Review

Molecular Detection of Membrane Damage: Lessons from Bacteria

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Review

Molecular Detection of Membrane Damage: Lessons from Bacteria

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Abstract

Mammalian cells contain many membranous organelles, among which endosomes are the initial destination for endocytosed materials. Drugs and pathogens, such as bacteria, are internalized by cells and transported to endosomes or phagosomes, then to lysosomes for degradation. Internalized drugs must escape from endosomes into the cytosol before undergoing degradation in lysosomes. However, endosomal escape is often inefficient in artificial drug delivery systems (DDSs). In contrast, many pathogens are phagocytosed and subsequently escape into the cytosol to proliferate. The studies on phagosomal escape of pathogens have revealed the molecular mechanisms through which host cells detect organelle membrane damage. In this review, we first provide an overview of bacterial endosomal and phagosomal escape, focusing on *Shigella flexneri* as a model organism. We then describe the current knowledge on the cellular machinery involved in sensing and repairing membrane damage, including galectins, ESCRTs, sphingomyelin, stress granules, PI4P in membrane contact sites, and Annexins. We further discuss the roles of secretory MVBs in plasma membrane repair in the Annexins and Future Perspectives sections. Research on membrane damage not only advances our understanding of cellular responses to damage caused by pathogens and artificial nanoparticles, but also informs the design of more effective DDSs.

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1. Introduction

Mammalian cells internalize nutrients, solutes, growth factors, or hormones that bind to specific receptors on the plasma membrane through endocytosis. Early endosomes are the first organelles that internalized materials reach, which sort and transport these materials to their respective destinations. The endocytic pathways have been well reviewed elsewhere [1–4]. Briefly, after internalization, cargo is sorted either to lysosomes for degradation, or to early or recycling endosomes for transporting back to the plasma membrane. There is a retrograde transport route from early endosomes to the Golgi apparatus, which leads to the endoplasmic reticulum (ER).

Internalized materials first reach early endosomes with a pH of 6.0-6.3, then are further acidified to a pH of 5.3 in late endosomes; subsequently, proteins are degraded in lysosomes with a pH of 5.2-5.3 [5–7]. The bulk flow route is thought to be the degradation pathway, as maturation of early endosomes to late endosomes occurs rather than direct transport from pre-existing early endosomes to late endosomes [3,8]. However, transmembrane receptors and lipids undergo cargo sorting processes in early endosomes and can be transported to recycling pathways leading to the plasma membrane, the Golgi apparatus, or the degradation pathway [9,10]. In addition, there are other uncharacterized endosomal pathways [11–13].

In this review, we first overview *Shigella flexneri,* a well-studied bacterium that escapes from endosomes; then, we focus on molecular machineries in mammalian cells. After endosomal escape, autophagy is crucial for preventing *Shigella* proliferation [14]. Autophagy is a process in which

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cytosolic components are degraded after being sequestered within a double-membrane structure marked by lipidated LC3 [15,16]. However, in this review, we do not discuss autophagy in detail, as it has already been extensively covered in other articles. Moreover, multiple pathways can induce autophagy (see Sections 3-1, 3-2, 3-3, and 3-5). Instead, we focus on the earlier phase of damaged organelle detection rather than on downstream autophagy process.

2. Phagosomal Escape by Shigella Species

2.1. Overview of Infection by Shigella spp.

Shigella species are Gram-negative bacteria belonging to the Enterobacteria that cause intestinal infection [17,18]. *Shigella* was first discovered by Dr. Kiyoshi Shiga in 1897 as the cause of epidemic dysentery, later named *S. dysenteriae* [19]. The genus *Shigella* comprises *S. dysenteriae, S. flexneri*, *S. boydii,* and *S. sonnei*. Among them, *S. flexneri* is the most extensively studied. M cells, specialized epithelial cells that continuously uptake gut antigens and transfer them to the associated lymphoid tissue, Peyer's patches via transcytosis, have been identified as the initial uptake site of *S. flexneri* [20,21]. *S. flexneri* crosses the epithelial layer via transcytosis of M cells, then kills macrophages that have engulfed *S. flexneri* [22]. *S. flexneri,* which harbors a 220 kb plasmid, kills macrophages via apoptosis, but non-invasive *S. flexneri* strains or *Listeria monocytogenes*—a Gram-positive bacterium that invades the cellular cytoplasm—do not cause apoptosis. Therefore, the invasion of *S. flexneri* is important for macrophage apoptosis, and not all invasive bacteria induce apoptosis. After escaping from macrophages, *S. flexneri* is internalized by epithelial cells (ECs) and proliferates within them [23–25]. In experiments, *S. flexneri* is phagocytosed by non-professional phagocytes, such as Henle epithelial cells or HeLa cells, and this process is blocked by phagocytosis inhibitors [24]. *S. flexneri* utilizes the actin cytoskeleton of ECs to move [26] and invades adjacent ECs to evade immune responses outside the cells [27].

The invasion activity is encoded in a 30-37 kb region of the 220 kb plasmid of *S. flexneri* [28–30]. This region encodes the Type III secretion system (T3SS) and its effector proteins [17]. The T3SS comprises about 20 proteins forming a needle-like structure that crosses from the bacterial surface, including the peptidoglycan layer, to the host membrane [18,31]. Effector proteins are secreted by the T3SS to manipulate host cell activities.

Shiga toxin is secreted into the culture medium by *S. dysenteriae* [32], as well as by *S. flexneri and S. sonnei* in smaller amounts [33,34]. Shiga toxin inhibits protein synthesis in host cells [35,36]. The pathogenesis of *S. dysenteriae* is primarily related to its invasive phenotype, rather than Shiga toxin production [37]. However, Shiga toxin exacerbates mucosal damage via vasculitis [38], as infections with *S. dysenteriae* strains harboring mutations in the Shiga toxin gene in macaque monkeys lack the phenotypes of bloody stools, intestinal ischemia, and leukocyte decrease [39].

2.2. Phagosomal lysis

We overview the entry of *S. flexneri* in Figure 1.

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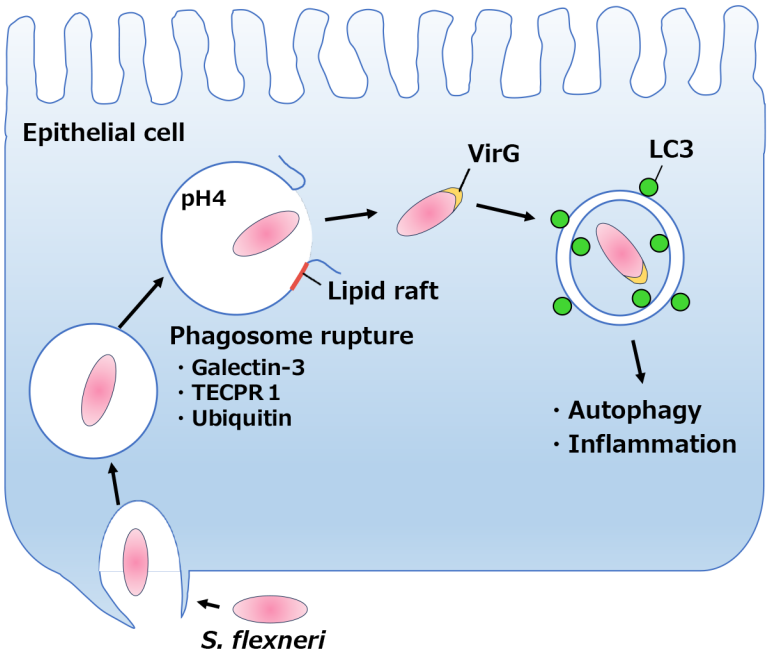

Figure 1. Shigella entry into epithelial cells.

