment and degradation of apoptotic cells

2.1.1. Two partially redundant pathways that control the engulfment of apoptotic cells—The nematode *C. elegans*, a small, free-living, round worm, is an excellent model organism for studying apoptosis-related events due to its simple anatomy, known cell lineage, well-established genetics, and easily distinguishable apoptotic cell

morphology (Metzstein et al., 1998; Reddien and Horvitz, 2004). Apoptotic cells, often referred to as "cell corpses," are recognized within living animals under the Nomarski Differential Interference Contrast microscope (Sulston and Horvitz, 1977; Sulston et al., 1983). In *C. elegans*, cell corpses are rapidly engulfed and degraded by multiple types of neighboring cells (Sulston and Horvitz, 1977; Sulston et al., 1983; Zhou et al., 2001).

Seven *C. elegans* genes, *ced-1* (cell death abnormal), *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-10*, and *ced-12*, were identified from genetic screens for viable mutants that contain persistent cell corpses (Reddien and Horvitz, 2004). These genes act in two partially redundant pathways to control the engulfment of apoptotic cells (Ellis et al., 1991; Mangahas and Zhou, 2005), which are briefly reviewed here because both pathways were recently found to have additional functions in the subsequent degradation of apoptotic cells (Yu et al., 2008). In one pathway, CED-2, CED-5, and CED-12 act together to regulate CED-10, a Rac GTPase, which promotes cytoskeletal reorganization during engulfment (Reddien and Horvitz, 2004). In recent years, additional players involved in this pathway have also been reported. An UNC-73 (Trio homolog)–MIG-2 (RhoG homolog) signaling module was reported to activate CED-10 through CED-12 (deBakker et al., 2004), although conflicting observations were also reported (Lundquist et al., 2001). In addition, the Wnt pathway and an integrin-controlled pathway were found to control engulfment through this pathway (Cabello et al., 2010; Hsu and Wu, 2010).

The other pathway is composed of CED-1, CED-6, and CED-7. CED-1 is an engulfing cell-specific, type I transmembrane protein that acts as a phagocytic receptor (Zhou et al., 2001). CED-1 recognizes cell corpses, clusters to the region of the plasma membrane facing the cell corpse, and initiates pseudopod extension in response to extracellular "eat me" signals, one of which is phosphatidylserine (Venegas and Zhou, 2007; Zhou et al., 2001). CED-7, a homolog of mammalian ABC1 transporters, is implicated in presenting the "eat me" signals (Venegas and Zhou, 2007; Wu and Horvitz, 1998; Zhou et al., 2001). CED-6 acts downstream of CED-1, possibly as an adaptor for CED-1, to activate the fusion of intracellular organelles to phagocytic cups and the expansion of pseudopods during engulfment (Liu and Hengartner, 1998; Su et al., 2002; Yu et al., 2006; Zhou et al., 2001). An additional activity of CED-1 and CED-6 in actin reorganization mediated by CED-10 has also been proposed (Kinchen et al., 2005).

A new *ced* gene, *dyn-1*, which encodes the *C. elegans* large GTPase dynamin (DYN-1), was identified from a genetic screen for a new class of mutants that were embryonic lethal and contained persistent cell corpses (Yu et al., 2006). Dynamins are evolutionarily conserved factors mediating multiple aspects of intracellular vesicle trafficking (Praefcke and McMahon, 2004). *C. elegans* DYN-1 acts downstream of CED-6 to control both the engulfment and degradation of apoptotic cells (Yu et al., 2006, 2008). In Section 2.5, we discuss the molecular mechanisms that support these important roles of DYN-1.

2.1.2. Time-lapse imaging technique for monitoring the dynamics of multiple cellular events during the removal of apoptotic cells—

The study of apoptotic cell removal traditionally focused on the engulfment process. Recently developed techniques have helped reveal new functions in phagosome maturation for multiple factors, including several factors previously thought to function only in engulfment (see sections below). A time-lapse fluorescent microscopic imaging protocol has recently been developed that allows the observation of the engulfment and phagosome maturation processes, as well as the dynamic changes of the subcellular localization of GFP- or mRFP-tagged proteins, in living *C. elegans* embryos (He et al., 2010; Lu et al., 2009, 2011; Mangahas et al., 2008; Yu et al., 2006, 2008). The embryonic development of *C. elegans* follows the same cell division lineage, making it possible to comparatively analyze the same apoptotic cells in wild-type

and mutant backgrounds (Sulston et al., 1983). Thus, any defects observed in a mutant can be unambiguously attributed to the genetic difference instead of individual variability of different apoptotic cells or engulfing cells. In wild-type embryos, the engulfment process lasts only 4–6min, whereas it takes 40–60min for a nascent phagosome to be degraded (He et al., 2010; Yu et al., 2006, 2008). In developing embryos, what was learned through observing the dynamics of multiple events occurring during phagocytosis and phagosome maturation indicate that a largely similar series of cellular events occur during the maturation of phagosomes containing apoptotic cells in *C. elegans* and phagosomes containing opsonized cells in mammalian systems (Kinchen and Ravichandran, 2008; Vieira et al., 2002; Zhou and Yu, 2008), although significant differences in the dynamics of certain phagosome maturation events have also been observed (Yu et al., 2006, 2008).

2.1.3. Genetic approaches that identified a large number of novel phagosome maturation factors—In the past 5 years, genetic screens conducted in *C. elegans* for mutants defective in the removal of apoptotic cells have led to the identification of a number of factors essential for promoting phagosome maturation (Table 8.1). These screens include several classical random mutagenesis screens (Guo et al., 2010; Li et al., 2009; Lu et al., 2008; Mangahas et al., 2008; Nieto et al., 2010; Yu et al., 2006), one genome-wide RNA interference (RNAi) screen (Almendinger et al., 2011; Kinchen and Ravichandran, 2010; Kinchen et al., 2008), and a couple of smaller-scale, candidate gene screens (Lu et al., 2011; Xiao et al., 2009). Further, reverse genetic approaches, which focused on examining the null mutant phenotypes of the *C. elegans* homologs of known mammalian phagosome maturation factors, further determined the functions of a few factors in whole-animal and developmental contexts (Kinchen et al., 2008; Yu et al., 2008). Genetic analyses, together with cell biological characterizations, further ordered the phagosome maturation factors in pathways. The genetic studies carried out in *Drosophila*, zebra fish, and mammals also expanded the troops of phagosome maturation factors that regulate apoptotic cell degradation. Below is a detailed discussion that integrates the knowledge learned from these model organisms.

2.2. Rab GTPases: Running in order

Rab proteins are a large group of conserved small GTPases that act as tethering factors to promote the homotypic and/or heterotypic fusion between intracellular organelles or between an intracellular organelle and the plasma membrane (Stenmark, 2009). Rab GTPases switch between an “inactive” GDP-bound state and an “active” GTP-bound state (Stenmark, 2009). The conversion from the GDP-bound to the GTP-bound state is catalyzed by guanine nucleotide exchange factors (GEFs), whereas the GTPase-activating proteins (GAPs) stimulate the intrinsic GTPase activity of Rabs, converting the active form of a Rab to its inactive form (Stenmark, 2009). Rab GTPases regulate a wide range of membrane trafficking events by ensuring the delivery of cargos to the appropriate subcellular destinations and by coordinating intracellular signaling events with membrane trafficking (Stenmark, 2009). The diverse functions of Rab GTPases are mediated by Rab effector proteins, a large group of heterogeneous proteins recruited to the target membrane by interacting with GTP-bound Rabs (Grosshans et al., 2006).

Proteomic approaches have identified more than a dozen of Rab GTPases that are associated with purified phagosomes containing latex beads or bacteria (Garin et al., 2001; Smith et al., 2007; Stuart et al., 2007), suggesting that other Rabs in addition to Rab5 and Rab7 might also be involved in phagosome maturation. Recent studies of the apoptotic cell removal in *C. elegans* not only revealed the conserved essential functions of RAB-5 and RAB-7 in the degradation of apoptotic cells (Kinchen et al., 2008; Yu et al., 2008) but also uncovered novel functions of RAB-2 and RAB-14 in promoting phagosome maturation (Fig. 8.3, Table

8.1) (Guo et al., 2010; Lu et al., 2008; Mangahas et al., 2008). Further, these studies identified multiple regulators of RAB GTPases that control the localization and activation of RABs on phagosome membrane (Kinchen and Ravichandran, 2010; Li et al., 2009; Nieto et al., 2010). Together, these studies have revealed that different RABs associate with phagosomes at different stages of maturation and act to facilitate the docking and fusion of intracellular organelles of different identities to phagosomes.

2.2.1. Functions of RAB GTPases in the maturation of apoptotic cell-containing phagosomes

RAB-5: Mammalian Rab5 plays an essential role in the endocytic pathway, facilitating the tethering and fusion of endocytic vesicles with early (sorting) endosomes as well as the homotypic fusion between early endosomes (Bucci et al., 1992; Gorvel et al., 1991). Rab5 also promotes the fusion of endo-somes to phagosomes containing latex beads or microbes, both *in vitro* and *in vivo* (Alvarez-Dominguez et al., 1996; Duclos et al., 2000; Jahraus et al., 1998). Recent studies have found that the incorporation of early endosomes into phagosomes is critical for the degradation of apoptotic cells (Yu et al., 2006), and that RAB-5 is important for promoting the maturation of phagosomes containing apoptotic cells (Kinchen et al., 2008; Kitano et al., 2008). It is thus plausible that RAB-5 specifically tethers early endosomes to phagosomes that contain apoptotic cells through its conserved tethering activity, regardless of the identity of the phagocytic cargos. Analogous with its function on early endosomes (Christoforidis et al., 1999; Murray et al., 2002; Shin et al., 2005), RAB-5 might also participate in the recruitment and activation of class III PI3K VPS-34, one of the RAB-5's effector proteins, that produces PtdIns(3)P on phagosomes (Fig. 8.4B).

RAB-7: Inactivating *C. elegans rab-7* by gene deletion or RNAi results in the arrest of phagosome maturation (Kinchen et al., 2008; Yu et al., 2008). Like its mammalian homolog, *C. elegans* RAB-7 plays essential roles in the recruitment and fusion of lysosomes into phagosomes (Harrison et al., 2003; Yu et al., 2008). During phagosome maturation, lysosomes meet phagosomes in two alternative routes: random collision or phagosomal tubule-aided attachment (Yu et al., 2008). Phagosomes frequently extend membrane tubules that capture and recruit lysosomes from cytosol to phagosomal surfaces (Harrison et al., 2003; Yu et al., 2008). In both *C. elegans* and mammalian cells, inactivating RAB-7 diminishes phagosomal tubules, resulting in an inefficient recruitment of lysosomes (Harrison et al., 2003; Yu et al., 2008). Further, in *C. elegans*, inactivating RAB-7 impairs the fusion between phagosomes and lysosomal particles that associate with phagosomal membranes, regardless whether the particle is brought to phagosomal surfaces by membrane tubules or not (Yu et al., 2008). The fusogenic activity of RAB-7 is reminiscent of its yeast ortholog Ypt7, which is required for homotypic vacuole fusion (Cai et al., 2007). Together, the severe defects in the recruitment and fusion of lysosomes to phagosomes resulted from *rab-7* deletion block the formation of phagolysosome and the consequential apoptotic cell degradation (Fig. 8.4C; Table 8.1) (Yu et al., 2008).

RAB-2: RAB-2 (another name: UNC-108) was the first novel RAB GTPase for phagosome maturation identified by forward genetic screens in *C. elegans* (Lu et al., 2008; Mangahas et al., 2008). Like RAB-7, RAB-2 plays an important role in the recruitment and fusion of lysosomes to phagosomes; however, unlike RAB-7, RAB-2 is also required for the acidification of phagosomal lumen (Table 8.1) (Mangahas et al., 2008; Yu et al., 2008). RAB-2 and RAB-7 might control lysosome–phagosome fusions in parallel; alternatively, they might act at different stages in a series of membrane trafficking events that lead to lysosome–phagosome fusions. Proteomic studies in *Drosophila* and mammals have identified Rab2 as a component of phagosomes (Garin et al., 2001; Stuart et al., 2007). It

remains to be elucidated whether mammalian or *Drosophila* Rab2 plays a conserved role in the maturation of phagosomes.

RAB-14: Like Rab2, mammalian and *Drosophila* Rab14 were also detected on phagosomes by proteomic studies (Garin et al., 2001; Stuart et al., 2007). Both mammalian and *Dictyostelium* Rab14 were known to be involved in phagosome maturation. *Dictyostelium* Rab14 promotes the homotypic fusion between phagosomes (Harris and Cardelli, 2002), whereas on phagosomes that contain pathogen *Mycobacterium tuberculosis*, mammalian Rab14 stimulates the fusion between phagosomes and early endosomes (Kyei et al., 2006). Genetic studies in *C. elegans* identified the role of RAB-14 in the degradation of apoptotic cells (Guo et al., 2010). Similar to RAB-2, RAB-14 is required for the incorporation of lysosomes to phagosomes and for the acidification of phagosomal lumen (Table 8.1) (Guo et al., 2010; Lu et al., 2008; Mangahas et al., 2008).

RAB-2, RAB-7, and RAB-14—functional relationships: The *rab-2;rab-14* double mutants contained more persistent cell corpses than any single mutants, suggesting RAB-2 and RAB-14 act in a partially redundant manner in degrading apoptotic cells (Guo et al., 2010). By examining the differential roles of RAB-2, RAB-14, and RAB-7 in the formation of phagolysosomes, Guo et al. (2010) concluded that RAB-2 and RAB-14 play partially redundant roles in recruiting lysosomes and tethering lysosomes to phagosomes, whereas RAB-7 acts subsequently to promote the lysosome–phagosome fusion (Guo et al., 2010). This cooperative action is consistent with the concurrent enrichment of these three RAB proteins on the phagosomes (Fig. 8.4A) (Guo et al., 2010; Mangahas et al., 2008; Yu et al., 2008). On the other hand, further investigation is needed to integrate the lysosomes-recruitment activities of RAB-2 and RAB-14 proposed by Guo et al. (2010) with the membrane fusion activities observed from *C. elegans* RAB-2 and the mammalian and *Dictyostelium* Rab14 (Harris and Cardelli, 2002; Kyei et al., 2006; Mangahas et al., 2008).

