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Let's play a game of chutes and ladders

Lysosome fusion with the epithelial plasma membrane

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In non-polarized cells, calcium-induced exocytosis of "conventional" lysosomes is important in diverse processes like membrane repair after exposure to pore-forming toxins and clearance of cellular debris. Resealing of torn membranes is especially critical for barrier epithelia that directly interact with pathogens and toxins, which can result in membrane microdisruptions and lesions. However, whether lysosomes participate in membrane repair in polarized epithelia has been an open question. We recently reported that in polarized Madin-Darby canine kidney (MDCK) cells, localized influx of calcium induces lysosomes to fuse with the basolateral membrane. This spatial segregation of exocytosis depends on an intact actin cytoskeleton, membrane cholesterol and restricted distribution of fusion machinery such as the t-SNARE syntaxin 4. Our data show that the polarity of syntaxin 4 (which is regulated by the clathrin adaptor protein AP-1) dictates whether lysosomes parachute down to the basolateral membrane or take a ladder up to the apical membrane. Here, we speculate about additional machinery (such as the lysosomal calcium sensor synaptotagmin VII and the v-SNARE VAMP7) that could be involved in polarized fusion of lysosomes with the epithelial membrane. We also discuss the potential importance of lysosome exocytosis in maintaining membrane integrity in the retinal pigment epithelium, the primary tissue affected in blinding diseases such as age-related macular degeneration.

Keywords: polarized epithelia, membrane repair, fusion machinery, SNAREs, synaptotagmins, retinal pigment epithelium, age-related disease

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Lysosome-Plasma Membrane Fusion: Multiple Modes and Roles

Lysosomes are morphologically heterogeneous, acidic organelles that are part of the highly dynamic endocytic network of the cell.[1,2] Lysosomes receive acid hydrolases and membrane proteins from the biosynthetic route and degrade cargo acquired by endocytosis, phagocytosis or autophagy. Cells such as melanocytes and platelets have lysosome-related organelles (LROs) that carry out specialized functions. Lysosomes and LROs are critical for maintaining cellular homeostasis because they function as signaling platforms and are involved in cholesterol trafficking, immune response and plasma membrane repair.[2,3] Consequently, defective lysosome function can contribute to the pathogenesis of numerous age-related diseases including Alzheimer disease, Parkinson disease and age-related macular degeneration.[4,5] A central requirement for lysosomes to carry out their diverse functions is their ability to fuse with late endosomes and autophagosomes to form hybrid organelles or with the plasma membrane for exocytosis.[3] Although lysosomes are considered to be terminal degradative compartments, exocytosis of secretory and conventional lysosomes has been observed in several cell types.

A growing body of work now suggests that lysosome exocytosis operates in diverse paradigms that control cell health and disease as detailed below:

Immunity and pigmentation. In cytotoxic T lymphocytes and natural killer cells, secretory lysosomes fuse with the

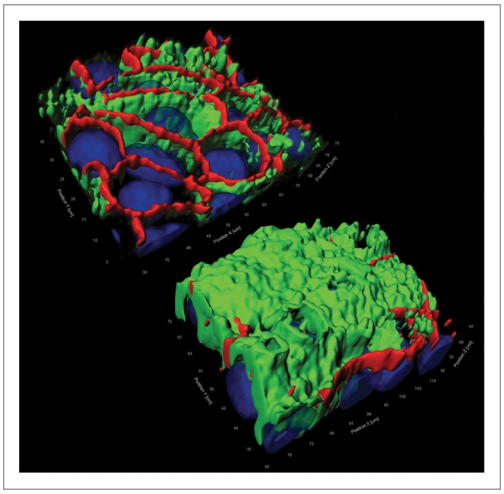

Figure 1. The epithelial-specific clathrin adaptor AP-1B regulates the polarity of calcium-induced lysosome exocytosis. 3-D rendering of surface LAMP2 staining in polarized wild-type MDCK cells (top left) and cells lacking the μ subunit of AP-1B (μ1BKD, bottom right) after a short exposure to the calcium ionophore ionomycin. The nuclear stain DAPI is in blue, and the tight junction marker ZO-1 is in red. In wild-type cells, LAMP2 is restricted to the basolateral domain (below ZO-1), whereas in μ1BKD cells, LAMP2 is on both the apical and basolateral domains, indicating non-polar lysosome exocytosis.