After being internalized, *S. flexneri* escapes from the acidic environment of phagosomes. This escape is dependent on lipid rafts and involves the recruitment of Gal-3 and TECPR1. Once in cytosol, *S.flexneri* is captured by the autophagosome and is eventually degraded.

Multiplication of *S. flexneri* requires the activity of phagosomal lysis. A non-invasive strain of *S. flexneri* was phagocytosed; however, phagosomal lysis was not observed [40]. Invasion plasmid antigen B (IpaB), an effector secreted by the T3SS, is essential for phagosomal lysis by *S. flexneri* [41]. IpaB binds to CD44, a hyaluronan receptor on the cell surface [42]. CD44 localizes to the plasma membrane and induces actin foci at the entry site of *S. flexneri.* An Anti-CD44 antibody inhibits internalization of *S. flexneri*. The IpaB and CD44 complex is partitioned into detergent-resistant membranes (DRMs), and *S. flexneri* entry is inhibited by methyl-β-cyclodextrin (MβCD), a reagent that extracts cholesterol from membranes, suggesting that the entry of *S. flexneri* is dependent on lipid rafts—membrane microdomains enriched in cholesterol and sphingomyelin [43].

IpaB forms K^+ ion channels in an acidic environment (pH 4), suggesting that IpaB forms pores in endosomes or lysosomes rather than in the plasma membrane [44]. Reduced activity of IpaB at pH 7.0 might be beneficial for *S. flexneri*. The feature of pH- and cholesterol- dependent pore formation is found in pore-forming toxins (PFTs) of Gram-positive bacteria, such as Listeriolysin O (LLO) from *L. monocytogenes*. *L. monocytogenes* is taken up through phagocytosis, then it perforates the phagosomal membrane through secretion of LLO and the phospholipases PlcA and PlcB [45,46]. LLO is a protein belonging to the cholesterol-dependent cytolysin (CDC) family [47], which is secreted as a soluble monomer that binds to cholesterol-rich lipid rafts on the phagosomal membrane, forming oligomers and maturing into transmembrane pores [48]. LLO exhibits maximum activity in an acidic environment (pH 5.5–6.0), allowing *L. monocytogenes* to escape the phagosome before it fuses with the lysosome [49]. Bacteria expressing LLO replicate more than bacteria expressing perfringolysin O (PFO), another CDC family PFT that lacks pH sensitivity [50,51]. Conceivably, reduced activity of LLO at pH 7.0 may protect host cells from plasma membrane damage, allowing for bacterial replication in the cytoplasm without inducing host cell death [51,52].

S. flexneri utilizes VirG for its movement by host cell actin [53,54]. VirG induces autophagy of *S. flexneri* by binding to ATG5, an essential protein for autophagosome formation [14,15]. IcsB secreted from *S. flexneri* binds to VirG to compete against ATG5 binding to VirG; therefore, wild-type *S. flexneri*

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prevents autophagy. *S. flexneri* Δ*icsB* can induce autophagy, and LC3 is recruited to *S. flexneri* [14] (See also Section 3-3-2). However, LC3 can be recruited to damaged membranes via membrane remnants damaged by internalized Latex beads [55] or LLOMe treatment [56]. In this case, LC3 recruitment would be independent of bacteria-secreted VirG.

Membrane remnants damaged by *S. flexneri* also recruit inflammasome components, including Ipaf (NLRC4) and ASC with Caspase-1; furthermore, inhibition of the autophagy pathway exacerbates proinflammatory responses, such as cytokine and reactive oxygen species (ROS) production and, eventually, necrosis [57]. In addition, The NLRC4 complex with NAIPs binds to the components of the T3SS [58,59]. Therefore, Immune reaction and autophagy events could be caused by several different pathways, and whether the effects originate from the damaged membrane of host cells or bacterial proteins, including VirG or T3SS, should be carefully assessed.

3. Molecular Detection of Membrane Damage in Mammalian Cells

We list the key factors to be recruited to damaged organelles in Table 1 following this review.

This table lists the molecules described in this review. The timing column indicates when each molecule is recruited to damaged organelles after damaging-inducing treatments, based on the published literature. Much earlier time points may be identified in future studies using improved live-cell imaging or advanced microscopy.

Table 1. Molecules recruited to damaged organelles.

molecule	recognition	Recruitment upon membrane damage	timing	reference
Galectin-3	lumenal glycosylation	lysosomes, endosomes	10-30min	[55–57,60]
Galectin-8	lumenal glycosylation	lysosomes, endosomes	30-60min	[73]
Galectin-9	lumenal glycosylation	lysosomes,endosomes	5-10min	[75–77]
LC3		lysosomes, endosomes, autophagosomes	30-60min	[55–57,60,73]
Ubiquitin		lysosomes, endosomes, plasma membrane	30-60min	[67,103,158]
UBE2QL1		lysosomes	30-60min	[67]
ALIX	ALG2 binding under Ca^{2+}, Gal-3 binding	lysosomes, endosomes, plasma membrane	30sec ~	[71,103,108,119,120,127]
ALG2	Ca^{2+}	lysosomes, endosomes, plasma membrane	20sec ~	[108,112,206]
CHMP4B	ALIX/TSG101	lysosomes, endosomes, plasma membrane	60sec ~	[71,108,119,120,127]
IST1	ALG2 binding under Ca^{2+}	lysosomes	60sec ~	[118,126]
SPG20	Peroxidized lipids, IST1	lysosomes	5min ~	[126]

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ITCH	SPG20 binding	lysosomes	15min	[126]
Lysenin	Sphingomyelin exposure to cytosol	lysosomes, endosomes, plasma membrane	5-40min	[138,139]
EqtSM	Sphingomyelin exposure to cytosol	lysosomes, endosomes, plasma membrane	2min ~	[142,147]
TECPR1	Sphingomyelin exposure to cytosol	bacteria-positive autophagosomes, lysosomes	10-30min	[144,145]
G3BP1/2		Stress granules, Slightly overlap with damaged lysosomes	30sec ~	[148,151,153]
Rabaptin-5	(Rab4,Rab5)[a]	endosomes	30min	[158]
PI4KIIA		lysosomes	10min	[169]
SidM	PI4P	lysosomes	5-10min	[168,178]
ORP9-11	PI4P	lysosomes	10min	[169]
OSBP	PI4P, (Arf1)[a]	lysosomes	6-30min	[168,169]
ATG2A		lysosomes	10 min	[169]
ATG9A	Ca^{2+}	lysosomes, plasma membrane	15-45min	[117,178]
Annexin A1,2,4,5,6,7	Ca^{2+} , negative-charged lipids	plasma membrane	2-50 sec	[204–208]
Annexin A1, 2, 6,7	Ca^{2+} , negative-charged lipids	MVBs, lysosomes, plasma membrane after fusion	10-30min	[209,211,212,221]

[a] ( ) binding sites known in normal condition.