2.2.2. Regulation of RAB GTPase during phagosome maturation

2.2.2.1. RAB proteins that are sequentially recruited to the surfaces of phagosomes: RAB-5, RAB-2, RAB-14, and RAB-7 are sequentially recruited to phagosomes undergoing phagosome maturation (Guo et al., 2010; He et al., 2010; Mangahas et al., 2008; Yu et al., 2008) (Fig. 8.4A). RAB-5 is detectable on phagosomal membranes immediately after the sealing of phagocytic cups (He et al., 2010), followed by the nearly simultaneous recruitment of RAB-2 and RAB-14 (Guo et al., 2010; Mangahas et al., 2008). RAB-7 is then recruited to phagosome membrane, slightly lagging behind RAB-2 and RAB-14 (Yu et al., 2008). Once recruited, RAB-7 maintains its association with a phagosome until the engulfed apoptotic cell is completely degraded (Yu et al., 2008). In comparison, the localization patterns of RAB-5, RAB-2, and RAB-14 on phagosomes are relatively transient (Fig. 8.4A) (Guo et al., 2010; He et al., 2010; Mangahas et al., 2008). How the localization patterns and activities of these RAB proteins are regulated is the theme of the following subsections.

2.2.2.2. Regulation of Rab5 on phagosomes: Although Rab5 is one of the best-characterized Rab GTPases, how it is regulated during phagosome maturation is just begun to be understood. Genetic studies in *C. elegans* demonstrated that the large GTPase DYN-1 plays a key role in recruiting RAB-5 (He et al., 2010; Kinchen et al., 2008). The inactivation of DYN-1 severely delays the recruitment of RAB-5 onto phagosomes (He et al., 2010; Kinchen et al., 2008). In cultured NIH/3T3 fibroblasts, Kinchen et al. (2008) observed that a dominant-negative form of dynamin 2 prevented Rab5 from associating with phagosomes, and that mammalian Vps34, the class III PI 3-kinase, interacted with both Dyn2 and $Rab5^{GDP}$. Kinchen et al. proposed that Vps34 acted as a bridging molecule to facilitate the recruitment of Rab5 by dynamin 2. This model appears to be different from the mechanism

of Rab5 action learned from endocytosis, in which the active form of Rab5 ($Rab5^{GTP}$) recruits Vps34 onto early endosomes through interacting with Vps34-interacting protein Vps15 (Christoforidis et al., 1999; Murray et al., 2002; Shin et al., 2005). Further genetic and biochemical studies are needed to integrate these two models.

In the search for the GEF protein(s) of Rab5, Kitano et al. (2008) identified Gapex-5 that specifically activates Rab5 on phagosomes that degrade apoptotic cells (Kitano et al., 2008). Kitano et al. further observed that microtubules and EB1, a microtubule-tip-associating protein, interacted with Gapex-5 and were involved in the activation of Rab5 on phagosomes. These observations led to the model that the dynamic microtubule network delivers Gapex-5 to phagosomes to activate Rab5 (Fig. 8.4B). Interestingly, inactivating three *C. elegans* Vps9 domain-containing candidate GEFs for RAB-5 (RME-6, RABX-5, and TAG-333), either individually or in combination, did not appear to affect the removal of apoptotic cells, suggesting the existence of a novel GEF that activates RAB-5 on *C. elegans* phagosomes (Kinchen et al., 2008).

After a transient association with nascent phagosomes, RAB-5 rapidly dissociates (He et al., 2010; Kitano et al., 2008). The physiological importance of inactivating RAB-5 is manifested by the observation that overexpression of RAB-5 (Q78L), the constitutively active form of RAB-5 that associates with phagosomes for a prolonged period, prolongs the cell-corpse removal process (Li et al., 2009). Most GAPs for Rabs contain a Tre/Bub2/Cdc16 (TBC) domain that accelerates the GTP hydrolysis on Rab GTPases (Bernards, 2003). *C. elegans* TBC-2 was recently identified as a GAP of RAB-5 (Fig. 8.3) (Chotard et al., 2010; Li et al., 2009). Inactivating *tbc-2* by genetic mutations does not affect the recruitment of RAB-5 onto phagosomes; rather, it delays the dissociation of RAB-5 from phagosomal surfaces (Table 8.1) (Li et al., 2009). In *tbc-2* mutants, several phagosome maturation events, including the recruitment of RAB-7, the formation of phagolysosomes, and the acidification of phagosomal lumen, are all defective (Table 8.1) (Li et al., 2009). As a consequence, cell corpses are not properly degraded. This study highlights the importance of the timely dissociation of RAB-5 from phagosome maturation.

Chotard et al. (2010) independently found that TBC-2 was able to specifically catalyze GTP hydrolysis on RAB-5 *in vitro* and inactivate RAB-5 on endosomes in *C. elegans*, which further confirmed that TBC-2 acted as a RAB-5 GAP. Intriguingly, constitutive activation of RAB-5 appeared to impair the recruitment of RAB-7 onto phagosomes but resulted in hyperactivation of RAB-7 on endosomes (Chotard et al., 2010; Li et al., 2009), suggesting that as a RAB-5 GAP, TBC-2 differently regulates endosome maturation and phagosome maturation.

2.2.2.3. RAB-5 to RAB-7 conversion: Rab5 and Rab7, which are enriched on early and late phagosomes/phagolysosomes, respectively, were proposed to act coordinately to mediate the early-to-late transition of phagosome maturation (Vieira et al., 2002). Recently, three groups independently identified a protein complex that promoted the transition of endosomes and phagosomes from the RAB-5(+) to the RAB-7(+) stage in *C. elegans* (Kinchen and Ravichandran, 2010; Nieto et al., 2010; Poteryaev et al., 2010). This complex is composed of two components, SAND-1 and CCZ-1, the *C. elegans* homologs of yeast and mammalian Mon1 and Ccz1, respectively. The Mon1–Ccz1 complex was first identified in the budding yeast, in which it mediated homotypic vacuole fusion (Wang et al., 2002, 2003). Inactivation of *C. elegans sand-1* or *ccz-1* significantly increases the number of undegraded germ apoptotic cells; in addition, in the above mutants, the number of RAB-5(+) phagosomes is substantially increased while RAB-7(+) phagosomes are hardly observable (Kinchen and Ravichandran, 2010; Nieto et al., 2010). Similarly, inactivating *sand-1* results in the arrest of endosomes at a RAB-5(+) but RAB-7(−) stage and causes the accumulation of endocytosed

but undigested yolk protein in enlarged endosomes, a phenotype resembling that caused by *rab-7* (RNAi) (Poteryaev et al., 2007, 2010). Together, these phenotypes indicate that the functions of SAND-1 and CCZ-1 are necessary for phagosomes to release RAB-5 and acquire RAB-7 on phagosomes and endosomes (Fig. 8.4D).

SAND-1 and its mammalian homolog Mon1 were detectable on phagosomes (Kinchen and Ravichandran, 2010). Mammalian Mon1 concurrently interacts with Ccz1 and the GTP-bound form of Rab5 ($Rab5^{GTP}$), suggesting that $Rab5^{GTP}$ might play a role in recruiting Mon1–Ccz1 complex (Fig. 8.4D) (Kinchen and Ravichandran, 2010). How does the SAND-1–CCZ-1 complex regulate RAB-5/RAB-7 conversion on phagosomes? SAND-1 is frequently detected on phagosomes that possess both RAB-5 and RAB-7, suggesting that SAND-1 may act as a bridging molecule that simultaneously interacts with two RABs (Kinchen and Ravichandran, 2010). Indeed, in addition to interacting with $Rab5^{GTP}$, the mammalian Mon1–Ccz1 complex, but not the Mon1 or Ccz1 subunits alone, is able to interact with $Rab7^{GTP}$ or $Rab7^{GDP}$ (Kinchen and Ravichandran, 2010). The mammalian Mon1–Ccz1 complex facilitates the dissociation of GDP-dissociation inhibitor from Rab7, and thus might be directly involved in the recruitment of Rab7 onto phagosomes (Fig. 8.4D) (Kinchen and Ravichandran, 2010). In addition, the yeast Mon1–Ccz1 complex was recently found to possess a novel GEF activity toward Ypt7, the yeast homolog of Rab7 (Nordmann et al., 2010), indicating a similar GEF activity of this complex on phagosomes (Fig. 8.4D).

SAND-1/Mon1 is known to inactivate RAB-5 on endosomes by promoting the dissociation of its GEF protein RABX-5 from endosomes (Poteryaev et al., 2010). However, RABX-5 and its mammalian ortholog Rabex-5 are not required for phagosome maturation (Kinchen et al., 2008; Kitano et al., 2008). Therefore, it is currently unclear how the SAND-1–CCZ-1 complex inactivates RAB-5 during phagosome maturation.

2.2.2.4. HOPS complex: A candidate RAB-7 effector: The homotypic fusion and protein sorting (HOPS) complex, composed of six subunits Vps11, Vps16, Vps18, Vps33, Vps39, and Vps41, were first identified in the budding yeast as a complex that promotes homotypic vacuole fusion (Nickerson et al., 2009). In *C. elegans*, inactivating each of the six subunits of the HOPS complex affects the degradation of apoptotic cells (Kinchen et al., 2008; Xiao et al., 2009). Further characterization of a *vps-18* deletion mutant strain has suggested that VPS-18 is required for the biogenesis of endosomes and lysosomes and for the fusion of phagosomes with lysosomes (Xiao et al., 2009). Similarly, in fruit flies carrying a null mutation of the *full-of-bacteria* (*fob*) gene, which encodes *Drosophila* Vps16B, phagosomes containing engulfed bacteria fail to fuse with lysosomes, rendering flies hypersensitivity to infections with nonpathogenic bacteria (Akbar et al., 2011). Although it was reported that Vps39p possesses a GEF activity for Ypt7p (yeast Rab7) (Wurmser et al., 2000), the disruption of HOPS complex was shown to arrest phagosome maturation at a RAB-7(+) stage (Akbar et al., 2011; Kinchen et al., 2008). Likewise, *vps-39* (RNAi) does not affect the enrichment of RAB-7 on intestinal endosomes of *C. elegans* (Chotard et al., 2010). These observations suggest that the HOPS complex is likely to act at a step downstream of RAB-7 during phagosome maturation (Fig. 8.4C). Consistently, the HOPS complex is known as an Ypt7p effector (Nickerson et al., 2009). In the yeast vacuole fusion system, HOPS facilitates membrane tethering and promotes *trans*-SNARE complex formation, while Ypt7p contributes to HOPS membrane association (Collins and Wickner, 2007; Hickey and Wickner, 2010; Hickey et al., 2009). Whether RAB-7 and HOPS complex act in a similar manner to promote the fusion of lysosomes with phagosomes needs to be tested.

2.3. Acidification of phagosomal lumen and vacuolar-type ATPase

The progressive acidification of phagosomal lumen is a hallmark and critical step of phagosome maturation, as an acidic environment is optimal for the activities of hydrolytic enzymes that degrade phagosomal contents (Beyenbach and Wieczorek, 2006; Steinberg et al., 2007). The requirement of a few Rab GTPases for acidifying phagosomes containing apoptotic cells has been recently examined in *C. elegans*. Mangahas et al. (2008) have established an assay that measures the level of phagosome acidification using Lysosensor blue/yellow DND-160, a dual-emission wavelength dye that displays strong yellow fluorescence at pH≤5.0. Using this assay, it was found that a loss-of-function mutation of *rab-2* but not that of *rab-7* impaired the acidification of phagosomes (Mangahas et al., 2008; Yu et al., 2008). The above results were confirmed by similar acidification assays using Lysosensor green (Guo et al., 2010). Because RAB-7 is essential for phagosome–lysosome fusion, these observations suggest that the acidification of phagosomes that contain apoptotic cells does not appear to depend on efficient phagosome–lysosome fusion, a conclusion consistent with a previous observation that phagosomal lumen is dramatically acidified (pH~5) before phagosomes fuse with lysosomes (McNeil et al., 1983).

Although the function of RAB-7 is largely dispensable for the acidification of phagosomes in *C. elegans*, the inactivation of mammalian Rab7, by overexpressing a dominant-negative form of Rab7, inhibits acidification of phagosomes that contain latex beads (Harrison et al., 2003). Consistently, mammalian Mon1, a positive regulator of Rab7, is required for phagosome acidification; whereas the inactivation of SAND-1, the *C. elegans* counterpart of Mon1, does not affect the phagosomal acidification (Kinchen and Ravichandran, 2010). These observations suggest that the regulation of phagosome maturation varies in different contexts and might depend on the identity of phagocytes and/or phagosomal cargos.

Like *rab-2* mutants, *C. elegans rab-14* mutants displayed a defect in phagosome acidification, which is further compromised in *rab-2;rab-14* double mutants, suggesting again that RAB-2 and RAB-14 act partially redundantly to promote phagosome acidification (Guo et al., 2010).

Notably, the role of the vacuolar-type ATPase (V-ATPase) in the acidification of phagosome containing apoptotic cells or the degradation of apoptotic cells has not been thoroughly tested. The only reported work examined a V-ATPase subunit in the degradation of apoptotic cells in zebrafish. By directly visualizing the removal process of apoptotic neurons in the intact brain of living zebrafish, Peri and Nusslein-Volhard (2008) found that the a1 subunit within the V0-complex of the V-ATPase is essential for the degradation of apoptotic neurons by microglia cells, resident brain phagocytes. Surprisingly, the depletion of the a1 subunit by morpholino injection did not affect phagosome acidification; rather, it impaired the efficient fusion between phagosomes and lysosomes (Peri and Nusslein-Volhard, 2008). A role of the V0-ATPases in promoting membrane fusion has been previously reported for synaptic transmission and yeast vacuolar fusion (Bayer et al., 2003; Hiesinger et al., 2005; Liegeois et al., 2006). Thus, V-ATPase might have additional functions besides acidifying phagosomes during phagosome maturation.