plasma membrane to release perforin, granzyme and other molecules involved in the immune response and target cell apoptosis. In activated monocytes, ATP triggers calcium influx and subsequent exocytosis of secretory lysosomes, which also release pro-inflammatory cytokines such as interleukin IL-1β.[6] Impaired exocytosis of secretory lysosomes in immune cells and melanocytes causes numerous autosomal human genetic diseases characterized by immune dysfunction and pigmentation defects.[7]

Membrane repair. Fusion of conventional lysosomes with the plasma membrane has been documented extensively in fibroblasts and other non-polarized cells.[8,9] Upon mechanical membrane damage or exposure to pore-forming toxins, calcium from the extracellular milieu rushes into the injured cell and triggers rapid resealing of membrane holes. This $[Ca^{2+}]i$-regulated exocytosis is an ubiquitous process critical for maintaining membrane integrity and for ensuring cell survival. What could be the source of the intracellular membrane required for patching tears after cell injury? Nearly two decades ago, Andrews and colleagues observed that during *Trypanosoma cruzi* infection, binding of the parasite to the cell membrane triggers calcium influx and lysosome fusion with the plasma membrane.[10] Subsequently, it was confirmed that calcium ionophores and pore-forming toxins like streptolysin O also lead to lysosome exocytosis within minutes of calcium influx.[8,9] Exocytosis can be monitored by the appearance of lysosome-associated membrane proteins (LAMP1 or LAMP2) on the plasma membrane in non-permeabilized cells and by the release of lysosomal hydrolases (β-hexosaminidase, acid sphingomyelinase, etc) into the extracellular medium.

Pathogen entry and infection. Studies show that pathogens exploit lysosome exocytosis for invasion and infection: *T. cruzi,* which causes Chagas disease, recruits lysosomes to the plasma membrane and the gradual fusion of lysosomes forms the parasitophorous vacuole that envelopes the parasite. The acidic environment of the lysosome disrupts the vacuole and allows the parasite to replicate in the cytosol. Thus, lysosomes act as safe havens for the trypomastigotes within the cell and lysosome exocytosis facilitates successful *T. cruzi* infection.[11] Contrary to its role in Chagas disease, lysosome exocytosis is protective in tuberculosis:[12] infection of macrophages with *Mycobacterium tuberculosis* causes microdisruptions on the plasma membrane, leading to necrosis that promotes bacterial replication and infection. Lysosomes and vesicles derived from the Golgi apparatus participate in resealing these membrane lesions and prevent macrophage necrosis. Membrane repair in this scenario depends on prostaglandin E2, which regulates syntaptotagmin VII, the lysosomal calcium sensor that is required for lysosome-plasma membrane fusion. *M. tuberculosis* blocks prostaglandin E2 synthesis by inducing the production of lipoxin A4; this in turn prevents membrane repair and facilitates necrosis.[12]

Debris removal. In lysosomal storage disorders, it is conceivable that fusion of lysosomes with the plasma membrane can help clear accumulated debris. In mucolipidosis type IV, an autosomal recessive disorder caused by mutations in mucolipin-1, calcium-induced exocytosis of lysosomes is inhibited.[13] In metachromatic leukodystrophy, another lysosomal storage disease caused by arylsulfatase A deficiency, fusion of lysosomes with the plasma membrane helps remove intralysosomal inclusions.[14]

Molecular Machinery of Lysosome Exocytosis in Non-Polarized Cells

Several studies have identified the molecular machinery required for lysosome-plasma membrane fusion in non-polarized cells, including the lysosomal calcium sensor synaptotagmin VII, the v-SNARE VAMP7 and t-SNAREs syntaxin 2, syntaxin 4 and SNAP 23.[8,15,16] Work by Andrews and colleagues showed that calcium causes a conformational change in synaptotagmin VII, which facilitates the formation of the four-helical bundle between t- and v-SNAREs and subsequent fusion between the lysosomal and plasma membranes. Studies have also shown that actin is a barrier to exocytosis, whereas microtubule-mediated long-range transport is essential to dock lysosomes at or near the membrane, but that microtubules are not involved in lysosome exocytosis per se.[8,17]