3.1. Galectins

3.1.1. Galectin-3

S. flexneri escapes from phagosomes and replicates in the cytosol by regulating the actin cytoskeleton of host cells [17,54]. During the entry of *S. flexneri* into HeLa, J774 macrophage, or CHO cells, Galectin-3 (Gal-3) is recruited to the membrane surrounding the bacterium [57,60]. Galectins are glycan-binding proteins, and Gal-3 preferentially binds to LacNAc (Galβ(1-4)GlcNAc), which is present internally in glycan residues [61,62]. Gal-3 is recruited to the phagosomal membrane, encapsulating the bacterium and a tubular structure connected to the limiting membrane of phagosomes. The glycan-binding ability of Gal-3 is required for this localization, suggesting that it binds to glycans on the internal leaflet of phagosomes rather than to lipopolysaccharide (LPS) that *S. flexneri* has on its cell wall [60]. Gal-3 can be recruited to membrane remnants of endosomes or lysosomes damaged by a wide variety of methods. Gal-3 is recruited to damaged phagosomes via internalized latex beads coated with transfection reagents and Salmonella-containing vacuoles (SCVs) by internalized *Salmonella Typhimurium* (*S. Typhimurium*) [55]. Early endosomes damaged by magnetic nanoparticles under a magnetic field can recruit Gal-3 [63]. Damaged lysosomes with L-

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leucyl-L-leucine methyl ester (LLOMe) also recruit Gal-3 [56]. As a non-bacterial damaging agent, LLOMe is often used for lysosomal membrane damage. LLOMe is a lysosome-damaging reagent that destabilizes the lysosomal lipid bilayer by forming a polymer from a dipeptide of Leucine in a Cathepsin C-dependent manner [64].

Gal-3 colocalizes with internalized *S. flexneri* together with ubiquitin, p62 and LC3 [57]. p62 is an autophagy adaptor that binds to polyubiquitin and bridges it to LC3[65,66]. Upon LLOMe treatment, K48- and K63-linked ubiquitin chains are conjugated to damaged lysosomes [67]. Screening of E2 ubiquitin ligases identified UBE2QL1, and both UBE2QL1 and K48- and K63-linked ubiquitin are detected 30-60 min after LLOMe treatment. Depletion of UBE2QL1 abolishes ubiquitin signals as well as the recruitment of LC3 and p97, which is required for clearance of damaged lysosomes [67,68]. Interestingly, Gal-3 recruitment to damaged lysosomes is enhanced by UBE2QL1 depletion, indicating that Gal-3 is recruited independently of ubiquitination, but Gal-3 accumulation is indirectly influenced by impaired clearance of damaged lysosomes.

Gal-3 interacts with TRIM16 to recruit ATG16L to damaged lysosomes [69]. ATG16L is a component of the autophagy machinery that is recruited to ubiquitin-positive *Salmonella* and ubiquitin-positive Latex-beads in a manner dependent on ubiquitin and FIP200 [55]. ATG16L determines the localization of LC3[70]. Depletion of TRIM16 inhibits ubiquitination of damaged lysosomes and partially reduces LC3 recruitment [69]. Likewise, depletion of Gal-3 partially decreases LC3 recruitment upon LLOMe treatment [71]. Thus, Gal-3 and TRIM16 contribute to LC3 recruitment and promote autophagy of damaged organelles, at least in part. However, autophagy can be triggered by several different signals, not only by Gal-3, as described in sections 3-2-3, 3-3-2 and 3-5.

Gal-3 KO mice were more susceptible to infection by *Mycobacterium tuberculosis* [69]. However, Gal-3 has also been reported to downregulate antibacterial autophagy [72]. Deletion of Gal-3 resulted in increased LC3 recruitment to phagosomes in *L. monocytogenes*-infected macrophages, which implies that Gal-3 plays a role in suppressing LC3 recruitment to phagosomes in this case [72]. Depletion of Gal-3 may inhibit the repair process of phagosomes, then LC3 recruitment could be increased. Whether Gal-3 inhibits or promotes antibacterial autophagy may depend on various conditions.

3.1.2. Galectin-8, 9

At 1 hour after infection with the Gram-negative bacteria *Salmonella Typhimurium* (*S. Typhimurium*) in HeLa cells, Gal-3, -8, and -9 were recruited to Salmonella-containing Vacuoles (SCVs) [73]. Only Gal-8 depletion increased proliferation of *S. Typhimurium,* suggesting that Gal-3 and Gal-9 do not play roles in inhibiting the proliferation of *S. Typhimurium.* Gal-8 binds similarly to LacNAc as Gal-3 [62], but binds to another autophagy adaptor, NDP-52 rather than p62 [74]. Depletion of NDP-52 increased proliferation of *S. Typhimurium*[73], supporting the roles of NDP-52 and Gal-8 in antibacterial activity against *S. Typhimurium.* After 4 hours, Gal-8-independent recruitment of NDP-52 was observed, indicating that Gal-8 is an early detector for *S. Typhimurium.*

Gal-9 was reported to be recruited to damaged endosomes/lysosomes by damaging agents—namely, chloroquine and siramesine—more than Gal-3 or Gal-8 [75]. Gal-9 was used for high-throughput screening of efficient lipid nanoparticle formulation [76]. In cells, Gal-9 depletion resulted in less ubiquitination of damaged lysosomes [77]. Through LLOMe treatment, Gal-9 binds to the deubiquitinating enzyme, USP9X, to inhibit deubiquitination to induce downstream effects of ubiquitination such as autophagy [77]. Depletion of Gal-9 inhibited ubiquitination of lysosomes via LLOMe treatment and inhibited LC3 lipidation [77].

Although Gal-9 has been used to detect endosomal escape, it may also affect immune system. Gal-9 is expressed in a wide variety of immune cells, and soluble Gal-9 binding to Tim-3 on the surface of $CD4^+$ T cells suppresses T cell activity [78]. Gal-9 binding to Tim-3 downregulates coreceptors of $CD4^+$ T cells, and upregulates p21 to inhibit HIV infection of $CD4^+$ T cells. In contrast,

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Gal-9 binding to secreted PDI on the surface of $CD4^+$ T cells enhances HIV infection to resting $CD4^+$ T cells [79]. The studies of Gal-9 highlight important roles of Gal-9 in immune regulation.

3.2. ESCRTs

3.2.1. Overview of ESCRT Proteins

Endosomal sorting complexes required for transport (ESCRT) proteins have been identified as class E vacuolar protein sorting (Vps) proteins, including ESCRT-0, I, II, III, and the Vps4 ATPase [80–85]. Studies on ESCRT proteins in the trafficking of the epidermal growth factor receptor (EGFR), a well-known receptor tyrosine kinase [86–88], have deepened our understanding of the roles of the ESCRT complexes. Upon EGF binding, EGFR is ubiquitinated [89–93], and Hrs and STAM complexes and ESCRT-0 components bind to the ubiquitinated EGFR and transfer EGFR to ESCRT-I, II, and III, inducing inward budding of the limiting membrane to produce intraluminal vesicles (ILVs) that form multi-vesicular bodies (MVBs) [2,94,95]. Through inward budding, the phosphorylated cytosolic region of EGFR—which binds signaling complexes such as Grb2 [96,97]—is incorporated into ILVs and degraded in lysosomes together with the luminal region of EGFR [87,88]. CHMP4A and CHMP4B, which are ESCRT-III proteins, form filaments [98], and ESCRT-III mediates membrane scission to form ILVs [99,100]. ESCRT-III assembles at the bud neck and constricts the membrane with Vps4 to sever the remaining membrane [101,102]. Thus, ESCRT proteins form vesicles by pushing the membrane from the cytosol into the lumen, which is an inverse mechanism compared to coat proteins such as clathrin or COPI, which invaginate vesicles by pulling the membrane from the lumen to the cytosol. At present, ESCRT proteins are known to have many functions beyond ILV formation, and their unique membrane scission activities plays important roles in diverse cellular events [95].