2.4. Lipid second messenger PtdIns(3)P and its effector proteins: Lipid–protein interactions

2.4.1. PtdIns(3)P and its production on phagosomes—As a derivative of phosphatidylinositol (PtdIns), PtdIns(3)P plays important signaling roles in intracellular membrane trafficking events, which include endocytic trafficking, retrograde trafficking, autophagy, and phagosome maturation (Backer, 2008; Di Paolo and De Camilli, 2006). During the removal of apoptotic cells in *C. elegans*, PtdIns(3)P is dynamically enriched on

maturing phagosomes (Kinchen et al., 2008; Mangahas et al., 2008; Yu et al., 2008). In *C. elegans* embryos, PtdIns(3)P appears on phagosomal surfaces in two consecutive waves: the initial burst of PtdIns(3)P, which appears upon the closure of a phagocytic cup and dissipates after 10–15min, and a subsequent reappearance of a relatively weaker PtdIns(3)P signal ~10min later, which lasts until an apoptotic cell is fully degraded (Mangahas et al., 2008; Yu et al., 2008). A similar pattern of PtdIns(3)P oscillation has also been observed on the surfaces of phagosomes containing pathogens (Chua and Deretic, 2004; Pattni et al., 2001). However, the physiological significance of this oscillation phenomenon remains unclear. Intracellular PtdIns(3)P pool enriched in endosomes, phagosomes, and autophagosomes is thought to be largely produced by class III PI 3-kinase Vps34, which phosphorylates the 3-hydroxyl group of the inositol ring of PtdIns (Backer, 2008; Vanhaesebroeck et al., 2001). Inactivation of PI 3-kinases in mammalian phagocytes by using inhibitors wortmannin or LY294002, or specific inactivation of Vps34 through injecting anti-Vps34 inhibitory antibody, greatly reduces the level of PtdIns(3)P on phagosomes and inhibits latex bead phagosomes from acquiring late endosome and lysosome markers, highlighting the important role of PtdIns(3)P and Vps34 in phagosome maturation (Fratti et al., 2001; Vieira et al., 2001). Similarly, RNAi knockdown of *C. elegans vps-34* causes the accumulation of undegraded germ cell corpses in worm gonad, suggesting that PtdIns(3)P is also required for the maturation of apoptotic cell-containing phagosomes (Kinchen et al., 2008). However, animals treated with *vps-34* RNAi or carrying a null mutation in *vps-34* only display a mild defect in the degradation of cell corpses (Kinchen et al., 2008; Li et al., 2009). In addition, inactivating *vps-34* does not completely deplete PtdIns(3)P on phagosomes (N. Lu and Z. Zhou, unpublished results). These observations suggest that in addition toVPS-34, there must be another PI 3-kinase(s) that generates PtdIns(3)P on phagosomes.

2.4.2. Novel PtdIns(3)P effectors that promote the degradation of apoptotic cells

2.4.2.1. Targeted genetic screens for PtdIns(3)P effectors: As a common feature, PtdIns(3)P recruits effector proteins with PtdIns(3)P-binding modules, such as the FYVE or PX (Phox homology) domains, to particular membrane compartments enriched with PtdIns(3)P (Birkeland and Stenmark, 2004). EEA1 and Hrs1 are two well-studied PtdIns(3)P effectors that play essential roles in endosomal trafficking (Saksena et al., 2007; Sasaki et al., 2007). Mammalian EEA1 and Hrs1 were reported to participate in the maturation of phagosomes containing latex beads or pathogens (Fratti et al., 2001; Vieira et al., 2004). However, inactivating genes encoding EEA-1 or HGRS-1, the *C. elegans* homologs of mammalian EEA1 or Hrs1, respectively, did not result in any apparent defect in the removal of apoptotic cells (Kinchen et al., 2008; Lu et al., 2011), suggesting that EEA-1 and HGRS-1 are not required for the maturation of phagosomes containing apoptotic cells.

Guided by the hypothesis that each PtdIns(3)P-regulated cellular process is mediated through a specific set of PtdIns(3)P effectors, a candidate genetic screen was carried out for the identification of PtdIns(3)P effectors specific for cell-corpse removal. This screen systematically examined the functional requirement of each *C. elegans* protein predicted to possess FYVE or PX domains and identified SNX-1, SNX-6, and LST-4/SNX-9, three PX-domain-containing proteins that belong to the sorting nexin family (Fig. 8.3), whose null mutations caused the accumulation of persistent cell corpses in both embryos and adult gonads (Lu et al., 2011). Both fluorescence microscopy and transmission electron microscopy studies indicate that null mutations of these genes specifically impair the degradation but not the engulfment of apoptotic cells (Lu et al., 2011). These three factors were also identified as apoptotic cell removal factors in independent targeted RNAi-based screens (Almendinger et al., 2011; Chen et al., 2010).

2.4.2.2. Three sorting nexins that regulate multiple phagosome maturation events as PtdIns(3)P effectors: As expected, *C. elegans* SNX-1 and LST-4/SNX-9 both interact with PtdIns(3)P *in vitro* and are transiently localized on phagosomes in a manner dependent on both their PX domains and the phagosomal PtdIns(3)P, indicating that they act as PtdIns(3)P effectors (Lu et al., 2011). Interestingly, SNX-6 specifically interacts with SNX-1 and relies on SNX-1 for association with phagosomes (Lu et al., 2011). Consistently, genetic epistasis analysis revealed that these three sorting nexins act in two parallel genetic pathways to promote phagosome maturation: SNX-1 and SNX-6 in one pathway, whereas LST-4/SNX-9 in the other pathway (Lu et al., 2011).

The three novel PtdIns(3)P effectors contribute to multiple molecular events involved in phagosome maturation. First, they are essential for the phagolysosome formation. Mutations in *snx-1* or *lst-4* significantly reduce the efficiency of the delivery of lysosomes into phagosomes, which is nearly completely blocked in *snx-1;lst-4* double mutants (Lu et al., 2011). This phenotype is partially caused by defects in the generation of phagosomal tubules, which act to capture and recruit lysosomes in the cytosol to the surface of phagosomes (Harrison et al., 2003; Yu et al., 2008). The ability of SNX-1 and LST-4/SNX-9 to promote phagosomal tubule formation can be attributed to their BAR (Bin-Amphiphysin-Rvs) domains (Fig. 8.3). SNX-1, SNX-6, and LST-4/SNX-9 all belong to the SNX-BAR subfamily of sorting nexins that each possesses a BAR domain, a banana-shaped dimer capable of interacting with negatively charged phospholipid head groups of lipid membranes and able to sense and induce membrane curvature (Gallop et al., 2006; Peter et al., 2004). Mutations of a few conserved basic residues in the BAR domain of SNX-1 or LST-4/SNX-9, predicted to locate on the membrane-contacting surface, render a partial loss of activities in phagosome maturation, suggesting that these proteins are likely to promote the extension of membrane tubules from phagosomal surface through the functions of BAR domains (Fig. 8.4E) (Lu et al., 2011). Consistent with this model, SNX-1 and LST-4/SNX-9 associate with phagosomal surfaces and are particularly enriched on phagosomal tubules and the base of the tubules (Lu et al., 2011).

In addition to this tubule-generating activity, LST-4/SNX-9 is also needed for phagosomes to acquire GTPases such as RAB-5 and RAB-7 (Almendinger et al., 2011; Lu et al., 2011). The molecular basis for this function can be attributed to LST-4/SNX-9's physical interaction with DYN-1, the *C. elegans* large GTPase dynamin, which is an essential organizer for many phagosome maturation events (see Section 2.5.). LST-4/SNX-9, like its mammalian homolog SNX-9, SNX-18, and SNX-33, contains an SH3 domain on its N-terminus and interacts with DYN-1 (Fig. 8.3) (Haberg et al., 2008; Lu et al., 2011; Lundmark and Carlsson, 2004; Shin et al., 2007; Soulet et al., 2005). LST-4 and DYN-1 colocalize on phagosomes (Almendinger et al., 2011). In addition, LST-4 and DYN-1 depend on each other for their phagosomal attachment: in *dyn-1*(*en9*) mutants, in which a DYN-1 point mutation abolishes DYN-1's self-assembly activity and consequentially its phagosome association, LST-4 fails to be recruited to phagosomes; and vice versa, in *lst-4* null mutants, a premature dissociation of DYN-1 from phagosomes is frequently observed (He et al., 2010; Lu et al., 2011). Since DYN-1 plays key roles in regulating the production of PtdIns(3)P and the recruitment of RAB-5 and RAB-7 onto phagosomes (see Section 2.5), the phagosome maturation arrest phenotype observed in *lst-4* mutants is likely partly caused by the reduced DYN-1 activity on nascent phagosomes, in addition to the lack of phagosomal tubule extension (Lu et al., 2011). On the other hand, the dissociation of LST-4 from phagosomal surfaces relies on DYN-1's GTP hydrolysis activity (Almendinger et al., 2011). These observations suggest a mutual regulatory mechanism for DYN-1 and LST-4/SNX-9 regarding their phagosome association and functions (Fig. 8.4E). In summary, these three sorting nexins facilitate phagosome maturation through regulating phagosomal membrane curvature and stabilizing DYN-1's attachment to phagosomal surfaces.

2.4.2.3. Do *C. elegans* SNX-1 and SNX-6 act as components of the retromer complex to promote the removal of apoptotic cells?: Mammalian SNX-1 and SNX-6, homologs of *C. elegans* SNX-1 and SNX-6, respectively, are components of the evolutionarily conserved retromer complex (Bonifacino and Hurley, 2008). A mammalian retromer consists of two subcomplexes, a membrane-deformation subcomplex composed of SNX-1 (or SNX-2) and SNX-5 (or SNX-6), and a cargo-recognition subcomplex composed of VPS26, VPS29, and VPS35, which recognizes the sorting motif within the cytoplasmic tail of membrane cargos (Bonifacino and Hurley, 2008; Wassmer et al., 2009). The retromer complex mediates endosome-to-Golgi retrieval of transmembrane receptors and other trafficking-related proteins in mammals and worms (Coudreuse et al., 2006; Pan et al., 2008; Shi et al., 2009; Verges et al., 2007; Yang et al., 2008). Recently, two groups independently examined whether *C. elegans* SNX-1 and SNX-6 act as components of the retromer complex to promote the removal of apoptotic cells and reported contradicting experimental results that led to opposite conclusions (Chen et al., 2010; Lu et al., 2011). Chen et al. (2010) concluded that SNX-1 and SNX-6 act in the *C. elegans* retromer complex to recycle phagocytic receptor CED-1 from phagosomal surfaces back to the plasma membrane, and in this manner facilitate apoptotic cell engulfment. This conclusion is made mainly based on the following experimental observations: (1) *snx-1* null mutant animals are primarily defective in the engulfment of apoptotic cells; (2) a CED-1::GFP reporter persists for a longer period of time on phagosomal surfaces in *snx-1* mutant embryos than in wild-type embryos, whereas CED-1 protein level is much lower in *snx-1* mutant embryos than in wild-type embryos; (3) SNX-1 is able to directly interact with the cytoplasmic tail as well as the extracellular domain of CED-1 (CED-1Ex); (4) overexpression of CED-1 is able to fully rescue the apoptotic cell removal of *snx-1* null mutants; and (5) inactivating each of VPS-26, VPS-29, or VPS-35, the subunits of the *C. elegans* cargo-recognition subcomplex caused defective removal of germ cell corpses, although the defects are milder than that displayed by the *snx-1* or *snx-6* single mutants (Chen et al., 2010).

Many key results addressing the same issue, obtained by Lu et al. (2011) under highly comparable experimental conditions, are different from those reported by Chen et al. (2010) and do not support the above model.

First of all, Lu et al. (2011) found that, in contrast to Chen et al.'s observation, the functions of VPS-26 and VPS-35 are not needed for the removal of germ apoptotic cells in the worm gonad. In addition, both Chen et al. and Lu et al. have reported that the cargo-selective complex is dispensable for the clearance of somatic apoptotic cells in developing embryos. These results strongly indicate that the retromer complex is unlikely involved in the removal of apoptotic cells.

Second, as mentioned earlier, by both fluorescence and transmission electronic microscopy, Lu et al. (2011) determined that the functions of both SNX-1 and LST-4/SNX-9 are specifically needed for promoting phagosome maturation, not for the internalization of apoptotic cells. This conclusion is consistent with the roles of SNX-1 and LST-4 as PtdIns(3)P effectors, as PtdIns(3)P is not detected on phagosomal surfaces until engulfment is complete and phagosomes are sealed (Mangahas et al., 2008; Yu et al., 2008). The engulfment and degradation of phagocytic targets are two different cellular events that are executed through distinct mechanisms (Caron, 2001; Vieira et al., 2002). Determining accurately which of these two events the PtdIns(3)P effectors are involved in is pivotal for further understanding their molecular functions.

More importantly, Lu et al. (2011) have reported different findings regarding whether SNX-1 and SNX-6 mediate the recycling of CED-1 during apoptotic cell removal and whether such recycling is necessary for CED-1's apoptotic cell removal activities. As a

type-I transmembrane protein, CED-1 performs two essential functions for the removal of apoptotic cells: it acts as a phagocytic receptor that recognizes apoptotic cells and initiates their engulfment and as a phagosome maturation initiator that triggers their phagosomal degradation (see Section 2.6.1) (Yu et al., 2008; Zhou et al., 2001). Like Chen et al. (2010), Lu et al. (2011) also observed the prolonged existence of CED-1::GFP on the surface of phagosomes in *snx-1* null mutant embryos. However, in contrast to Chen et al., Lu et al. found that this prolonged presence of CED-1 on the phagosome did not appear to affect the level of CED-1 on the plasma membrane of the host cells. Further, in developing embryos, in particular during early to mid-embryogenesis, when most cell death events occur, the overall levels of CED-1::GFP in entire *snx-1*, *snx-6*, or *lst-4* single mutant embryos are similar to that in wild-type embryos, observed in stage-by-stage comparisons (Lu et al., 2011). Just as importantly, Lu et al. observed that the overexpression of CED-1 failed to produce any rescuing activity toward the corpse removal defect of *snx-1* or *snx-6* mutant embryos, in contrast to the full rescuing activity reported by Chen et al. (2010). Together, these results indicate that the level of CED-1 on engulfing cell surfaces is not primarily controlled by SNX-1 or SNX-6.

Chen et al. (2010) reported the direct interaction between SNX-1 and the cytoplasmic tail of CED-1, as an evidence to support the model that the retromer complex acts to recycle CED-1 from phagosomal surfaces. However, the same protein–protein interaction assay also showed that SNX-1 and SNX-6 each interacts with the CED-1Ex (Chen et al., 2010). Given that SNX-1 and SNX-6 are cytoplasmic proteins (not secreted proteins) and are thus not expected to be in the extracellular space like CED-1Ex, which is extracellular during engulfment and in the phagosomal lumen after engulfment, the observed interactions between SNX-1 or SNX-6 and CED-1Ex raise the concern about the significance of this particular assay.