Molecular Machinery of Lysosome Exocytosis in Polarized Epithelia

Although fusion of lysosomes with the plasma membrane of non-polarized cells has been extensively documented, little is known about the mechanism of lysosome

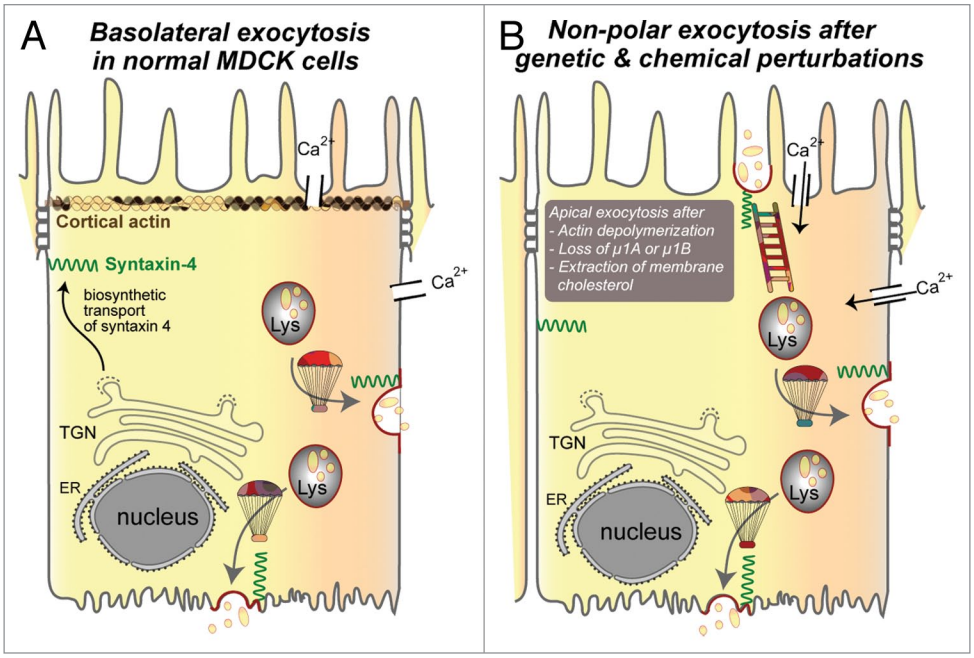

Figure 2. "Chutes and ladders" model of lysosome exocytosis in polarized epithelia. (**A**) In MDCK cells, lysosome exocytosis occurs preferentially at the basolateral membrane in response to localized calcium influx. Polarized expression of the t-SNARE syntaxin 4 at the basolateral membrane (mediated by the clathrin adaptor AP-1) and cortical actin at the apical membrane spatially restrict fusion of lysosomes to the basolateral domain. (**B**) In cells lacking the μ subunit of AP-1 or in cells treated with cytochalasin D to depolymerize the actin cytoskeleton or methyl-b-cyclodextrin to deplete membrane cholesterol, syntaxin 4 is misdirected to the apical membrane. This non-polar distribution of the t-SNARE induces lysosome exocytosis at both the basolateral (chutes) and apical (ladders) membranes.

exocytosis in polarized epithelia. We recently conducted a detailed analysis of lysosome exocytosis in Madin-Darby canine kidney (MDCK) cells, the canonical polarized epithelial cell model.[18] Our results show that in these cells, exposure to the calcium ionophore ionomycin or the pore-forming toxin Streptolysin O induces lysosomes to fuse predominantly with the basolateral membrane. This is in agreement with previous studies showing that *T. cruzi* infects polarized MDCK cells from the basolateral surface only.[19] In polarized MDCK, the t-SNARE syntaxin 4 is restricted to the basolateral domain and depletion of syntaxin 4 inhibits exocytosis. These data indicate that the basolateral localization of syntaxin 4 is critical for dictating the polarity of lysosome exocytosis. The epithelial-specific clathrin adaptor protein AP-1B has been shown to participate in biosynthetic delivery of syntaxin 4 to the basolateral membrane.[20] In MDCK cells lacking the μ subunit of either AP-1B (μ1B) or the ubiquitously-expressed AP-1A (μ1A), syntaxin 4 is non-polar and as a result of this mispolarization, lysosomes fuse with the both the apical and basolateral membranes (**Fig. 1**).