3.2.2. ESCRT in Plasma Membrane Damage

When the plasma membrane was damaged by a UV laser, CHMP4B—an ESCRT-III component—was recruited to the wound before the initiation of repair [103]. The depletion of CHMP4B decreased cell survival after wounding, indicating that the ESCRT machinery functions in the wound repair of the plasma membrane [103]. ALIX, which binds to TSG101 and CHMP4B to connect Tsg101/ESCRT-I to ESCRT-III [104–107], is recruited to damaged plasma membrane [103,108]. ALIX was originally found as a protein binding to Apoptosis-linked-gene2 (ALG2), and thus was named ALG-2 interacting protein 1 or X (AIP1/ALIX) [109,110]. Overexpression of ALIX is known to induce apoptosis [111]. ALIX binds to ALG2 in a Ca^{2+}-dependent manner, and ALG-2 is known to show oscillatory localization from cytosol to ER-exit site dependent on Ca^{2+} [109,110,112,113]. In steady state, ALIX localizes in endosomes dependent on lysobisphosphatidic acid (LBPA) [114–116]. By inducing plasma membrane damage, ALIX is recruited to the damaged area, and mutant ALIX with defective Ca^{2+} binding has been impaired in recruitment to the lesion of the plasma membrane, suggesting that ALIX binds to the damaged area in a Ca^{2+}-dependent manner[103,108]. Depletion of Ca^{2+} also reduces CHMP4B, indicating that ALIX bypasses ESCRT-II and connects TSG101 to CHMP4B directly [104–107]. Indeed, the ESCRT-0 proteins Hrs and STAM, the ESCRT-I protein Vps37, and the ESCRT-II protein Vps25 are not detected at the damaged plasma membrane [103,108]. ALG2 is required for recruiting ALIX and CHMP4B [108], suggesting that Ca^{2+}-dependent binding of ALG2 to the damaged area is critical for the detection of plasma membrane damage. Ubiquitin is also observed at damaged plasma membrane, but only after CHMP4B, indicating that ubiquitin conjugation is a later event than ESCRT recruitment[103]. It has been proposed that ESCRT-III and Vps4 remove damaged membrane by constricting the undamaged membrane, leading to shedding of the damaged membrane as vesicles outside the cell [103]. In recent research, ALIX is not always required for the repair of plasma membrane damage, whereas TSG101 plays an essential role [117]. ALG-2 can also bind to another protein IST1, which forms a complex with CHMP1, a component of ESCRT-III [85,118] (see Section 3-2-3). Furthermore, there are other Ca^{2+}-dependent proteins such as

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IQGAP1 (see Section 3-6-2) and Annexins (see Section 3-7). Therefore, there may be several pathways for plasma membrane repair.

3.2.3. ESCRT in Lysosomal Membrane Damage

ESCRTs repair damaged lysosomal membranes as well [119,120]. The lysosome-damaging agents, LLOMe, and glycyl-L-phenylalanine 2-naphthylamide (GPN)—which is a substrate of Cathepsin C and causes osmotic lysis of lysosomes specifically [121–123]—have been shown to recruit the ESCRT proteins ALIX and CHMP4B, preceding the recruitment of Gal-3, ubiquitin, and LC3. The combined depletion of ALIX and TSG101 inhibited the recovery of lysosomes after the washout of damaging reagents [119,120]. These results indicate that, similarly to lesions in the plasma membrane, ALIX, TSG101, and ESCRT-III are recruited to damaged lysosomes. In addition, Ca^{2+} helps to recruit CHMP4B [120]. Ca^{2+} is required for the recruitment of ALIX to damaged lysosomes in the early phase, such as 10 minutes after lysosomal damage [71]. After 30 minutes of lysosomal damage, Gal-3 depletion decreased ALIX and CHMP4B recruitment to damaged lysosomes, as did Ca^{2+} chelation. Gal-3 is known to interact with ALIX [124,125], and forms a complex with ALIX and CHMP4B at 30 minutes after lysosomal damage [71]. Therefore, the initial recruitment of ALIX is mainly dependent on Ca^{2+}; meanwhile, in later phases, ALIX can be recruited to damaged lysosomes by Gal-3 as well as by Ca^{2+}.

Lysosomes damaged by LLOMe or silica nanoparticle, recruit IST1 and SPG20 [126]. IST1 is an ESCRT-III-like protein with a CHMP-like domain [118]. IST1 binds to ALG-2 in a Ca^{2+}-dependent manner and forms a complex with CHMP1A and B [118], suggesting that IST1 acts as another Ca^{2+}-dependent adaptor linking ALG-2 to ESCRT-III. Although IST1 binds to SPG20, deletion of the IST1-binding domain in SPG20 only partially reduces SPG20 recruitment to damaged lysosomes [126]. SPG20 has an SC domain that binds to the lipid loosely packed due to peroxidation. Lipid peroxidation induces SPG20 recruitment to damaged lysosomes, whereas Ca^{2+} release without lipid-packing defects—induced by ML-SA1, an agonist of lysosomal Ca^{2+} channel TRPML1— does not recruit SPG20, even though IST1 can be recruited by this condition [126]. These findings indicate that SPG20 interacts with both defective lipids and IST1. SPG20 forms a complex with ubiquitin ligase ITCH, which is recruited to damaged lysosomes. Depletion of ITCH reduces K63-linked ubiquitination in lysosomes, leading to decreases in LC3 recruitment and cell survival rates [126].

3.2.4. ESCRT in Endosomal Membrane Damage

Recruitment of ALIX, CHMP4B, and CHMP1B has been observed in damaged early endosomes, and the depletion of ALIX and TSG101 inhibits CHMP4B recruitment [127]. The rupture of early endosomes was achieved via hypertonic shock, and the reduction in membrane tension in early endosomes was correlated with the recruitment of ESCRT proteins [127]. Membrane tension was detected by a tension probe, Flipt-R, whose fluorescent lifetime decreases when membrane tension decreases [128,129]. Mercier et al. proposed that a decrease in membrane tension in early endosomes, caused by membrane damage due to hypertonic shock or by the fusion of many endocytic vesicles with endosomes upon EGF treatment, would trigger ESCRT recruitment [127].

3.2.5. ESCRT in Bacteria-Containing Vacuoles

Coxiella burnetii—a Gram-negative bacterium that proliferates in lysosomes—also caused ESCRT recruitment to lysosomes [119,120]. *Salmonella*-containing vacuoles (SCVs) are specific organelles in which *Salmonella* proliferate without escaping into the cytosol. The depletion of CHMP3, an ESCRT-III protein, induced the enlargement of SCVs and significantly increased the proliferation of *Salmonella Typhimurium* in the cytosol [130]. ESCRT proteins likely play roles in controlling the shape and size of SCVs to inhibit bacterial proliferation.