The direct evidence that SNX-1 acts downstream of CED-1 comes from the observation that, in *snx-1* mutant embryos, the dynamic phago-somal enrichment patterns of DYN-1, PtdIns(3)P, and RAB-5, three phagosome maturation factors that are dependent on CED-1 for their enrichment on phagosomal surfaces, occur in the normal manner (Lu et al., 2011). These observations, together with our finding that *snx-1* mutants are not defective in cell-corpse engulfment, indicate that the function of CED-1 is not affected by the null mutation in *snx-1* (Lu et al., 2011). In addition, CED-1 controls the phagosomal recruitment of SNX-1, which is likely attributed to CED-1's ability to promote the PtdIns(3)P production on phagosomes (Lu et al., 2011; Yu et al., 2008). The above results have established that the SNX-1/SNX-6 complex acts downstream of CED-1 to control phagosome maturation (Fig. 8.5). Recently, several reports have revealed cases in which mammalian SNX1 regulates membrane trafficking and membrane remodeling in retromer-independent mechanisms (Gullapalli et al., 2006; Nisar et al., 2010; Prosser et al., 2010). Lu et al.'s finding that the SNX-1/SNX-6 complex promotes the incorporation of lysosomes into phagosomes through regulating phagosomal curvature and tubule formation has revealed a novel retromer-independent function of the SNX-1/SNX-6 complex, a function regulated by the phagocytic receptor and phagosome maturation initiator CED-1.

2.5. Dynamin, key organizer of phagosome maturation events

2.5.1. How does DYN-1 regulate the removal of apoptotic cells?—Dynamin is a conserved large GTPase that plays pivotal roles in many membrane-related cellular processes (Praefcke and McMahon, 2004). Dynamin and dynamin-related proteins displayed a number of molecular functions, the best-studied being the membrane fission activity of dynamin essential for the release of endocytic vesicles from plasma membranes (Schmid and Frolov, 2011). In addition, dynamin and dynamin-related proteins also act to promote

membrane fusion events during the fusion of mitochondria and yeast vacuoles (Hoppins and Nunnari, 2009; Peters et al., 2004). A genetic screen for *C. elegans* mutants that contain a large number of persistent cell corpses led to the isolation of 14 loss-of-function alleles of *dyn-1*, which encode dynamin (Yu et al., 2006). Phenotypic and molecular characterization revealed that *dyn-1* is essential for promoting both the engulfment and degradation of apoptotic cells. During the engulfment of apoptotic cells, DYN-1 is transiently enriched on phagocytic cups, where it promotes the recruitment and fusion of early endosomes to extending pseudopods, providing lipid materials to support the rapid expansion of phagocyte membrane along the surface of dying cells (He et al., 2010; Yu et al., 2006). Prior to the discovery of DYN-1's engulfment role, it was reported that the expression of a dominant-negative mutant form of mammalian dynamin 2 (K44A) in macrophages prevents the internalization of IgG-coated particles (Gold et al., 1999). Together, these results demonstrate an evolutionarily conserved role of dynamins in membrane fusion during engulfment.

Another function of DYN-1 essential for the removal of apoptotic cells, the one that will be discussed here in detail, is its pivotal role in phagosome maturation (Kinchen et al., 2008; Yu et al., 2006, 2008). In *dyn-1* mutants, phagosome maturation is greatly delayed or even blocked (Kinchen et al., 2008; Yu et al., 2006, 2008). DYN-1 is required for the efficient recruitment and fusion of endosomes and lysosomes to phagosomes, critical events that drive the maturation of phagosomes (Yu et al., 2006, 2008). Two lines of evidence suggest that DYN-1 acts on the surface of phagosomes for this function. First, DYN-1 is transiently enriched on the surfaces of extending pseudopods and nascent phagosomes (Kinchen et al., 2008; Yu et al., 2006). Second, the specific inhibition of DYN-1's localization on phagosomes without affecting DYN's functions in other membrane trafficking events, as a result of inactivating CED-1 and CED-6, two upstream regulators of DYN-1 (see Section 2.6.1), causes strong phagosome maturation defect similar to that observed in *dyn-1* mutants (Yu et al., 2008).

Although a complete understanding of the molecular functions of DYN-1 in promoting phagosome maturation has not been reached, accumulative experimental evidence has revealed several specific mechanisms. First, DYN-1 plays an essential role in facilitating the production of PtdIns (3)P on the surfaces of phagosomes and consequentially the attraction of PtdIns(3)P effectors (Fig. 8.5). In *dyn-1* mutant embryos and adult gonads, the production of PtdIns(3)P on phagosomal surfaces is largely inhibited, sometimes even abolished (Kinchen et al., 2008; Yu et al., 2008). As described above, lacking phagosomal PtdIns(3)P results in a failure in recruiting SNX-1, SNX-6, and LST-4/SNX-9, three PtdIns(3)P effectors specific for phagosome maturation, to phagosomal surfaces (see Section 2.4.2). Consistent with this role, DYN-1 directly interacts with class III PI 3-kinase VPS-34, implying that it might recruit VPS-34 to the surfaces of phagosomes (Kinchen et al., 2008). In addition to PtdIns(3)P production, the recruitment of RAB-5 and RAB-7 also relies on DYN-1 (He et al., 2010; Kinchen et al., 2008; Yu et al., 2008). Further, as mentioned above (Section 2.4.2.2), DYN-1 on phagosomal surfaces acts together with PtdIns(3)P to attract LST-4/SNX-9, one of the three PtdIns (3)P effectors, to the phagosomal surfaces. By regulating the production and/or recruitment of multiple important regulators onto phagosomes, DYN-1 becomes a key organizer of multiple signaling cascades that initiate a variety of phagosome maturation events targeting intracellular organelles to phagosomes (Fig. 8.5). This function of DYN-1 is a novel one independent of its documented membrane fission or fusion activities.

In addition to acting as an organizer of phagosome maturation pathways, dynamin might also directly participate in membrane remodeling events during phagosome maturation. In the engulfing cells of *dyn-1* RNAi-treated *C. elegans*, morphologically aberrant vesicles are

often observed in the proximity of membranes that contact apoptotic cells (Yu et al., 2006). Some of these vesicles remain connected to the membrane of phagocytic cups and phagosomes while others are either abnormally large or interconnected via tubular structures (Yu et al., 2006), indicating that loss of DYN-1 activity affects membrane fission and/or fusion. Thus, DYN-1 might directly contribute to the fusion of intracellular vesicles to phagocytic cups and phagosomes or facilitate the scission of vesicles from phagosomes as they gradually shrink during degradation process.

2.5.2. Dynamic association of DYN-1 with phagosomes—Upstream regulators and an autoregulatory loop—The dynamic association of DYN-1 to extending pseudopods and nascent phagosomes is important for DYN-1's functions in the removal of apoptotic cells (Yu et al., 2006). A recent report revealed how the coordinated actions of DYN-1's two different molecular activities establish the transient association of DYN-1 with its target membranes (He et al., 2010). Dynamins are composed of five domains: an N-terminal GTPase domain, a middle domain, a pleckstrin homology domain, a GTPase effector domain, and a C-terminal proline-rich domain (Fig. 8.3). Using a number of well-established *in vitro* assays, He et al. (2010) have determined that a particular mutation (I401F) in the middle domain specifically abolishes DYN-1's self-assembly activity, assembly-dependent liposome association, and assembly-stimulated GTPase activity. In contrast, mutations in the GTPase domain specifically eliminate DYN-1's GTP binding and thus the GTP hydrolysis activities (He et al., 2010). Interestingly, the GTP binding and hydrolysis activities are dispensable for the association of DYN-1 to lipid bilayer; rather, they are essential for the disassembly of DYN-1 polymers and the dissociation of DYN-1 from phagosomal surfaces (He et al., 2010). Using a bimolecular fluorescence complementation assay, the self-assembly of DYN-1 monomers has been detected, for the first time, in living animals (He et al., 2010). Consistent with the molecular activity of each domain revealed *in vitro*, live-cell imaging studies performed in *C. elegans* embryos have discovered that the middle domain of DYN-1 is essential for DYN-1's association with phagosomal membranes, whereas the GTPase domain is needed for the subsequent dissociation of DYN-1 from phagosomes (He et al., 2010). Phenotypic characterizations have revealed that both domains are equally important for DYN-1's function in animal's viability and for phagosome maturation (He et al., 2010). The above observations lead to a model that DYN-1's transient localization on target membranes, including that of phagosomes, is likely to be controlled by an autoregulatory loop (Fig. 8.4F) (He et al., 2010). According to this model, in response to an upstream signal from phagocytic receptor CED-1 (see Section 2.6.1), DYN-1 monomers form polymers, which are subsequently targeted to phagosomes; on the surfaces of nascent phagosomes, the self-assembly of DYN-1 stimulates its GTPase activity, which hydrolyzes GTP and stimulates the disassembly of DYN-1 polymers and the consequential dissociation of DYN-1 from phagosome membranes. Such an autoregulatory mechanism ensures the precise timing and duration of DYN-1's phagosomal association (Fig. 8.4F). Moreover, recent studies have shown that the association of dynamin polymers with membranes stabilizes membranes of high curvature (Bashkirov et al., 2008). The disassembly of dynamin helix is also known to promote membrane fission (Bashkirov et al., 2008; Pucadyil and Schmid, 2008). The self-assembly and GTP hydrolysis activities thus might be the basis that supports DYN-1's function in remodeling phagosomal membranes.

2.6. CED-1 and Draper: Phagocytic receptors that initiate phagosome maturation

2.6.1. *C. elegans* CED-1—What is the upstream signal(s) that recruits DYN-1? *dyn-1* acts in the same genetic pathway as *ced-1*, *ced-6*, and *ced-7* (Yu et al., 2006). Although CED-1 is only transiently localized to the surfaces of nascent phagosomes, it coexists with DYN-1 for a period of time (Fig. 8.4A) (Yu et al., 2006). Moreover, the localization of

DYN-1 on phagocytic cups and nascent phagosomes is dependent on CED-1, CED-6, and CED-7, indicating that CED-1, CED-6, and CED-7 are positive upstream regulators of DYN-1 (Fig. 8.4F) (Yu et al., 2006). Therefore, in addition to their functions in engulfment, CED-7, CED-1, and CED-6 may also promote the degradation of apoptotic cells by promoting the localization of DYN-1 on phagosomes (Yu et al., 2006). To test this hypothesis, using the time-lapse recording technique, Yu et al. (2008) monitored the engulfment and degradation of cell corpses in living *C. elegans* embryos and found that the null mutation in *ced-1* not only greatly reduced engulfment efficiency but also significantly delayed the degradation of engulfed apoptotic cells (Yu et al., 2008). Further, similar to *dyn-1* mutants, both *ced-1* and *ced-6* mutants displayed the defects in the incorporation of endosomes and lysosomes to phagosomes (Yu et al., 2006, 2008). Last, a number of DYN-1-mediated phagosome maturation events, including the production of PtdIns(3)P, the recruitment of PtdIns(3)P effectors SNX-1 and LST-4/SNX-9, as well as the recruitment of RAB-5 and RAB-7, are dependent on the functions of CED-1 and CED-6 (Lu et al., 2011; Yu et al., 2006, 2008; Z. Zhou, unpublished results). These observations established that the phagocytic receptor CED-1 and its adaptor CED-6 are not only required for the engulfment but also required for the subsequent degradation of engulfed apoptotic cells through recruiting DYN-1, the key phagosome maturation organizer, which in turn triggers a signaling cascade that promote phagosome maturation (Fig. 8.5).

2.6.2. *Drosophila* Draper—The novel function of phagocytic receptors in initiating phagosome maturation appears to be evolutionarily conserved. Draper, the *Drosophila* ortholog of CED-1, plays an essential role in the engulfment of multiple targets, including apoptotic cells and pruned axon fragments (Fig. 8.3) (Awasaki et al., 2006; MacDonald et al., 2006; Manaka et al., 2004; Ziegenfuss et al., 2008). Recently, Kurant et al. (2008) reported that inactivating Draper caused the accumulation of apoptotic neurons inside glia cells, the resident phagocytes in the central nervous system, indicating that Draper is also needed for the degradation of apoptotic cells. Kurant et al. (2008) further identified a novel *Drosophila* phagocytic receptor called six-microns-under (SIMU) that mediated the recognition and engulfment of apoptotic neurons. SIMU is a transmembrane protein that contains an N-terminal EMI domain, an domain also found in CED-1 and Draper; however, unlike CED-1 or its *Drosophila* and mammalian homologs, SIMU possesses four Nimrod (NIM) repeats rather than the tandem repeats of a CED-type atypical EGF-like motif in its extracellular domain (Fig. 8.3) (Callebaut et al., 2003; Kurant et al., 2008). The genetic and cell biological characterization of *draper* and *simu* single mutants as well as the *draper;simu* double mutants led Kurant et al. to propose that whereas in *C. elegans*, the functions of recognizing, engulfing, and degrading apoptotic cells are all carried out by a single phagocytic receptor CED-1, in *Drosophila*, which possesses a more complex phagocytic system, these tasks are taken over by more than one receptor.

2.6.3. Intact apoptotic cells versus apoptotic cell bodies—The functions of mammalian CED-1-like proteins in the removal of apoptotic cells are somewhat elusive. Recently, Jedi-1 and MEGF10, two mammalian homologs of CED-1 (Fig. 8.3), have been implicated in the engulfment of apoptotic neurons (Hamon et al., 2006; Wu et al., 2009). It remains unknown whether Jedi-1 and MEGF10, like CED-1, also participate in the degradation of engulfed apoptotic cells.

A recent study performed in the mammalian dendritic cell, an antigen-presenting phagocyte, showed that intact apoptotic cells and apoptotic cell bodies generated through fragmentation of apoptotic cells are degraded at different rates inside dendritic cells (Peng and Elkon, 2011). Dendritic cells isolated from wild-type mice efficiently engulfed intact apoptotic cells and degraded them in phagosomes through a classical lysosomal-mediated pathway (Delamarre et al., 2005; Erwig and Henson, 2008; Trombetta and Mellman, 2005). In

contrast, dendritic cells isolated from mice deficient for MFG-E8, a secreted protein that acts as a bridging molecule mediating the recognition of apoptotic cells by phagocytic receptor integrins $\alpha_v\beta_5$ and $\alpha_v\beta_3$ (Hanayama et al., 2002), were not able to swiftly engulf intact apoptotic cells. As a consequence, the unengulfed apoptotic cell became fragmented apoptotic cell bodies, which were somehow efficiently ingested by MFG-E8-deficiency dendritic cells (Peng and Elkon, 2011). Peng and Elkon (2011) further observed that phagosomes containing apoptotic cell bodies underwent a much slower maturation process and failed to fuse with lysosomes after a longer period of time, in comparison to those containing intact apoptotic cells. In *mfg-e8(−/−)* mice, the delayed phagosome maturation correlated with the increased autoimmunity, which was attributed by authors to the preservation of partially processed self-antigen for the access to the cross-presentation machinery (Peng and Elkon, 2011). The engulfment of apoptotic cell bodies, which appears to be independent of MFG-E8, is likely to involve a different mechanism from the engulfment of intact apoptotic cells. The different fates of phagosomes containing intact apoptotic cells versus apoptotic cell bodies further imply that different ligand–receptor pairs on phagosomes govern the subsequent phagosome maturation modes.