In wild-type MDCK cells, ~4–6% of total cellular β-hexosaminidase is released at the basolateral surface, indicating that calcium-induced lysosome exocytosis is a local event that mostly involves lysosomes that are already pre-docked at the plasma membrane.[8] Indeed, microtubule depolymerization did not affect the polarity or extent of lysosome exocytosis in MDCK cells as reported previously for non-polarized cells.[9] On the other hand, actin depolymerization induced lysosome fusion at the apical membrane, possibly by dispersing syntaxin 4 clusters from the basolateral to the apical membrane. Depletion of membrane cholesterol had a similar effect on syntaxin 4 dispersal, leading to non-polar exocytosis. On the other hand, overloading lysosomes with cholesterol completely inhibited exocytosis, possibly due to decreased lysosome motility[21] or abnormal sequestration of target and vesicular SNAREs[22] (**Fig. 2**).

Relevance of Lysosome Exocytosis in Polarized Epithelia

Epithelial cells line body cavities and tubular structures, forming a physical barrier between the organism and the external environment. Lysosome exocytosis is therefore likely to be a major player in maintaining membrane integrity after exposure to pathogens and toxins. Our data show that lysosome exocytosis is restricted to the basolateral domain in epithelia that express the clathrin adaptor AP-1B. In these cells, apical membrane tears could be patched with membrane from primary cilia or microvilli.[23,24] In highly metabolically active epithelial cells such as hepatocytes and the retinal pigment epithelium, lysosome exocytosis could also function as a conduit to rid the cell of unwanted debris. For instance, in rat hepatocytes, secretion of copper-laden lysosomes into bile is one mechanism by which cells deal with excess copper.[25] Apical lysosome exocytosis into bile is in agreement with studies showing that hepatocytes do not express AP-1B[26] and underscores the role of this adaptor

protein in spatially restricting lysosome fusion with the basolateral membrane.

In the eye, the post-mitotic cells of the retinal pigment epithelium (RPE) perform many functions indispensable for vision including the daily phagocytosis and degradation of shed photoreceptor outer segment discs.[27] Damage to the RPE is an initiating factor in many blinding diseases such as age-related macular degeneration.[28] With age, lipid-protein aggregates accumulate within RPE lysosomes (called lipofuscin) and in the RPE basement membrane (called drusen). Lysosome exocytosis in the RPE could potentially be a route for lipofuscin to exit the cell: indeed, exocytosis of undigested outer segment discs and latex particles at the basolateral surface of RPE has been documented in vitro.[29,30] The RPE is also susceptible to membrane damage as a result of chronic complement activation and deposition of sublytic membrane attack complexes.[31] Since these cells do not express AP-1B, lysosome exocytosis could potentially protect both the apical and basolateral surfaces from complement-mediated damage.