3.3. Sphingomyelin

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3.3.1. Sphingomyelin Exposure in Phagosomal Escape

Sphingomyelin is an important component of lipid rafts. Lipid rafts are membrane microdomains that are initially characterized by DRMs and are sensitive to MβCD, which eliminates cholesterol from the liquid-disordered phase of the membrane [131,132]. Sphingomyelin is synthesized from ceramide in the lumen of the Golgi apparatus [133] and is transported to the plasma membrane, endosomes, and lysosomes [134–137]. Sphingomyelin resides in the luminal leaflet of organelles or the outer leaflet of the plasma membrane [134]. Ellison et al. reported that the bacterial membrane damage caused by *S. Typhimurium*, *S. flexneri*, *L. monocytogenes*, and *Streptococcus pyogenes* exposes sphingomyelin to the cytosolic side [138]. The cytosolic presence of sphingomyelin is detected by the C-terminal region of Lysenin, a sphingomyelin-binding proteinaceous toxin isolated from the earthworm *Eisenia foetida* [139]. Lysenin recruitment to *S. Typhimurium* and *S. flexneri* preceded that of Gal-8. Through electron microscopy, damaged membranes positive only for Lysenin were shown to have 100-200 nm gaps, whereas those double-positive for Lysenin and Gal-8 had large gaps and no longer enclose *S. Typhimurium*, suggesting that sphingomyelin exposure to the cytosol is an earlier event than Galectin recruitment [138].

3.3.2. Sphingomyelin Receptor in Membrane Damage

Sphingomyelin can also be detected by equinatoxin II (EqtII), a PFT isolated from *Actinia equina* [140], and EqtII-GFP detects organelles after permeabilization [141,142]. Interestingly, Lysenin and EqtII do not colocalize completely [142]. In the presence of glycolipids that are miscible with sphingomyelin, the binding of Lysenin to SM was decreased [143], indicating that Lysenin binds to highly clustered sphingomyelin but not to sphingomyelin mixed with glycolipids. Lysenin stained large puncta in the plasma membrane as well as sphingomyelin in late endosomes, but not in the Golgi apparatus, early endosomes, or recycling endosomes [142]. In contrast, EqtII detects sphingomyelin in smaller puncta in the plasma membrane as well as in late endosomes and recycling endosomes [142]. EqtII can bind to sphingomyelin dispersed in glycolipids, thus EqtII can detect more sphingomyelin in diverse organelles.

TECPR1 was revealed as an endogenous receptor for *sphingomyelin* exposed to the cytosol [144]. TECPR1 is known to bind to ATG5 [145], and is localized in *S. flexneri* Δ*icsB*-positive membrane. IcsB, a protein encoded by the virulence plasmid in *S. flexneri*[146], binds to VirG[14]. IcsB usually inhibits the interaction between VirG and ATG5 to inhibit autophagy of *S.flexneri*, and depletion of IcsB in *S. flexneri* inhibited the escape of *S. flexneri* from the autophagosome. As such, *S. flexneri* Δ*icsB* was trapped in the autophagosome [14,146]. TECPR1 depletion inhibited the recruitment of LC3 to the *S. flexneri* Δ*icsB*-positive membrane, and replication of *S. flexneri* Δ*icsB* increased [145]. In addition, TECPR1 localized to damaged membranes caused by *S. typhimurium* and *L. monocytogenes*, as well as those damaged by osmotic shock and LLOMe [144]. TECPR1 promotes LC3 conjugation to sphingomyelin-containing liposomes with ATG5 and ATG12, demonstrating that sphingomyelin indeed participates in autophagy pathway involved in organelle damage [144].

3.3.3. Sphingomyelin Pathway in Membrane Damage

Sphingomyelin is exposed by Ca^{2+}-activated scramblase, rather than through simple exposure upon membrane damage [147]. EqtSM—an SM binding protein expressed in cytosol—is recruited to damaged organelles upon treatment with LLOMe or Streptolysin O (SLO), a PFT from *S. pyogenes, and the removal of* Ca^{2+} *by EGTA or BAPTA-AM reduced EqtSM recruitment to damaged organelles* [147]. *The depletion of TMEM16F—a calcium-activated scramblase localized in the plasma membrane—abolished EqtSM recruitment to SLO- and ionomycin-induced damaged membranes but not to lysosomes damaged by LLOMe, suggesting that TMEM16F plays a role in the plasma membrane but not in lysosomes. As BAPTA-AM also inhibited the recruitment of EqtSM to lysosomes damaged by LLOMe, there is likely another scramblase in lysosomes. The depletion of ESCRT proteins, including CHMP3, ALIX, and TSG101, did not inhibit EqtSM recruitment, suggesting that sphingomyelin exposure is independent of ESCRT proteins* [147].

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The cell survival after LLOMe treatment was reduced due to the depletion of either sphingomyelin synthase or ALIX and TSG101, indicating that the sphingomyelin pathway contributes to cell survival independent of the ESCRT pathway [147].

3.4. Stress Granules

Lysosomal damage induces stress granule formation. Various stress granule components were found to be associated with lysosomes via lysosomal IP after LLOMe treatment [148]. G3BP1 is a core protein to drive stress granule assembly by interacting with cytosolic RNAs by liquid–liquid phase separation [149,150]. Interestingly, stress granule formation occurs in a different location than lysosomes. After their formation, stress granules associate with damaged lysosomes [148]. The stress granule protein, NUFIP2, supports the inactivation of mTOR upon lysosomal damage to induce autophagy. Depletion of NUFIP2 keeps mTOR active, resulting in maintained autophagy pathway inactive [148]. G3BP1 forms condensates in damaged artificial vesicles with poly(A)-RNA at low pH, implicating the mixing of lower osmolarity and low pH solution with RNA leading to condensation of G3BP1[151]. Depletion of G3BP1 and 2 increased replication of *M. tuberculosis*, suggesting that stress granule plugs restrict bacterial proliferation in host cells [151]. Stress granule formation is triggered by various stresses, leading to phosphorylation of eIF2α and inhibiting global translation, thus resulting in the accumulation of untranslated mRNAs [152]. A search for proteins binding to eIF2α under LLOMe treatment identified PKR and its activator PACT as upstream kinases upon lysosomal damage to eIF2α [153]. ALIX binds to PKR and PACT under LLOMe treatment, and depletion of Ca^{2+} or ALIX inhibits phosphorylation of eIF2α, suggesting that Ca^{2+} leakage and recruitment of ALIX/ALG2 lead to phosphorylation of eIF2α for stress granule formation. The stress granule protein, NUFIP2, binds to Gal-8; however, depletion of Gal-8 does not inhibit stress granule formation [148]. On the other hand, depletion of Gal-3 increases stress granule formation, indicating that the Gal-3 pathway contributes to the repair of lysosomal damage [153]. Stress granule formation can be induced by various pathogenic lysosomal damage, including *M. tuberculosis*, Adenovirus infection, SARS-Cov-2 ORF3a protein, malarial pigment hemozoin, silica crystals, and Tau aggregates [148,153], indicating the importance of stress granule formation in attenuating the effects of lysosomal damage.

3.5. Rabaptin-5 in Endosomal Damage

Rabaptin-5 is a protein that binds to the small GTPases Rab5 and Rab4 and localizes to early and recycling endosomes [154–157]. Early and recycling endosomes are damaged by Chloroquine and Monensin [158]. Chloroquine accumulates in acidic compartments, where it absorbs protons and induces swelling of endosomes or lysosomes through osmotic water influx. Monensin is an ionophore that perturbs the exchange of Na^+ and H^+, thereby preventing acidification, primarily in recycling endosomes and the Golgi apparatus. Chloroquine treatment induces recruitment of Rabaptin-5, Gal-3, Gal-8, ubiquitin and LC3 to endosomes [158]. Rabaptin-5 binds to the autophagy initiators FIP200 and ATG16L, and depletion of Rabaptin-5 inhibited LC3 recruitment to early or recycling endosomes [158]. Depletion of Rabaptin-5 increased the survival of Salmonellae internalized in HeLa and HEK293A cells, suggesting Rabaptin-5 play a role in restriction of Salmonellae survival.