2.6.4. Involvement of phagocytic receptors in the initiation of phagosome maturation—

The discovery of the novel function of phagocytic receptors in initiating phagosome maturation has several important implications. First, this discovery reveals that phagosome maturation is not a spontaneous process that occurs once a phagocytic target is internalized into an engulfing cell; rather, it needs to be initiated by phagosomal-surface receptors that recognize specific “molecular patterns” of engulfed particles, such as the “eat me” signals on the surfaces of apoptotic cells. Consistent with this hypothesis, phagocytic receptor CED-1 is transiently present on nascent phagosomes after apoptotic cells are fully engulfed. Second, unlike other known phagosome maturation factors such as Rab GTPases, PtdIns(3)P and its effectors, or dynamin, phagocytic receptors have an intrinsic ability to distinguish “self” and “non-self” molecular patterns displayed on the surface of phagocytic targets, such as the pathogen-associated molecular patterns on pathogens or “eat me” signals on apoptotic cells (Blander and Medzhitov, 2006). Therefore, the engagement of different phagocytic receptors in the recognition of pathogens or apoptotic cells could potentially initiate different cellular signaling and elicit different inflammatory responses. Phagocytic receptors for apoptotic cells might contribute to the induction of anti-inflammatory response by acting on the surfaces of phagosomes. In addition to phagocytic receptors, certain specific phagosomal-surface receptors have also been implicated for such a role. Mammalian toll-like receptors (TLRs), which are not phagocytic receptors but able to recognize the common cell-surface pattern of many microorganisms, are enriched on the surfaces of phagosomes containing bacteria and induce the proinflammatory responses of the host cells (Akira et al., 2006; Blander and Medzhitov, 2006). In summary, transmembrane receptors that are able to recognize the distinct molecular natures of phagocytic targets, including phagocytic receptors, might be key determinants of different inflammatory responses elicit by host cells. CED-1’s mammalian homologs might act to specifically label phagosomes that contain apoptotic cells and elicit apoptotic cell-specific immune responses in mammals.

3. Concluding Remarks

In the past two decades, extensive studies of phagosome maturation conducted in cultured mammalian cells have discovered a number of protein and lipid factors that promote phagosome maturation. In the past 5 years, investigations using model organisms, in particular the nematode *C. elegans*, have not only revealed multiple novel molecular mechanisms that control the action of the known phagosome maturation factors, but, more importantly, through systematic genetic approaches, identified numerous novel factors that

mediate phagosome maturation and further placed them in genetic pathways that mediate phagosome maturation (Fig. 8.5). These new studies have established the evolutionary conservation of phagosome maturation mechanisms from simple to complex organisms, have implied the involvement of the mammalian homologs of these novel factors in phagosome maturation, and have demonstrated that studying the degradation of apoptotic cells is effective in revealing basic molecular mechanisms that apply to the maturation of phagosomes containing all sorts of cargos. These studies will also further our understanding of membrane trafficking events utilized in multiple cellular events in addition to phagosome maturation.

Further studies of the newly identified apoptotic cell-degradation factors are required for answering many remaining questions regarding the basic molecular mechanisms of phagosome maturation and membrane trafficking. For instance, the identification of the differential functions of four Rab GTPases, RAB-2, RAB-5, RAB-7, and RAB-14, in phagosome maturation cries for further investigation of the function and regulation of the Rab GTPases, regarding aspects such as the identity of the mysterious GEF for RAB-5, the effectors for RAB-7 (the HOPS complex as one promising candidate), and the phagosome-specific regulatory proteins for RAB-2 and RAB-14, which have been less studied. In addition, considering the idea that different Rab GTPases act as membrane tethering factors for intracellular organelles of different identities, it is important to know that, among the 29 RAB GTPases in *C. elegans*, how many are involved in phagosome maturation and their differential roles in phagosome maturation.

Regarding PtdIns(3)P, the lipid second messenger and essential initiator of phagosome maturation, its production, turnover, and effectors all need to be further studied. Besides the class III PI(3) kinase VPS-34, PtdIns(3)P production on phagosomal surfaces is likely to involve a yet-to-be identified PI 3-kinase(s). Just as importantly, almost nothing is known about how PtdIns(3)P is downregulated during phagosome maturation or the physiological significance of PtdIns(3)P turnover for phagosome maturation. Moreover, the molecular mechanisms utilized by SNX-1, SNX-6, and LST-4/SNX-9, three PtdIns(3)P effectors, to specifically promote phagosome maturation, in particular those utilized by LST-4/SNX-9, which display multiple activities in generating and stabilizing membrane curvature and in interacting with DYN-1, need to be further investigated. In addition, there might exist additional effectors of PtdIns(3)P that act in phagosome maturation. Last but not least, as an upstream regulator of PtdIns (3)P and the Rab GTPases, how DYN-1 acts to organize multiple phagosome maturation events is far from being understood and needs to be continuously investigated.

On the other hand, accumulative evidence also revealed the differential regulatory mechanisms of phagosome maturation applied to phagosomes containing different kinds of cargos, and occurring in phagocytes of different identities and in different organisms. These distinct mechanisms are likely to have profound physiological significance. In this sense, the finding that phagocytic receptors CED-1 and Draper act to initiate phagosome maturation in addition to initiating engulfment provides a new clue for understanding the molecular mechanisms behind the differential regulation of phagosome maturation and possibly behind the differential immune responses elicited by phagocytes internalizing different targets. The studies focusing on the degradation of apoptotic cells will continue to reveal unique mechanisms underlying the differential processing of different phagocytic targets and by different phagocytes.

Recently, phagocytic receptors CED-1 and Draper were shown to function in other physiological contexts in addition to the removal of apoptotic cells. CED-1 has been reported to contribute to the innate immunity of *C. elegans* against pathogen infection by

regulating genes that control the unfolded protein response (Haskins et al., 2008). *Drosophila* Draper was recently found to play a cell-autonomous role in inducing autophagy in dying salivary gland cells (McPhee et al., 2010). Although the molecular mechanisms of CED-1 and Draper in these events are largely unknown, it is conceivable that a cell-surface receptor might recognize a diverse range of ligands through its different recognition domains or through different coreceptors and, in this manner trigger distinct cellular responses. The continuous investigations of the molecular mechanisms of phagocytic receptors in different biological contexts will help us understand how phagocytes process their meals in host defense and homeostasis.

In addition to the intracellular organelle/phagosome fusion events that are essential for driving phagosome maturation, a recent report indicates that components of autophagosomes (but not the intact autophagosomes) are associated with maturing phagosomes, and that the autophagy machinery is involved in TLR-mediated phagosome maturation of pathogens (Sanjuan et al., 2007). A comprehensive study of this phenomenon will reveal the relationship between autophagy and phagosome maturation. Further, other cellular events in addition to membrane trafficking might also be involved in the degradation of apoptotic cells. For example, the rearrangement of the actin cytoskeleton was found to play important roles in the maturation of phagosomes that contain pathogens, opsonized red blood cells, or latex beads (Bohdanowicz et al., 2010; Marion et al., 2011). *C. elegans* CED-5, which was implicated in actin reorganization, was also found to regulate the lysosomes/phagosome fusion during the degradation of apoptotic cells (Table 8.1) (Yu et al., 2008). Given that CED-5, CED-2, and CED-12 are in a complex that activates the CED-10/Rac1 GTPase, it is possible that actin reorganization might play active roles in the degradation of apoptotic cells.

Acknowledgments

We apologize to all authors whose relevant work was not cited due to space constraints. This work was supported by grants from NIH (GM067848) and the March of Dimes Foundation (FY10-434).

References

Akbar MA, Tracy C, Kahr WH, Kramer H. The full-of-bacteria gene is required for phagosome maturation during immune defense in Drosophila. J Cell Biol. 2011; 192:383–390. [PubMed: 21282466]

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006; 124:783–801. [PubMed: 16497588]

Almendinger J, Doukoumetzidis K, Kinchen JM, Kaech A, Ravichandran KS, Hengartner MO. A conserved role for SNX9-family members in the regulation of phagosome maturation during engulfment of apoptotic cells. PLoS One. 2011; 6:e18325. [PubMed: 21494661]

Alvarez-Dominguez C, Barbieri AM, Beron W, Wandinger-Ness A, Stahl PD. Phagocytosed live Listeria monocytogenes influences Rab5-regulated in vitro phagosome-endosome fusion. J Biol Chem. 1996; 271:13834–13843. [PubMed: 8662791]

Awasaki T, Tatsumi R, Takahashi K, Arai K, Nakanishi Y, Ueda R, et al. Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during Drosophila metamorphosis. Neuron. 2006; 50:855–867. [PubMed: 16772168]

Backer JM. The regulation and function of Class III PI3Ks: novel roles for Vps34. Biochem J. 2008; 410:1–17. [PubMed: 18215151]

Bashkirov PV, Akimov SA, Evseev AI, Schmid SL, Zimmerberg J, Frolov VA. GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. Cell. 2008; 135:1276–1286. [PubMed: 19084269]

Bayer MJ, Reese C, Buhler S, Peters C, Mayer A. Vacuole membrane fusion: V0 functions after trans-SNARE pairing and is coupled to the Ca2+-releasing channel. J Cell Biol. 2003; 162:211–222. [PubMed: 12876274]

Bernards A. GAPs galore! A survey of putative Ras superfamily GTPase activating proteins in man and Drosophila. Biochim Biophys Acta. 2003; 1603:47–82. [PubMed: 12618308]

Beyenbach KW, Wieczorek H. The V-type H+ ATPase: molecular structure and function, physiological roles and regulation. J Exp Biol. 2006; 209:577–589. [PubMed: 16449553]

Birkeland HC, Stenmark H. Protein targeting to endosomes and phagosomes via FYVE and PX domains. Curr Top Microbiol Immunol. 2004; 282:89–115. [PubMed: 14594215]

Blander JM, Medzhitov R. On regulation of phagosome maturation and antigen presentation. Nat Immunol. 2006; 7:1029–1035. [PubMed: 16985500]

Bohdanowicz M, Cosio G, Backer JM, Grinstein S. Class I and class III phosphoinositide 3-kinases are required for actin polymerization that propels phagosomes. J Cell Biol. 2010; 191:999–1012. [PubMed: 21115805]

Bonifacino JS, Hurley JH. Retromer. Curr Opin Cell Biol. 2008; 20:427–436. [PubMed: 18472259]

Boulais J, Trost M, Landry CR, Dieckmann R, Levy ED, Soldati T, et al. Molecular characterization of the evolution of phagosomes. Mol Syst Biol. 2010; 6:423. [PubMed: 20959821]

Bucci C, Parton RG, Mather IH, Stunnenberg H, Simons K, Hoflack B, et al. The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. Cell. 1992; 70:715–728. [PubMed: 1516130]

Cabello J, Neukomm LJ, Gunesdogan U, Burkart K, Charette SJ, Lochnit G, et al. The Wnt pathway controls cell death engulfment, spindle orientation, and migration through CED-10/Rac. PLoS Biol. 2010; 8:e1000297. [PubMed: 20126385]

Cai H, Reinisch K, Ferro-Novick S. Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. Dev Cell. 2007; 12:671–682. [PubMed: 17488620]

Callebaut I, Mignotte V, Souchet M, Mornon JP. EMI domains are widespread and reveal the probable orthologs of the *Caenorhabditis elegans* CED-1 protein. Biochem Biophys Res Commun. 2003; 300:619–623. [PubMed: 12507493]

Caron, E.; Hall, A. Phagocytosis. In: Marsh, M., editor. Endocytosis. Oxford University Press; Oxford: 2001. p. 58-77.

Chen D, Xiao H, Zhang K, Wang B, Gao Z, Jian Y, et al. Retromer is required for apoptotic cell clearance by phagocytic receptor recycling. Science. 2010; 327:1261–1264. [PubMed: 20133524]

Chotard L, Mishra AK, Sylvain MA, Tuck S, Lambright DG, Rocheleau CE. TBC-2 regulates RAB-5/RAB-7-mediated endosomal trafficking in *Caenorhabditis elegans*. Mol Biol Cell. 2010; 21:2285–2296. [PubMed: 20462958]

Christoforidis S, Miaczynska M, Ashman K, Wilm M, Zhao L, Yip SC, et al. Phosphatidylinositol-3-OH kinases are Rab5 effectors. Nat Cell Biol. 1999; 1:249–252. [PubMed: 10559924]

Chua J, Deretic V. Mycobacterium tuberculosis reprograms waves of phosphatidylinositol 3-phosphate on phagosomal organelles. J Biol Chem. 2004; 279:36982–36992. [PubMed: 15210698]

Clarke M, Kohler J, Arana Q, Liu T, Heuser J, Gerisch G. Dynamics of the vacuolar H(+)-ATPase in the contractile vacuole complex and the endosomal pathway of Dictyostelium cells. J Cell Sci. 2002; 115:2893–2905. [PubMed: 12082150]

Collins KM, Wickner WT. Trans-SNARE complex assembly and yeast vacuole membrane fusion. Proc Natl Acad Sci USA. 2007; 104:8755–8760. [PubMed: 17502611]

Coudreuse DY, Roel G, Betist MC, Destree O, Korswagen HC. Wnt gradient formation requires retromer function in Wnt-producing cells. Science. 2006; 312:921–924. [PubMed: 16645052]

deBakker CD, Haney LB, Kinchen JM, Grimsley C, Lu M, Klingele D, et al. Phagocytosis of apoptotic cells is regulated by a UNC-73/TRIO-MIG-2/RhoG signaling module and armadillo repeats of CED-12/ELMO. Curr Biol. 2004; 14:2208–2216. [PubMed: 15620647]

Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. Science. 2005; 307:1630–1634. [PubMed: 15761154]

Desjardins M, Huber LA, Parton RG, Griffiths G. Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. J Cell Biol. 1994; 124:677–688. [PubMed: 8120091]

Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature. 2006; 443:651–657. [PubMed: 17035995]