Outstanding Questions

Much remains to be understood about the mechanism and relevance of lysosome exocytosis in polarized cells: what are the roles of the lysosomal calcium sensor synaptotagmin VII and the v-SNARE VAMP7 in apical vs. basolateral exocytosis? Does compensatory endocytosis after lysosome fusion occur with comparable kinetics in epithelial cells as in non-polarized cells? Is there any overlap in the machinery involved in regulated lysosome exocytosis and constitutive secretion of exosomes? Does the efficiency of lysosome exocytosis decline with age and could defective membrane repair contribute to diseases such as diabetes,[32] atherosclerosis[33] and age-related macular degeneration? It is tempting to speculate that an inability to repair membrane microdisruptions and sustained calcium influx could promote inflammation and aberrant signaling, which can in turn impact cell fate decisions, disease initiation and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. de Duve C. The lysosome turns fifty. Nat Cell Biol 2005; 7:847-9; PMID:16136179; http://dx.doi.org/10.1038/ncb0905-847
2. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat Rev Mol Cell Biol 2009; 10:623-35; PMID:19672277; http://dx.doi.org/10.1038/nrm2745
3. Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. Nat Rev Mol Cell Biol 2007; 8:622-32; PMID:17637737; http://dx.doi.org/10.1038/nrm2217
4. Nixon RA, Yang DS, Lee JH. Neurodegenerative lysosomal disorders: a continuum from development to late age. Autophagy 2008; 4:590-9; PMID:18497567
5. Lakkaraju A. Endo-lysosome function in the retinal pigment epithelium in health and disease. Adv Exp Med Biol 2012; 723:723-9; PMID:22183399; http://dx.doi.org/10.1007/978-1-4614-0631-0_92
6. Andrei C, Margiocco P, Poggi A, Lotti LV, Torrisi MR, Rubartelli A. Phospholipases C and A2 control lysosome-mediated IL-1 beta secretion: Implications for inflammatory processes. Proc Natl Acad Sci USA 2004; 101:9745-50; PMID:15192144; http://dx.doi.org/10.1073/pnas.0308558101
7. Stinchcombe J, Bossi G, Griffiths GM. Linking albinism and immunity: the secrets of secretory lysosomes. Science 2004; 305:55-9; PMID:15232098; http://dx.doi.org/10.1126/science.1095291
8. Jaiswal JK, Andrews NW, Simon SM. Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. J Cell Biol 2002; 159:625-35; PMID:12438417; http://dx.doi.org/10.1083/jcb.200208154
9. Rodríguez A, Webster P, Ortego J, Andrews NW. Lysosomes behave as Ca2+-regulated exocytic vesicles in fibroblasts and epithelial cells. J Cell Biol 1997; 137:93-104; PMID:9105039; http://dx.doi.org/10.1083/jcb.137.1.93
10. Rodríguez A, Samoff E, Rioult MG, Chung A, Andrews NW. Host cell invasion by trypanosomes requires lysosomes and microtubule/kinesin-mediated transport. J Cell Biol 1996; 134:349-62; PMID:8707821; http://dx.doi.org/10.1083/jcb.134.2.349
11. Andrade LO, Andrews NW. The Trypanosoma cruzi-host-cell interplay: location, invasion, retention. Nat Rev Microbiol 2005; 3:819-23; PMID:16175174; http://dx.doi.org/10.1038/nrmicro1249
12. Divangahi M, Chen M, Gan H, Desjardins D, Hickman TT, Lee DM, et al. Mycobacterium tuberculosis evades macrophage defenses by inhibiting plasma membrane repair. Nat Immunol 2009; 10:899-906; PMID:19561612; http://dx.doi.org/10.1038/ni.1758
13. LaPlante JM, Sun M, Falardeau J, Dai D, Brown EM, Slaugenhaupt SA, et al. Lysosomal exocytosis is impaired in mucolipidosis type IV. Mol Genet Metab 2006; 89:339-48; PMID:16914343; http://dx.doi.org/10.1016/j.ymgme.2006.05.016
14. Klein D, Büssow H, Fewou SN, Gieselmann V. Exocytosis of storage material in a lysosomal disorder. Biochem Biophys Res Commun 2005; 327:663-7; PMID:15649398; http://dx.doi.org/10.1016/j.bbrc.2004.12.054
15. Rao SK, Huynh C, Proux-Gillardeaux V, Galli T, Andrews NW. Identification of SNAREs involved in synaptotagmin VII-regulated lysosomal exocytosis. J Biol Chem 2004; 279:20471-9; PMID:14993220; http://dx.doi.org/10.1074/jbc.M400798200
16. Chen D, Lemons PP, Schraw T, Whiteheart SW. Molecular mechanisms of platelet exocytosis: role of SNAP-23 and syntaxin 2 and 4 in lysosome release. Blood 2000; 96:1782-8; PMID:10961877
17. Laulagnier K, Schieber NL, Maritzen T, Haucke V, Parton RG, Gruenberg J. Role of AP1 and Gadkin in the traffic of secretory endo-lysosomes. Mol Biol Cell 2011; 22:2068-82; PMID:21525240; http://dx.doi.org/10.1091/mbc.E11-03-0193
18. Xu J, et al. Mechanism of polarized lysosome exocytosis in epithelial cells. J Cell Sci 2013; In press; PMID:23038769
19. Schenkman S, Andrews NW, Nussenzweig V, Robbins ES. Trypanosoma cruzi invade a mammalian epithelial cell in a polarized manner. Cell 1988; 55:157-65; PMID:3139302; http://dx.doi.org/10.1016/0092-8674(88)90018-9
20. Reales E, Sharma N, Low SH, Fölsch H, Weimbs T. Basolateral sorting of syntaxin 4 is dependent on its N-terminal domain and the AP1B clathrin adaptor, and required for the epithelial cell polarity. PLoS ONE 2011; 6:e21181; PMID:21698262; http://dx.doi.org/10.1371/journal.pone.0021181
21. Lebrand C, Corti M, Goodson H, Cosson P, Cavalli V, Mayran N, et al. Late endosome motility depends on lipids via the small GTPase Rab7. EMBO J 2002; 21:1289-300; PMID:11889035; http://dx.doi.org/10.1093/emboj/21.6.1289
22. Fraldi A, Annunziata F, Lombardi A, Kaiser HJ, Medina DL, Spampanato C, et al. Lysosomal fusion and SNARE function are impaired by cholesterol accumulation in lysosomal storage disorders. EMBO J 2010; 29:3607-20; PMID:20871593; http://dx.doi.org/10.1038/emboj.2010.237
23. Los FC, Kao CY, Smitham J, McDonald KL, Ha C, Peixoto CA, et al. RAB-5- and RAB-11-dependent vesicle-trafficking pathways are required for plasma membrane repair after attack by bacterial pore-forming toxin. Cell Host Microbe 2011; 9:147-57; PMID:21320697; http://dx.doi.org/10.1016/j.chom.2011.01.005
24. Rondanino C, Poland PA, Kinlough CL, Li H, Rbaibi Y, Myerburg MM, et al. Galectin-7 modulates the length of the primary cilia and wound repair in polarized kidney epithelial cells. Am J Physiol Renal Physiol 2011; 301:F622-33; PMID:21677144; http://dx.doi.org/10.1152/ajprenal.00134.2011
25. Gross JB Jr., Myers BM, Kost LJ, Kuntz SM, LaRusso NF. Biliary copper excretion by hepatocyte lysosomes in the rat. Major excretory pathway in experimental copper overload. J Clin Invest 1989; 83:30-9; PMID:2910913; http://dx.doi.org/10.1172/JCI113873