3.6. Membrane Contact Sites in Membrane Repair

3.6.1. Phosphatidylinositol-4 Phosphate (PI4P) in Lysosomal Damage

Phosphatidylinositol-4 phosphate (PI4P) localizes in the Golgi, endosomes/lysosomes and the plasma membrane [159]. PI4P serves as a binding site of lipid transfer proteins, including Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) [159–161]. ORPs often localize in membrane contact sites in a PI4P-dependent manner at post-Golgi organelles where ORPs mediate the transfer of sterols or phosphatidylserine (PS) between post-Golgi organelles and the ER membrane. PI4P can be visualized using PI4P-binding probes such as the PD domain of OSBP (OSBP-

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PH) and SidM(P4M) [162,163]. Localization of OSBP-PH to the Golgi depends on both PI4P and Arf1, a small GTP-binding protein essential for vesicle budding at the Golgi[164]. Treatment of Brefeldin A (BFA), which inhibits the guanine nucleotide exchange factor required for Arf1 activation [165–167], disrupts Golgi localization of OSBP-PH [162]. This suggests that Golgi targeting of OSBP-PH requires not only PI4P but also Arf1. In contrast, SidM—a protein secreted by *Legionella pneumophila*—is solely dependent on PI4P, as SidM remains at the Golgi even after BFA treatment [163].

Proteomic and lipidomic analyses of damaged lysosomes revealed that PI4P is generated upon lysosomal injury [168,169]. Consistently, SidM is recruited to damaged lysosomes 5-10 min after LLOMe [168]. OSBP-PH is also recruited to damaged lysosomes[169]. Notably, OSBP-PH recruitment to damaged lysosomes after LLOMe treatment is independent of Arf proteins because BFA treatment does not inhibit the recruitment of OSBP-PH [169]. PI4P production at damaged lysosomes requires PI4KIIA, since PI4KIIA depletion abolishes OSBP-PH recruitment to damaged lysosomes [169]. PI4KIIA itself is recruited to damaged lysosomes about 10 min after LLOMe treatment [169]. Importantly, activation of the lysosomal Ca^{2+} channel TRPML1 by its agonist ML-SA1 is sufficient to trigger PI4KIIA recruitment, suggesting that Ca^{2+} leakage is a key signal for recruitment of PI4KIIA to damaged lysosomes [169]. Interestingly, PI4KIIA recruitment is independent of ESCRT machinery. Depletion of ALIX and TSG101 does not affect PI4P production in damaged lysosomes, and conversely, depletion of PI4KIIA does not impair the recruitment of ALIX, CHMP4B, and Gal-3 to damaged lysosomes [168].

Within 10 min after LLOMe treatment, ORP1L, ORP9, 10, 11, and OSBP are recruited to damaged lysosomes [168,169]. ORP9, OSBP, and ORP1L contain an FFAT motif that binds to VAP-A and VAP-B localized in the ER and form membrane contact sites [159,161]. LLOMe treatment extensively increased ER–lysosome membrane contact sites [168,169]. Depletion of VAP-A/B attenuates the formation of ER–lysosome membrane contact sites [168]. Similarly, ORP9, 10, 11, and OSBP quadruple knockout (QKO) diminished VAP-A clustering around damaged lysosomes [169], suggesting that VAP-A/B and ORP form membrane contact sites in lysosomes upon lysosomal damage. ORP9 and 11 transfer phosphatidylserine (PS) to PI4P liposomes in vitro, and LLOMe treatment increased PS in lysosomes [169]. PS accumulation is rescued by any of two ORPs, ORP9/10, 9/11, or 10/11, and reduced Gal-3 recruitment to damaged lysosomes, implicating that PS transfer to damaged lysosomes plays a role in the repair of lysosomes.

Cholesterol also plays a role in repairing damaged lysosomes. OSBP transfers cholesterol, and depletion of OSBP decreased the survival of cells after LLOMe treatment. Accumulation of cholesterol in lysosomes decreases Gal-3 recruitment to damaged lysosomes, indicating that the presence of cholesterol protects lysosomes from damage as well as PS [168].

3.6.2. ATG2-ATG9 in Membrane Damage

ATG2, originally discovered as a required component for autophagy in yeast [15,16,170,171], possesses a cavity to bind to lipids and, thus, has lipid transfer activity [172–174]. Similarly, the human homologs ATG2A and ATG2B have PS transfer activity [174,175]. ATG2A localizes in lysosomes after LLOMe treatment [169]. Expression of mutant ATG2A that lost lipid transfer activity of PS to PI4P-positive liposomes in vitro maintains Gal-3 puncta longer than WT ATG2A expression, suggesting that PS transfer of ATG2A to lysosomes helps to repair damaged lysosomes [169].

ATG9, another essential protein for autophagy [176,177], helps to repair the damaged plasma membrane [117] and lysosomes [178]. ATG9 is a multi-membrane spanning protein and cycles between TGN and late and recycling endosomes [179,180]. When the autophagy pathway is activated, ATG9 relocalizes to precursors of the autophagosome [180,181]. ATG9 forms a homotrimer complex [182] and has lipid scramblase activity [183]. ATG9 binds to ATG2 and is thought to supply phospholipids to the autophagosome for autophagosome membrane expansion [183,184]. ATG9 binds to many of the vesicle budding machineries. ATG9 binds to clathrin adaptors, AP-2 [185,186], AP-4 [187,188], AP-1 [189], and AP-3 [178], as well as retromer complex [185]. When AP-2 or AP-4 are depleted, ATG9 remains in the recycling endosomes or TGN even under starvation conditions,

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and the autophagy pathway is inhibited, suggesting proper transport of ATG9 is required for autophagosome formation [185–187].

When the plasma membrane was damaged by digitonin, saponin, SLO, or the endogenous pore-forming protein Gasdermin-D, plasma membrane permeabilization was exacerbated in ATG9-depleted cells indicating that ATG9 protects plasma membrane from damage [117]. ATG9 is recruited to the injured plasma membrane in a manner dependent on IQGAP1, which responds to Ca^{2+}, and depletion of Ca^{2+} outside of cells inhibits ATG9 recruitment to the injured plasma membrane [117]. IQGAP1 interacts with ATG9 and CHMP2A, ESCRT-III protein, and double knockout of ATG9 and CHMP2B does not exacerbate PM permeabilization compared to single knockout of ATG9 or CHMP2B, which indicates that ATG9 and CHMP2B function in the same pathway for plasma membrane repair.