Duclos S, Diez R, Garin J, Papadopoulou B, Descoteaux A, Stenmark H, et al. Rab5 regulates the kiss and run fusion between phagosomes and endosomes and the acquisition of phagosome leishmanicidal properties in RAW 264.7 macrophages. J Cell Sci. 2000; 113(Pt 19):3531–3541. [PubMed: 10984443]

Elliott MR, Ravichandran KS. Clearance of apoptotic cells: implications in health and disease. J Cell Biol. 2010; 189:1059–1070. [PubMed: 20584912]

Ellis RE, Jacobson DM, Horvitz HR. Genes required for the engulfment of cell corpses during programmed cell death in *Caenorhabditis elegans*. Genetics. 1991; 129:79–94. [PubMed: 1936965]

Ellson CD, Anderson KE, Morgan G, Chilvers ER, Lipp P, Stephens LR, et al. Phosphatidylinositol 3-phosphate is generated in phagosomal membranes. Curr Biol. 2001a; 11:1631–1635. [PubMed: 11676926]

Ellson CD, Gobert-Gosse S, Anderson KE, Davidson K, Erdjument-Bromage H, Tempst P, et al. PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40(phox). Nat Cell Biol. 2001b; 3:679–682. [PubMed: 11433301]

Erwig LP, Henson PM. Immunological consequences of apoptotic cell phagocytosis. Am J Pathol. 2007; 171:2–8. [PubMed: 17591947]

Erwig LP, Henson PM. Clearance of apoptotic cells by phagocytes. Cell Death Differ. 2008; 15:243–250. [PubMed: 17571081]

Erwig LP, McPhilips KA, Wynes MW, Ivetic A, Ridley AJ, Henson PM. Differential regulation of phagosome maturation in macrophages and dendritic cells mediated by Rho GTPases and ezrin-radixin-moesin (ERM) proteins. Proc Natl Acad Sci USA. 2006; 103:12825–12830. [PubMed: 16908865]

Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest. 1998; 101:890–898. [PubMed: 9466984]

Fratti RA, Backer JM, Gruenberg J, Corvera S, Deretic V. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. J Cell Biol. 2001; 154:631–644. [PubMed: 11489920]

Freire-de-Lima CG, Xiao YQ, Gardai SJ, Bratton DL, Schiemann WP, Henson PM. Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages. J Biol Chem. 2006; 281:38376–38384. [PubMed: 17056601]

Gallop JL, Jao CC, Kent HM, Butler PJ, Evans PR, Langen R, et al. Mechanism of endophilin N-BAR domain-mediated membrane curvature. EMBO J. 2006; 25:2898–2910. [PubMed: 16763559]

Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, et al. The phagosome proteome: insight into phagosome functions. J Cell Biol. 2001; 152:165–180. [PubMed: 11149929]

Gold ES, Underhill DM, Morrissette NS, Guo J, McNiven MA, Aderem A. Dynamin 2 is required for phagocytosis in macrophages. J Exp Med. 1999; 190:1849–1856. [PubMed: 10601359]

Gorvel JP, Chavrier P, Zerial M, Gruenberg J. Rab5 controls early endosome fusion *in vitro*. Cell. 1991; 64:915–925. [PubMed: 1900457]

Grosshans BL, Ortiz D, Novick P. Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci USA. 2006; 103:11821–11827. [PubMed: 16882731]

Gullapalli A, Wolfe BL, Griffin CT, Magnuson T, Trejo J. An essential role for SNX1 in lysosomal sorting of protease-activated receptor-1: evidence for retromer-, Hrs-, and Tsg101-independent functions of sorting nexins. Mol Biol Cell. 2006; 17:1228–1238. [PubMed: 16407403]

Guo P, Hu T, Zhang J, Jiang S, Wang X. Sequential action of *Caenorhabditis elegans* Rab GTPases regulates phagolysosome formation during apoptotic cell degradation. Proc Natl Acad Sci USA. 2010; 107:18016–18021. [PubMed: 20921409]

Haberg K, Lundmark R, Carlsson SR. SNX18 is an SNX9 paralog that acts as a membrane tubulator in AP-1-positive endosomal trafficking. J Cell Sci. 2008; 121:1495–1505. [PubMed: 18411244]

Hamon Y, Trompier D, Ma Z, Venegas V, Pophillat M, Mignotte V, et al. Cooperation between engulfment receptors: the case of ABCA1 and MEGF10. PLoS One. 2006; 1:e120. [PubMed: 17205124]

Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. Nature. 2002; 417:182–187. [PubMed: 12000961]

Harris E, Cardelli J. RabD, a Dictyostelium Rab14-related GTPase, regulates phagocytosis and homotypic phagosome and lysosome fusion. J Cell Sci. 2002; 115:3703–3713. [PubMed: 12186956]

Harrison RE, Bucci C, Vieira OV, Schroer TA, Grinstein S. Phagosomes fuse with late endosomes and/or lysosomes by extension of membrane protrusions along microtubules: role of Rab7 and RILP. Mol Cell Biol. 2003; 23:6494–6506. [PubMed: 12944476]

Haskins KA, Russell JF, Gaddis N, Dressman HK, Aballay A. Unfolded protein response genes regulated by CED-1 are required for *Caenorhabditis elegans* innate immunity. Dev Cell. 2008; 15:87–97. [PubMed: 18606143]

He B, Yu X, Margolis M, Liu X, Leng X, Etzion Y, et al. Live-cell imaging in *Caenorhabditis elegans* reveals the distinct roles of dynamin self-assembly and guanosine triphosphate hydrolysis in the removal of apoptotic cells. Mol Biol Cell. 2010; 21:610–629. [PubMed: 20016007]

Henson PM, Hume DA. Apoptotic cell removal in development and tissue homeostasis. Trends Immunol. 2006; 27:244–250. [PubMed: 16584921]

Hickey CM, Wickner W. HOPS initiates vacuole docking by tethering membranes before trans-SNARE complex assembly. Mol Biol Cell. 2010; 21:2297–2305. [PubMed: 20462954]

Hickey CM, Stroupe C, Wickner W. The major role of the Rab Ypt7p in vacuole fusion is supporting HOPS membrane association. J Biol Chem. 2009; 284:16118–16125. [PubMed: 19386605]

Hiesinger PR, Fayyazuddin A, Mehta SQ, Rosenmund T, Schulze KL, Zhai RG, et al. The v-ATPase V0 subunit a1 is required for a late step in synaptic vesicle exocytosis in Drosophila. Cell. 2005; 121:607–620. [PubMed: 15907473]

Hoppins S, Nunnari J. The molecular mechanism of mitochondrial fusion. Biochim Biophys Acta. 2009; 1793:20–26. [PubMed: 18691613]

Hsu TY, Wu YC. Engulfment of apoptotic cells in *C. elegans* is mediated by integrin alpha/SRC signaling. Curr Biol. 2010; 20:477–486. [PubMed: 20226672]

Jahraus A, Tjelle TE, Berg T, Habermann A, Storrie B, Ullrich O, et al. *In vitro* fusion of phagosomes with different endocytic organelles from J774 macrophages. J Biol Chem. 1998; 273:30379–30390. [PubMed: 9804802]

Kanai F, Liu H, Field SJ, Akbary H, Matsuo T, Brown GE, et al. The PX domains of p47phox and p40phox bind to lipid products of PI(3)K. Nat Cell Biol. 2001; 3:675–678. [PubMed: 11433300]

Kawane K, Fukuyama H, Kondoh G, Takeda J, Ohsawa Y, Uchiyama Y, et al. Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver. Science. 2001; 292:1546–1549. [PubMed: 11375492]

Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, et al. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature. 2006; 443:998–1002. [PubMed: 17066036]

Kinchen JM, Ravichandran KS. Phagosome maturation: going through the acid test. Nat Rev Mol Cell Biol. 2008; 9:781–795. [PubMed: 18813294]

Kinchen JM, Ravichandran KS. Identification of two evolutionarily conserved genes regulating processing of engulfed apoptotic cells. Nature. 2010; 464:778–782. [PubMed: 20305638]

Kinchen JM, Cabello J, Klingele D, Wong K, Feichtinger R, Schnabel H, et al. Two pathways converge at CED-10 to mediate actin rearrangement and corpse removal in *C. elegans*. Nature. 2005; 434:93–99. [PubMed: 15744306]

Kinchen JM, Doukoumetzidis K, Almendinger J, Stergiou L, Tosello-Trampont A, Sifri CD, et al. A pathway for phagosome maturation during engulfment of apoptotic cells. Nat Cell Biol. 2008; 10:556–566. [PubMed: 18425118]

Kitano M, Nakaya M, Nakamura T, Nagata S, Matsuda M. Imaging of Rab5 activity identifies essential regulators for phagosome maturation. Nature. 2008; 453:241–245. [PubMed: 18385674]

Kurant E, Axelrod S, Leaman D, Gaul U. Six-microns-under acts upstream of Draper in the glial phagocytosis of apoptotic neurons. Cell. 2008; 133:498–509. [PubMed: 18455990]

Kyei GB, Vergne I, Chua J, Roberts E, Harris J, Junutula JR, et al. Rab14 is critical for maintenance of *Mycobacterium tuberculosis* phagosome maturation arrest. EMBO J. 2006; 25:5250–5259. [PubMed: 17082769]

Li W, Zou W, Zhao D, Yan J, Zhu Z, Lu J, et al. *C. elegans* Rab GTPase activating protein TBC-2 promotes cell corpse degradation by regulating the small GTPase RAB-5. Development. 2009; 136:2445–2455. [PubMed: 19542357]

Liegeois S, Benedetto A, Garnier JM, Schwab Y, Labouesse M. The V0-ATPase mediates apical secretion of exosomes containing Hedgehog-related proteins in *Caenorhabditis elegans*. J Cell Biol. 2006; 173:949–961. [PubMed: 16785323]

Liu QA, Hengartner MO. Candidate adaptor protein CED-6 promotes the engulfment of apoptotic cells in *C. elegans*. Cell. 1998; 93:961–972. [PubMed: 9635426]

Lu Q, Zhang Y, Hu T, Guo P, Li W, Wang X. *C. elegans* Rab GTPase 2 is required for the degradation of apoptotic cells. Development. 2008; 135:1069–1080. [PubMed: 18256195]

Lu N, Yu X, He X, Zhou Z. Detecting apoptotic cells and monitoring their clearance in the nematode *Caenorhabditis elegans*. Methods Mol Biol. 2009; 559:357–370. [PubMed: 19609769]

Lu N, Shen Q, Mahoney TR, Liu X, Zhou Z. Three sorting nexins drive the degradation of apoptotic cells in response to PtdIns(3)P signaling. Mol Biol Cell. 2011; 22:354–374. [PubMed: 21148288]

Lundmark R, Carlsson SR. Regulated membrane recruitment of dynamin-2 mediated by sorting nexin 9. J Biol Chem. 2004; 279:42694–42702. [PubMed: 15299020]

Lundquist EA, Reddien PW, Hartwieg E, Horvitz HR, Bargmann CI. Three *C. elegans* Rac proteins and several alternative Rac regulators control axon guidance, cell migration and apoptotic cell phagocytosis. Development. 2001; 128:4475–4488. [PubMed: 11714673]

MacDonald JM, Beach MG, Porpiglia E, Sheehan AE, Watts RJ, Freeman MR. The Drosophila cell corpse engulfment receptor Draper mediates glial clearance of severed axons. Neuron. 2006; 50:869–881. [PubMed: 16772169]

Manaka J, Kuraishi T, Shiratsuchi A, Nakai Y, Higashida H, Henson P, et al. Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by Drosophila hemocytes/macrophages. J Biol Chem. 2004; 279:48466–48476. [PubMed: 15342648]

Mangahas PM, Zhou Z. Clearance of apoptotic cells in *Caenorhabditis elegans*. Semin Cell Dev Biol. 2005; 16:295–306. [PubMed: 15797839]

Mangahas PM, Yu X, Miller KG, Zhou Z. The small GTPase Rab2 functions in the removal of apoptotic cells in *Caenorhabditis elegans*. J Cell Biol. 2008; 180:357–373. [PubMed: 18227280]

Marion S, Hoffmann E, Holzer D, Le Clainche C, Martin M, Sachse M, et al. Ezrin promotes actin assembly at the phagosome membrane and regulates phago-lysosomal fusion. Traffic. 2011; 12:421–437. [PubMed: 21210911]

McNeil PL, Tanasugarn L, Meigs JB, Taylor DL. Acidification of phagosomes is initiated before lysosomal enzyme activity is detected. J Cell Biol. 1983; 97:692–702. [PubMed: 6885916]

McPhee CK, Logan MA, Freeman MR, Baehrecke EH. Activation of autophagy during cell death requires the engulfment receptor Draper. Nature. 2010; 465:1093–1096. [PubMed: 20577216]

Metzstein MM, Stanfield GM, Horvitz HR. Genetics of programmed cell death in *C. elegans*: past, present and future. Trends Genet. 1998; 14:410–416. [PubMed: 9820030]

Murray JT, Panaretou C, Stenmark H, Miaczynska M, Backer JM. Role of Rab5 in the recruitment of hVps34/p150 to the early endosome. Traffic. 2002; 3:416–427. [PubMed: 12010460]

Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. Cell. 2010; 140:619–630. [PubMed: 20211132]

Nickerson DP, Brett CL, Merz AJ. Vps-C complexes: gatekeepers of endolysosomal traffic. Curr Opin Cell Biol. 2009; 21:543–551. [PubMed: 19577915]

Nieto C, Almendinger J, Gysi S, Gomez-Orte E, Kaech A, Hengartner MO, et al. ccz-1 mediates the digestion of apoptotic corpses in *C. elegans*. J Cell Sci. 2010; 123:2001–2007. [PubMed: 20519582]

Nisar S, Kelly E, Cullen PJ, Mundell SJ. Regulation of P2Y1 receptor traffic by sorting Nexin 1 is retromer independent. Traffic. 2010; 11:508–519. [PubMed: 20070609]

Nordmann M, Cabrera M, Perz A, Brocker C, Ostrowicz C, Engelbrecht-Vandre S, et al. The Mon1-Ccz1 complex is the GEF of the late endosomal Rab7 homolog Ypt7. Curr Biol. 2010; 20:1654–1659. [PubMed: 20797862]

Pan CL, Baum PD, Gu M, Jorgensen EM, Clark SG, Garriga G. *C. elegans* AP-2 and retromer control Wnt signaling by regulating mig-14/Wntless. Dev Cell. 2008; 14:132–139. [PubMed: 18160346]