26. Schreiner R, Frindt G, Diaz F, Carvajal-Gonzalez JM, Perez Bay AE, Palmer LG, et al. The absence of a clathrin adapter confers unique polarity essential to proximal tubule function. Kidney Int 2010; 78:382-8; PMID:20531453; http://dx.doi.org/10.1038/ki.2010.166
27. Lakkaraju A, Finnemann SC, Rodriguez-Boulan E. The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proc Natl Acad Sci USA 2007; 104:11026-31; PMID:17578916; http://dx.doi.org/10.1073/pnas.0702504104
28. Bird AC. Therapeutic targets in age-related macular disease. J Clin Invest 2010; 120:3033-41; PMID:20811159; http://dx.doi.org/10.1172/JCI42437
29. Matsumura M, Ogino N, Shirakawa H, Yamakawa R, Tsukahara I. Exocytosis by retinal pigment epithelial cells. Ophthalmic Res 1985; 17:148-53; PMID:4011128; http://dx.doi.org/10.1159/000265366
30. Peters S, Reinthal E, Blitgen-Heinecke P, Bartz-Schmidt KU, Schraermeyer U. Inhibition of lysosomal degradation in retinal pigment epithelium cells induces exocytosis of phagocytic residual material at the basolateral plasma membrane. Ophthalmic Res 2006; 38:83-8; PMID:16352920; http://dx.doi.org/10.1159/000090268
31. Lueck K, Wasmuth S, Williams J, Hughes TR, Morgan BP, Lommatzsch A, et al. Sub-lytic C5b-9 induces functional changes in retinal pigment epithelial cells consistent with age-related macular degeneration. Eye (Lond) 2011; 25:1074-82; PMID:21597483; http://dx.doi.org/10.1038/eye.2011.109
32. Howard AC, McNeil AK, Xiong F, Xiong WC, McNeil PL. A novel cellular defect in diabetes: membrane repair failure. Diabetes 2011; 60:3034-43; PMID:21940783; http://dx.doi.org/10.2337/db11-0851
33. Haka AS, Grosheva I, Chiang E, Buxbaum AR, Baird BA, Pierini LM, et al. Macrophages create an acidic extracellular hydrolytic compartment to digest aggregated lipoproteins. Mol Biol Cell 2009; 20:4932-40; PMID:19812252; http://dx.doi.org/10.1091/mbc.E09-07-0559