ATG9 has also been reported to play a role in lysosomal repair as well [178]. In LLOMe-treated cells, ATG9 is recruited to damaged lysosomes [178]. Depletion of cellular Ca^{2+} inhibited ATG9 recruitment, indicating that Ca^{2+} promotes ATG9 recruitment [178]. ATG9 vesicles contain ARFIP2/Arfaptin 2, an Arf-GTP binding protein [164], and bind to PI4P and PI4PK2A [178,190]. Depletion of ARFIP2 increases ATG9, PI4KIIA, and PI4P localization in damaged lysosomes, and lysosomes are repaired faster [178]. ARFIP2 forms a complex with AP-3 and, when AP-3 is depleted, ATG9 increases in damaged lysosomes, implicating that AP-3 helps to retrieve ATG9 from the endolysosomal compartment to the Golgi and attenuates lysosomal repair [178]. ARFIP2 binds to ORP9 and interferes with the PS lipid transfer activity of ORP9/11 in vitro [178]. Depletion of ARFIP2 restricts *M. tuberculosis* and *Salmonella* infection, demonstrating that ARFIP2 inhibits lysosomal repair under normal conditions, while depletion of ARFIP2 promotes lysosomal repair [178]. These findings indicate that the transfer of PS and cholesterol at the expense to PI4P contributes to membrane repair, mediated by ORP9-11, OSBP, or ATG2-ATG9 complex. In contrast, clathrin adaptors and ARFIP2 restrict the repair process by regulating ATG9 trafficking.

3.7. Annexins

3.7.1. Annexins in Plasma Membrane Repair

Annexins are a family of Ca^{2+}-dependent phospholipid-binding proteins [191,192]. Annexins bind to negatively charged phospholipids, including PS, PI, and PI(4,5) P2, in a manner dependent on Ca^{2+} [193–199]. Among them, PS is most characterized and largely used for Ca^{2+}-dependent binding of Annexins to lipids. In addition, Annexin A2 binds to cholesterol independent of Ca^{2+}[200].

Annexins A1 and A2 associate with Dysferlin, whose mutation causes dysferlinopathy [201]. Dysferlin-deficient myotubules lose the ability to reseal the damaged plasma membrane [201]. Lack of Annexin A2 impairs repair of injury in a Cholesterol-dependent manner in muscle cells [202]. Annexins also affect plasma membrane repair in non-muscle cells. As shown via the scraping procedure in HeLa and kidney epithelial cells, Annexin A1 deficient for Ca^{2+} binding loses the ability to reseal plasma membrane injury [203]. Annexins A4, 5, 6, and 7 are reportedly also involved in plasma membrane repair [204–206].

Annexins A1, 2, and 6 translocate to the wound area in a wave-like manner and form a ring-like structure around the wound within 2-30 sec [207]. Annexins A1, 2, 5, and 6 localize in the repair cap as well after injury in 50 sec, dependent on Ca^{2+} and actin [208]. Annexin A5 forms a trimer to form a 2D array via self-assembly on PS-exposed membranes [205]. An Annexin A5 mutant that disrupt its trimer structure inhibited membrane repair; however, membrane binding was not inhibited [205], suggesting that self-assembly of Annexin A5 is required for membrane repair but not membrane binding.

Annexins A4 and A6 can regulate membrane curvature [204]. Annexin A4 binds to the edge of the membrane and rolls up the membrane. In contrast, Annexin A6 can constrict the planar membrane. As determined via live imaging, Annexin A6 was recruited to the damaged area before Annexin A4 and localized as a ring-like structure around the wound. It has been proposed that

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Annexin A6 binds to the edge and Annexin A4 rolls up the hole of the edge, following which Annexin A6 constricts to close the membrane. This process completed within 15 sec in a mammary carcinoma cell line, MCF7 cells [204].

Annexin A7 also plays a role in plasma membrane repair [206]. Annexin A7 binds to ALG2 and recruits ALG2 to the plasma membrane wound within 100 sec. ALG2 is accumulated at the edge of the membrane in an Annexin A7-dependent manner. A mutant of Annexin A7 that cannot be recruited to the lesion of the plasma membrane inhibited ALG2 recruitment. Interestingly, CHMP4B localized at the repair site, and shed vesicles were observed from the plasma membrane within 2-15 min. The ESCRT complex induces shedding of the plasma membrane as microvesicles/ectosomes for membrane repair. The findings of these studies indicate that Annexins function as first-line machinery for plasma membrane damage detection and repair. ESCRT-mediated membrane repair follows the repair initiated by Annexin A7.

3.7.2. Annexins in Endolysosomal Repair

Annexin A2 is recruited to damaged multi-vesicular bodies (MVBs) as well as lysosomes in dendritic cells (DCs) [209]. Ultra-high molecular weight polyethylene is released from joint replacement as wear debris, and induces inflammation when endocytosed by macrophages and DCs [210]. Wear debris comprising nanometer- to micrometer-sized particles are incorporated into MVBs and release lysosomal enzymes such as Cathepsin S and B [209,210], suggesting the internalization of wear debris damages MVBs and lysosomes. Depletion of Annexin A2 results in increases in the release of Cathepsin B and IL-1b [209]; therefore, Annexin A2 would repair damaged MVBs and lysosomes.

Annexins A1 and 2 play roles in lysosomal repair after LLOMe treatment in human osteosarcoma U2Os cells [211]. Although many Annexins are recruited to damaged lysosomes, only Annexins A1 and 2 have activity for lysosomal repair. Interestingly, Annexin A1/2 depletion slows down lysosomal repair more strongly than ALIX/TSG101 depletion, suggesting that the Annexin A1/2 repair pathway is independent of ESCRTs, in contrast to plasma membrane repair (see section 3-7-1). Annexins A1 and 2 are preferably recruited to damaged lysosomes that sustain larger injuries, sufficient to release 10-KDa dextran, whereas ESCRTs can be recruited to even to lysosomes with smaller injuries.

Annexin A7 has also been reported to be important for lysosomal repair in MCF7 and HeLa cells [212]. Depletion of Annexin A7 exacerbates Gal-3 recruitment to damaged lysosomes by LLOMe. Although Annexin A7 is known to bind to ALG2 [206], CHMP4B recruitment to damaged lysosomes is not affected in Annexin A7-depleted cells [212]. In addition, Annexin A7 depletion does not affect lysophagy or alter lipid composition, which is regulated by the PI4P pathway [168,169]. Thus, Annexin A7 facilitates lysosomal repair through other mechanisms.

We also note that the timing of Annexin recruitment to damaged lysosomes is slower than to damaged plasma membrane (Table 1). Together, these findings suggest that Annexin contribute to lysosomal repair via mechanisms distinct from those operating during plasma membrane repair.

3.7.3. Annexins for Secretory Lysosome/MVB Fusion to the Plasma Membrane

Upon plasma membrane damage, it is proposed that secretory lysosomes fuse with the damaged area of the plasma membrane in a Ca^{2+}-dependent manner to repair plasma membrane damage [213]. Annexins may facilitate such fusion for plasma membrane repair.

Annexin A2 has endosome fusion activity [214], and plays a role in exocytosis in chromaffin cells [215]. In Annexin A2-deficient chromaffin cells, exocytosis upon nicotine stimulation is inhibited [215]. Annexin A2 is translocated to the plasma membrane after exocytosis, in a manner dependent on lipid rafts. Therefore, it is proposed that Annexin A2 forms a lipid raft in the plasma membrane in a Ca^{2+}-dependent manner and mediates the fusion of secretory granules with the plasma membrane[215].

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Exosomes are endosome-derived extracellular vesicles, which are secreted by a wide variety of cells, including cancer cells and platelets [216]. When multivesicular bodies (MVBs) fuse with the plasma membrane, their internal luminal vesicles (ILVs) are released as exosomes [217–219]. The tetraspanin protein CD63 localizes in ILVs of MVBs and is secreted on exosomes [220]. Exosomal release can be induced by Ca^{2+} in HCT116, a human colon cancer cell line, and plasma membrane damage by streptolysin O (SLO) also stimulates exosome secretion [221].