Pattni K, Jepson M, Stenmark H, Banting G. A PtdIns(3)P-specific probe cycles on and off host cell membranes during Salmonella invasion of mammalian cells. Curr Biol. 2001; 11:1636–1642. [PubMed: 11676927]

Peng Y, Elkon KB. Autoimmunity in MFG-E8-deficient mice is associated with altered trafficking and enhanced cross-presentation of apoptotic cell antigens. J Clin Invest. 2011; 121:2221–2241. [PubMed: 21537078]

Peri F, Nusslein-Volhard C. Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion *in vivo*. Cell. 2008; 133:916–927. [PubMed: 18510934]

Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, et al. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science. 2004; 303:495–499. [PubMed: 14645856]

Peters C, Baars TL, Buhler S, Mayer A. Mutual control of membrane fission and fusion proteins. Cell. 2004; 119:667–678. [PubMed: 15550248]

Poteryaev D, Fares H, Bowerman B, Spang A. *Caenorhabditis elegans* SAND-1 is essential for RAB-7 function in endosomal traffic. EMBO J. 2007; 26:301–312. [PubMed: 17203072]

Poteryaev D, Datta S, Ackema K, Zerial M, Spang A. Identification of the switch in early-to-late endosome transition. Cell. 2010; 141:497–508. [PubMed: 20434987]

Praefcke GJ, McMahon HT. The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol. 2004; 5:133–147. [PubMed: 15040446]

Prosser DC, Tran D, Schooley A, Wendland B, Ngsee JK. A novel, retromer-independent role for sorting nexins 1 and 2 in RhoG-dependent membrane remodeling. Traffic. 2010; 11 (10):1347–1362. [PubMed: 20604901]

Pucadyil TJ, Schmid SL. Real-time visualization of dynamin-catalyzed membrane fission and vesicle release. Cell. 2008; 135:1263–1275. [PubMed: 19084268]

Reddien PW, Horvitz HR. The engulfment process of programmed cell death in *Caenorhabditis elegans*. Annu Rev Cell Dev Biol. 2004; 20:193–221. [PubMed: 15473839]

Saksena S, Sun J, Chu T, Emr SD. ESCRTing proteins in the endocytic pathway. Trends Biochem Sci. 2007; 32:561–573. [PubMed: 17988873]

Sanjuan MA, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature. 2007; 450:1253–1257. [PubMed: 18097414]

Sasaki T, Sasaki J, Sakai T, Takasuga S, Suzuki A. The physiology of phosphoinositides. Biol Pharm Bull. 2007; 30:1599–1604. [PubMed: 17827706]

Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature. 2000; 407:784–788. [PubMed: 11048729]

Schmid SL, Frolov VA. Dynamin: functional design of a membrane fission catalyst. Annu Rev Cell Dev Biol. 2011; 27:79–105. [PubMed: 21599493]

Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005; 6:1191–1197. [PubMed: 16369558]

Shi A, Sun L, Banerjee R, Tobin M, Zhang Y, Grant BD. Regulation of endosomal clathrin and retromer-mediated endosome to Golgi retrograde transport by the J-domain protein RME-8. EMBO J. 2009; 28:3290–3302. [PubMed: 19763082]

Shin HW, Hayashi M, Christoforidis S, Lacas-Gervais S, Hoepfner S, Wenk MR, et al. An enzymatic cascade of Rab5 effectors regulates phosphoinositide turnover in the endocytic pathway. J Cell Biol. 2005; 170:607–618. [PubMed: 16103228]

Shin N, Lee S, Ahn N, Kim SA, Ahn SG, YongPark Z, et al. Sorting nexin 9 interacts with dynamin 1 and N-WASP and coordinates synaptic vesicle endocytosis. J Biol Chem. 2007; 282:28939–28950. [PubMed: 17681954]

Shui W, Sheu L, Liu J, Smart B, Petzold CJ, Hsieh TY, et al. Membrane proteomics of phagosomes suggests a connection to autophagy. Proc Natl Acad Sci USA. 2008; 105:16952–16957. [PubMed: 18971338]

Smith AC, Heo WD, Braun V, Jiang X, Macrae C, Casanova JE, et al. A network of Rab GTPases controls phagosome maturation and is modulated by Salmonella enterica serovar Typhimurium. J Cell Biol. 2007; 176:263–268. [PubMed: 17261845]

Soulet F, Yarar D, Leonard M, Schmid SL. SNX9 regulates dynamin assembly and is required for efficient clathrin-mediated endocytosis. Mol Biol Cell. 2005; 16:2058–2067. [PubMed: 15703209]

Steinberg BE, Huynh KK, Grinstein S. Phagosomal acidification: measurement, manipulation and functional consequences. Biochem Soc Trans. 2007; 35:1083–1087. [PubMed: 17956285]

Stenmark H. Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol. 2009; 10:513–525. [PubMed: 19603039]

Stuart LM, Ezekowitz RA. Phagocytosis and comparative innate immunity: learning on the fly. Nat Rev Immunol. 2008; 8:131–141. [PubMed: 18219310]

Stuart LM, Boulais J, Charriere GM, Hennessy EJ, Brunet S, Jutras I, et al. A systems biology analysis of the Drosophila phagosome. Nature. 2007; 445:95–101. [PubMed: 17151602]

Su HP, Nakada-Tsukui K, Tosello-Trampont AC, Li Y, Bu G, Henson PM, et al. Interaction of CED-6/GULP, an adapter protein involved in engulfment of apoptotic cells with CED-1 and CD91/low density lipoprotein receptor-related protein (LRP). J Biol Chem. 2002; 277:11772–11779. [PubMed: 11729193]

Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev Biol. 1977; 56:110–156. [PubMed: 838129]

Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol. 1983; 100:64–119. [PubMed: 6684600]

Sun-Wada GH, Tabata H, Kawamura N, Aoyama M, Wada Y. Direct recruitment of H+-ATPase from lysosomes for phagosomal acidification. J Cell Sci. 2009; 122:2504–2513. [PubMed: 19549681]

Trombetta ES, Mellman I. Cell biology of antigen processing *in vitro* and *in vivo*. Annu Rev Immunol. 2005; 23:975–1028. [PubMed: 15771591]

Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. Annu Rev Immunol. 2002; 20:825–852. [PubMed: 11861619]

Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, et al. Synthesis and function of 3-phosphorylated inositol lipids. Annu Rev Biochem. 2001; 70:535–602. [PubMed: 11395417]

Vaux DL, Korsmeyer SJ. Cell death in development. Cell. 1999; 96:245–254. [PubMed: 9988219]

Venegas V, Zhou Z. Two alternative mechanisms that regulate the presentation of apoptotic cell engulfment signal in *Caenorhabditis elegans*. Mol Biol Cell. 2007; 18:3180–3192. [PubMed: 17567952]

Verges M, Sebastian I, Mostov KE. Phosphoinositide 3-kinase regulates the role of retromer in transcytosis of the polymeric immunoglobulin receptor. Exp Cell Res. 2007; 313:707–718. [PubMed: 17184770]

Vieira OV, Botelho RJ, Rameh L, Brachmann SM, Matsuo T, Davidson HW, et al. Distinct roles of class I and class III phosphatidylinositol 3-kinases in phagosome formation and maturation. J Cell Biol. 2001; 155:19–25. [PubMed: 11581283]

Vieira OV, Botelho RJ, Grinstein S. Phagosome maturation: aging gracefully. Biochem J. 2002; 366:689–704. [PubMed: 12061891]

Vieira OV, Bucci C, Harrison RE, Trimble WS, Lanzetti L, Gruenberg J, et al. Modulation of Rab5 and Rab7 recruitment to phagosomes by phosphatidylinositol 3-kinase. Mol Cell Biol. 2003; 23:2501–2514. [PubMed: 12640132]

Vieira OV, Harrison RE, Scott CC, Stenmark H, Alexander D, Liu J, et al. Acquisition of Hrs, an essential component of phagosomal maturation, is impaired by mycobacteria. Mol Cell Biol. 2004; 24:4593–4604. [PubMed: 15121875]

Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997; 390:350–351. [PubMed: 9389474]

Wang CW, Stromhaug PE, Shima J, Klionsky DJ. The Ccz1-Mon1 protein complex is required for the late step of multiple vacuole delivery pathways. J Biol Chem. 2002; 277:47917–47927. [PubMed: 12364329]

Wang CW, Stromhaug PE, Kauffman EJ, Weisman LS, Klionsky DJ. Yeast homotypic vacuole fusion requires the Ccz1-Mon1 complex during the tethering/docking stage. J Cell Biol. 2003; 163:973–985. [PubMed: 14662743]

Wassmer T, Attar N, Harterink M, van Weering JR, Traer CJ, Oakley J, et al. The retromer coat complex coordinates endosomal sorting and dynein-mediated transport, with carrier recognition by the trans-Golgi network. Dev Cell. 2009; 17:110–122. [PubMed: 19619496]

Wu Y, Horvitz HR. The *C. elegans* cell corpse engulfment gene *ced-7* encodes a protein similar to ABC transporters. Cell. 1998; 93:951–960. [PubMed: 9635425]

Wu HH, Bellmunt E, Scheib JL, Venegas V, Burkert C, Reichardt LF, et al. Glial precursors clear sensory neuron corpses during development via Jedi-1, an engulfment receptor. Nat Neurosci. 2009; 12:1534–1541. [PubMed: 19915564]

Wurmser AE, Sato TK, Emr SD. New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. J Cell Biol. 2000; 151:551–562. [PubMed: 11062257]

Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. Int Rev Cytol. 1980; 68:251–306. [PubMed: 7014501]

Xiao H, Chen D, Fang Z, Xu J, Sun X, Song S, et al. Lysosome biogenesis mediated by vps-18 affects apoptotic cell degradation in *Caenorhabditis elegans*. Mol Biol Cell. 2009; 20:21–32. [PubMed: 18923146]

Yang PT, Lorenowicz MJ, Silhankova M, Coudreuse DY, Betist MC, Korswagen HC. Wnt signaling requires retromer-dependent recycling of MIG-14/Wntless in Wnt-producing cells. Dev Cell. 2008; 14:140–147. [PubMed: 18160347]

Yoshida H, Kawane K, Koike M, Mori Y, Uchiyama Y, Nagata S. Phosphatidylserine-dependent engulfment by macrophages of nuclei from erythroid precursor cells. Nature. 2005a; 437:754–758. [PubMed: 16193055]

Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S. Lethal anemia caused by interferon-beta produced in mouse embryos carrying undigested DNA. Nat Immunol. 2005b; 6:49–56. [PubMed: 15568025]

Yu X, Odera S, Chuang CH, Lu N, Zhou Z. *C. elegans* Dynamin mediates the signaling of phagocytic receptor CED-1 for the engulfment and degradation of apoptotic cells. Dev Cell. 2006; 10:743–757. [PubMed: 16740477]

Yu X, Lu N, Zhou Z. Phagocytic receptor CED-1 initiates a signaling pathway for degrading engulfed apoptotic cells. PLoS Biol. 2008; 6:e61. [PubMed: 18351800]

Zhou Z, Yu X. Phagosome maturation during the removal of apoptotic cells: receptors lead the way. Trends Cell Biol. 2008; 18:474–485. [PubMed: 18774293]

Zhou Z, Hartwieg E, Horvitz HR. CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans*. Cell. 2001; 104:43–56. [PubMed: 11163239]

Ziegenfuss JS, Biswas R, Avery MA, Hong K, Sheehan AE, Yeung YG, et al. Draper-dependent glial phagocytic activity is mediated by Src and Syk family kinase signalling. Nature. 2008; 453:935–939. [PubMed: 18432193]

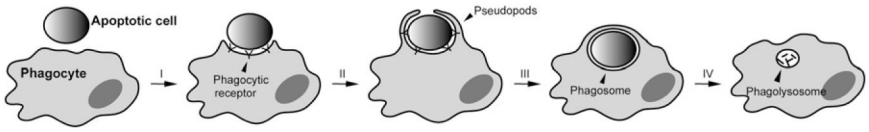

Figure 8.1.

Apoptotic cells are removed by phagocytes through four distinct stages: (I) recognition—apoptotic cells are recognized by phagocytic receptor(s) on the surface of phagocytes; (II) pseudopod extension—the activated phagocytic receptor(s) stimulates the expansion of plasma membrane and the reorganization of actin cytoskeleton (not shown), leading to the extension of pseudopods around apoptotic cells; (III) phagosome formation—the tips of pseudopods meet and fuse, completing the engulfment process, followed by the pinching off of a phagosome that contain an apoptotic cell from the plasma membrane; and (IV) phagosome maturation—the nascent phagosome subsequently undergoes the maturation process and eventually fuses with lysosomes, resulting in the complete degradation of apoptotic cells.

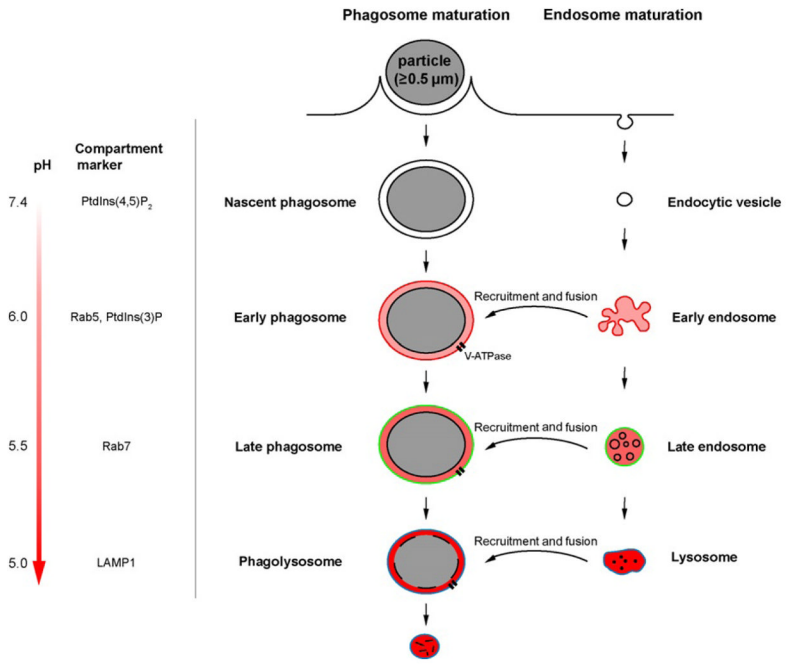

Figure 8.2.

Events occurring during phagosome maturation. The lumen of nascent phagosomes mimics the extracellular environment and has a neutral pH value. Multiple molecules, such as the lipid second messenger PtdIns(3)P and small GTPases Rab5 and Rab7, are produced/recruited on the surface of phagosomes at different stages, driving the stepwise phagosome maturation process. During phagosome maturation, nascent phagosomes sequentially fuse with early endosomes, late endosomes, and lysosomes, forming early phagosomes, late phagosomes, and phagolysosomes, respectively, and gradually acquire the membrane and luminal properties of these organelles, including a variety of hydrolases. The phagosomal lumen is continuously acidified owing to the activity of the V-ATPase, until reaching below 5.0, under which the hydrolysas are fully activated and result in the degradation of phagocytic cargos.

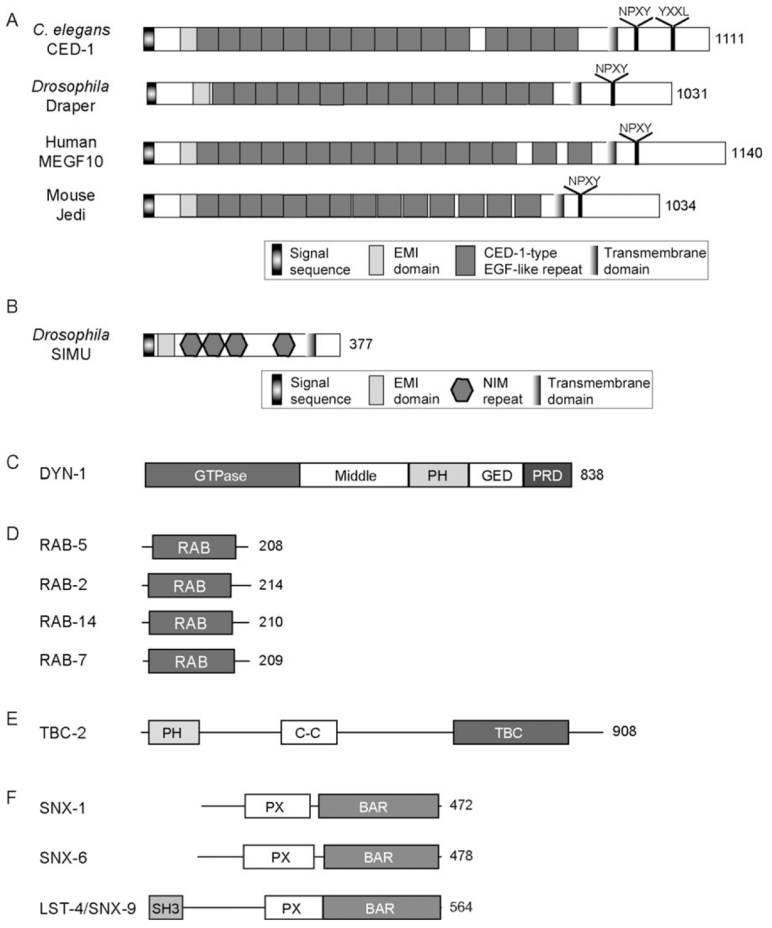

Figure 8.3.

Domain structures of the important proteins described in this chapter. (A) CED-1 and its homologs. EMI, *Emi*lin, and multimerlin domain; CED-1-like EGF-like repeats, a motif with the consensus sequence that include eight regularly positioned cysteines; NPXY and YXXL motifs, potential tyrosine phosphorylation sites. (B) SIMU. NIM repeat, Nimrod repeats. (C) DYN-1. PH, pleckstrin homology domain; GED, GTPase effector domain; PRD, proline-rich domain. (D) The RAB GTPase family. (E) TBC-2. C-C, coiled-coil domain; TBC, Tre-2/Bub2/Cdc16 domain. (F) Three SNX-BAR domain sorting nexins in *C. elegans*. SH3, Src homology 3; PX, phox homology; BAR, Bin-amphiphysin-Rvs.

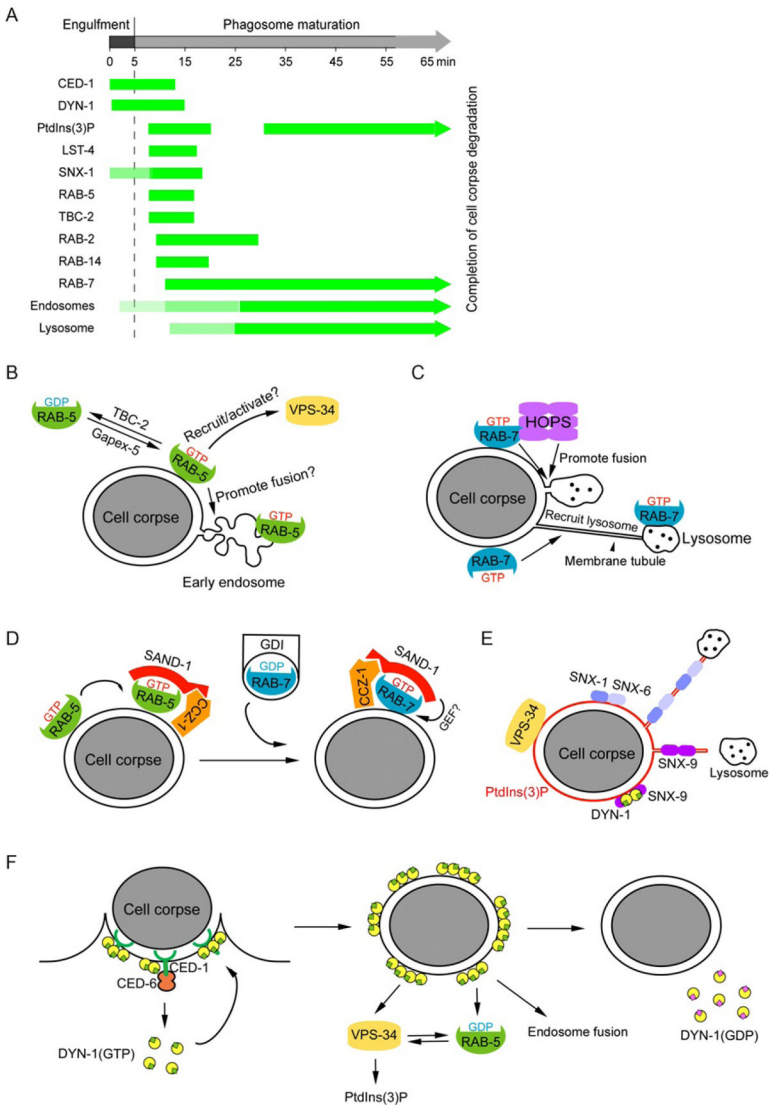

Figure 8.4.

Models depicting the novel molecular mechanisms that drive the maturation of phagosomes containing apoptotic cells. (A) The temporal order of the phago-some localization of multiple phagosome maturation factors and the incorporation of endosomes and lysosomes. Data represent mean durations of GFP- or mRFP-fused reporters localized on pseudopods or phagosomes, obtained from time-lapse imaging experiments monitoring the engulfment and degradation of multiple apoptotic cells. “0 min” represents the time point when engulfment begins. The transition from light to dark color indicates the gradual increase of signal intensity. (B–F) The functions and regulations of Rab GTPase (B–D), PtdIns(3)P effectors (E), and dynamin (DYN-1) (F) in the degradation of apoptotic cells. (B) A model illustrating the regulation of Rab5 during phagosome maturation and the functional relationship between Rab5 and Vps34 (see Section 2.2.2.2). (C) A model depicting that RAB-7 promotes the extension of phagosomal membrane tubules that recruit lysosomes. RAB-7 is also required for phagosome–lysosome fusion, possibly by recruiting the HOPS complex to phagosomes (see Sections 2.2.1 and 2.2.2.4). (D) A model proposing that the RAB-5-to-RAB-7 conversion on phagosomes is mediated by a SAND-1–CCZ-1 complex (see Section 2.2.2.3). (E) A model depicting the action of phagosomal PtdIns(3)P. PtdIns(3)P generated

on nascent phagosome recruits its effectors SNX-1, SNX-6, and LST-4/SNX-9, which promote the extension of phagosomal membrane tubules that recruit lysosomes. LST-4/SNX-9 also interacts with DYN-1 and stabilizes DYN-1's association with phagosomes (see Section 2.4). (F) The dynamic association of DYN-1 with phagosomes is controlled by the upstream regulators and an autoregulatory loop. DYN-1, transiently enriched on nascent phagosomes, serves as an organizer for multiple downstream phagosome maturation events (see Section 2.5).

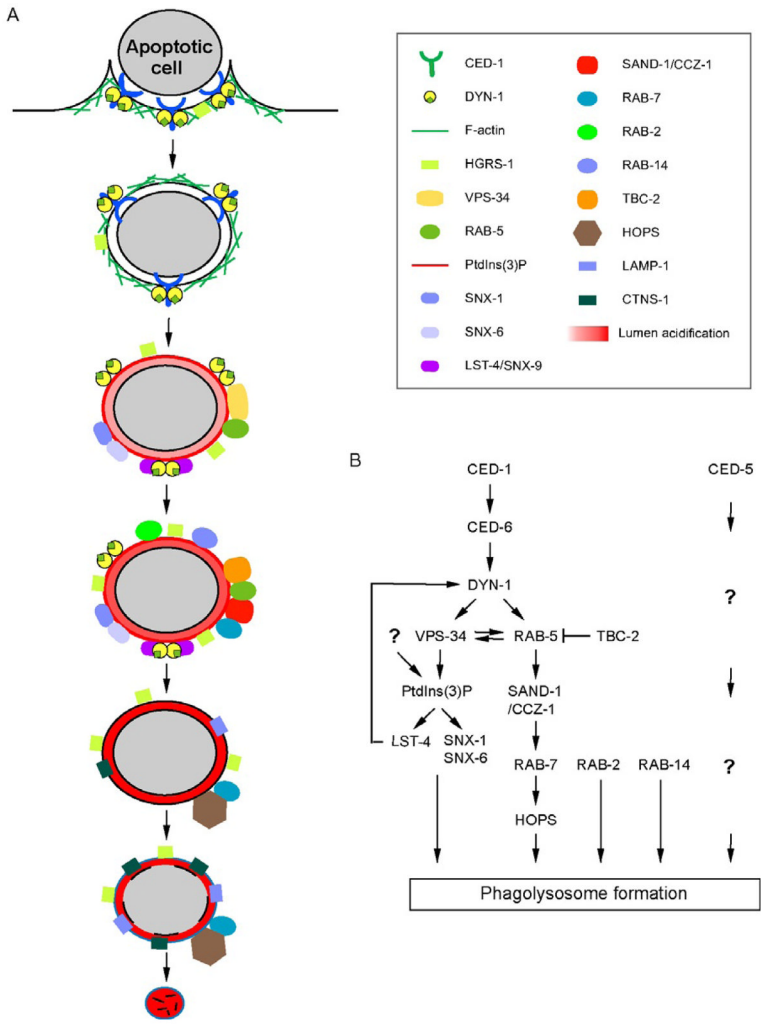

Figure 8.5.

The molecular mechanisms of phagosome maturation during the degradation of apoptotic cells in *C. elegans*. (A) The dynamic localizations of multiple maturation-promoting factors on phagosomes are illustrated to indicate their sequential and coordinated actions that drive the stepwise progression of phagosome maturation. (B) An updated signaling network that promotes the degradation of apoptotic cells, established by substantial genetic and cell biological characterizations. See text for detail.

Table 8.1

Defects in phagosome maturation events caused by the inactivation of *C. elegans* genes needed for the degradation of apoptotic cell

	Phagosomal enrichment of						Incorporation of		
Gene inactivated	**DYN-1**	**PtdIns(3)P**	**RAB-5**	**RAB-2**	**RAB-14**	**RAB-7**	**Endosomes**	**Lysosomes**	**Phagosome acidification**
ced-1[a,b]	Defective[1]	Defective[2]	Defective[13]	ND	ND	Defective[2]	Defective[1]	Defective[2]	ND
ced-5[a,b]	Normal[1]	Normal[2]	ND	ND	ND	Normal[2]	ND	Defective[2]	ND
dyn-1[a,b]	NA	Defective[2,3]	Defective[3,7]	ND	ND	Defective[2,3,5]	Defective[1]	Defective[2]	Defective[3]
vps-34[b]	Normal[3]	Defective[3]	Defective[3]	Defective[10]	Defective[10]	Defective[3]	ND	ND	Defective[3]
rab-5[b]	ND	Defective[3]	NA	Defective[10]	Defective[10]	ND	ND	ND	Defective[8]
tbc-2[a]	ND	Persistent[5]	Persistent[5]	ND	ND	Defective[5]	ND	Defective[5]	Defective[5]
rab-2[a]	ND	Normal[4,10]	Normal[10]	NA	Normal[10]	Normal[4,10]	Normal[4]	Defective[4]	Defective[4,10]
rab-14[a]	ND	Normal[10]	Normal[10]	Normal[10]	NA	Normal[10]	ND	Defective[10]	Defective[10]
rab-7[a,b]	ND	Normal[2,3]	Normal[3]	Normal[10]	Normal[10]	NA	Normal[2]	Defective[2]	Normal[2,4,10]
sand-1[a,b]	Normal[8]	Normal[8]	Persistent[8]	ND	ND	Defective[8]	ND	Defective[8]	Normal[8]
ccz-1[a,b]	ND	Normal[8,9]	Persistent[8,9]	ND	ND	Defective[8,9]	ND	Defective[8]	ND
vps-18[a]	ND	ND	Normal[3]	ND	ND	Normal[3]	ND	Defective[6]	Normal[6]
snx-1[a]	Normal[11]	Normal[11]	Normal[11]	ND	ND	Normal[11]	Defective[11]	Defective[11]	ND
lst-4[a]	Defective[11,12]	Defective[12]	Defective[12]	ND	ND	Defective[11,12]	Defective[11]	Defective[11]	ND

Only genes that have been reported for four or more categories of characterization are listed.

References are marked as superscript.

References: 1. Yu et al., 2006; 2. Yu et al., 2008; 3. Kinchen et al., 2008; 4. Mangahas et al., 2008; 5. Li et al., 2009; 6. Xiao et al., 2009; 7. He et al., 2010; 8. Kinchen and Ravichandran, 2010; 9. Nieto et al., 2010; 10. Guo et al., 2010; 11. Lu et al., 2011; 12. Almendinger et al., 2011; 13. Z. Zhou, unpublished results.

Abbreviations: NA, not applicable; ND, not determined.

[a]Gene inactivation by genetic mutations.

[b]Gene inactivation by RNA interference.