Annexins A2 and 6 have been identified as proteins recruited to CD63-positive MVBs in a Ca^{2+} dependent manner [221]. Although the role of Annexin A2 has not been examined, depletion of Annexin A6 inhibited MVB fusion and release of exosomes stimulated by Ca^{2+} ionophore [221]. Moreover, an antibody against Annexin A6 prevents SLO-stimulated exosome release, indicating that Annexin A6 mediates the fusion of MVBs to the damaged plasma membrane.

These findings suggest a model in which CD63-positive MVBs fuse with the damaged plasma membrane to facilitate plasma membrane repair, releasing exosomes. However, whether this mechanism represents a ubiquitous cellular response or is restricted to certain secretory or cancer cells remains to be determined (see Future Perspectives).

4. Future Perspective

Studies on bacteria have significantly advanced our understanding of how cells detect organelle membrane damage. Some molecules are shared between the detection of plasma membrane and lysosomal membrane damage, whereas others are specific to each organelle. The key molecules are summarized in Table 1, and Figure 2 illustrates their association with each organelle.

We summarize the timing of recruitment of each molecule, as reported in the studies discussed in this review in Table 1. Early recruitment events are largely dependent on calcium release, which triggers the recruitment of ALG2/ALIX with the ESCRT complex, ATG9 vesicles, and Annexins. These events occur at both the plasma membrane and damaged lysosomes, although not all molecules are universally present (Table 1 and Figure 2). Additional mechanisms for membrane damage detection include the exposure of sphingomyelin and glycans to the cytosol, which recruits TECPR1 and Galectins, thereby initiates autophagy. Stress granule formation appears to occur specifically upon lysosomal damage, resulting from the mixing of acidic, high-osmolarity lysosomal contents with the cytosol. Moreover, Rabaptin-5-mediated signaling from early or recycling endosomes to the autophagy pathway also seems to be unique to early/recycling endosomes, as Rabaptin-5 binds to Rab4 and Rab5, which are typically specific to early/recycling endosomes. Future studies should aim to reveal how specific each event is to each organelle.

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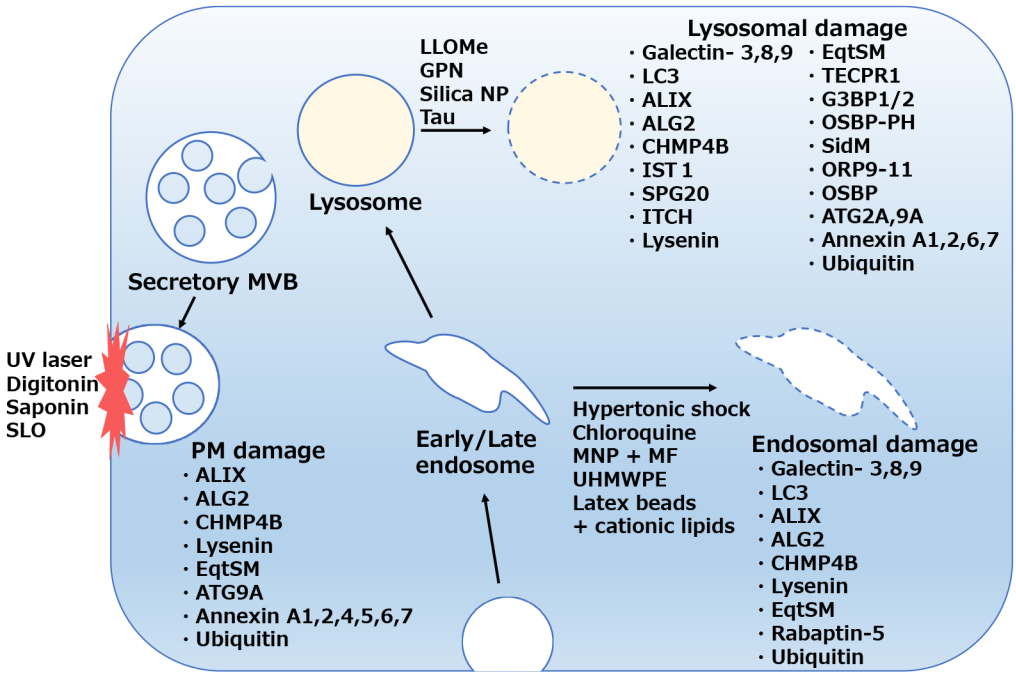

Figure 2. Schematic model of molecules recruited to damaged organelles and artificial damaging methods.

This figure illustrates the key factors involved in membrane damage detection in different organelles, as described in this review. Various artificial methods used to induce organelle damage are also indicated. Abbreviations not used in the main text: NP, nanoparticle; MNP, magnetic nanoparticle; MF, magnetic field; UHMWPE, Ultra-high molecular weight polyethylene.

Although secretory MVBs were not the focus of this review, the discussion of membrane damage detection and repair naturally converges on the question of how secretory MVBs contribute to plasma membrane repair. The hypothesis that secretory MVBs or lysosomes fuse with the plasma membrane to repair damage is attractive, but several issues remain unresolved. First, it is unclear whether this fusion occurs universally or only in specific cell types. CD63-positive MVBs are positive for Rab27, a secretory granule marker [222], and fusion with the plasma membrane can be stimulated by Ca^{2+} or even by histamine in HeLa cells, suggesting even non-classical secretory cells may harbor secretory granule-like MVBs [223]. In contrast, CD63 release has been reported to occur independently of Rab27 in HEK293 cells [224]. Thus, there may be cell types that lack “secretory” MVBs or lysosomes to fuse with the plasma membrane. Second, even if cells have secretory MVBs or lysosomes to fuse with the plasma membrane, these cells may have several different MVBs. It is known that there are MVBs that are only EGF-positive, only CD63-positive, and both-positive [225,226]. CD63-positive MVBs are considered secretory MVBs that release exosomes via plasma membrane fusion [221]. Our analyses of endosomal sorting by ArfGAP proteins, which hydrolyze Arf-GTP and regulate cargo sorting [227–229], revealed that EGF trafficking to lysosomes is regulated by ArfGAP3 [230], whereas CD63 to MVBs is regulated by ADAP1 or ARAP1, other ArfGAP proteins [226]. Therefore, even if cells have secretory MVBs, these may be distinct from normal “degradable” MVBs, which are typically EGF-positive. Third, it remains unclear whether secretory MVBs are completely different from degradable MVBs or not. EGF-positive MVBs can fuse with the plasma membrane when the lysosomal pathway is inhibited [231]. In addition, Rab7-positive MVBs/lysosomes, which are usually destined for degradation, can mature into Rab27-positive secretory MVBs through the formation of membrane contact sites [232]. Thus, it is possible that secretory MVBs are derived from normal degradable MVBs. Determining which cell type possess secretory MVBs, which MVBs are responsible for repairing plasma membrane damage, and how secretory MVBs are formed or diverge from degradable MVBs should be the focus of future work.

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Finally, membrane damage responses are increasingly recognized as integral components of broader cellular stress responses, with implications extending to exosome biology, autophagy, inflammation, and the development of Drug Delivery Systems (DDS). Continued discoveries are expected in this field to expand the list of key factors involved, thereby contributing to a broad range of biomedical research.

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