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Investigation of endosome and lysosome biology by ultra pH-sensitive nanoprobes☆

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ABSTRACT

Endosomes and lysosomes play a critical role in various aspects of cell physiology such as nutrient sensing, receptor recycling, protein/lipid catabolism, and cell death. In drug delivery, endosomal release of therapeutic payloads from nanocarriers is also important in achieving efficient delivery of drugs to reach their intracellular targets. Recently, we invented a library of ultra pH-sensitive (UPS) nanoprobes with exquisite fluorescence response to subtle pH changes. The UPS nanoprobes also displayed strong pH-specific buffer effect over small molecular bases with broad pH responses (e.g., chloroquine and NH_4Cl). Tunable pH transitions from 7.4 to 4.0 of UPS nanoprobes cover the entire physiological pH of endocytic organelles (e.g., early and late endosomes) and lysosomes. These unique physico-chemical properties of UPS nanoprobes allowed a 'detection and perturbation' strategy for the investigation of luminal pH in cell signaling and metabolism, which introduces a nanotechnology-enabled paradigm for the biological studies of endosomes and lysosomes.

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1. Introduction of endosome/lysosome biology

Endocytosis is involved in various important cellular processes, such as protein/lipid metabolism, antigen presentation and energy homeostasis [1,2]. Endocytosis process senses and regulates the interaction

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between a cell and its external environment. For example, the recycling and degradation of transmembrane receptors through endocytosis regulates the sensitivity of cells to their specific ligands [3]. Extracellular materials, including a variety of molecules, cargos and fluid-phase contents, are internalized by cells through endocytosis. They travel along the increasingly acidic endocytic pathway and end up in endosomes or lysosomes. pH homeostasis and maintenance of proton gradient across organelle membranes are essential to cell physiology [1]. For endocytic pathways, progressive acidification is essential to various aspects of different biochemical and physiological functions [4,5]. For instance, it compartmentalizes ligand–receptor uncoupling and activation of proteases for protein/lipid degradations into endosomes and lysosomes, respectively.

Lysosomes, as the 'end-point' of the endocytic pathway, were first discovered by Christian de Duve in 1955 [6]. Lysosomes have a highly acid lumen with various kinds of hydrolases/lipases for protein/lipid degradation and recycling of extracellular and intracellular components via endocytosis and autophagy, respectively. The resulting breakdown products become building blocks for protein/lipid synthesis and energy generation. More recently, lysosomes have been identified as signaling organelles that play an important role in nutrient sensing and response [7,8], and activate a master gene that regulates nutrient and energy metabolism [9,10].

1.1. Characteristics of endocytic organelles

After receptors and their ligands are internalized into cells through clathrin-coated pits (~100 nm diameter) [11] or early endosomes, they are disassociated from each other because of the acidic environment in the early endosomes. Most membrane-bound receptors are recycled back to cell surface for another round of delivery, while the ligands are destined for degradation in lysosomes. Many ligand-conjugated nanoparticle designs took advantage of receptor-mediated endocytosis for targeted delivery into endocytic organelles. For non-targeted nanoparticles, most are internalized into cells through micropinocytosis, or fluidic phase endocytosis. It occurs with cell membrane ruffling and forms a pocket (up to 5 μm diameter) [12] engulfing bulk of extracellular fluids and molecules. The macropinosomes then pinch off from cell membrane and fuse with endosomes and lysosomes.

Late endosomes (250–400 nm diameter) [5] are bigger than early endosomes in size. Some late endosomes have more complex internal vesicular structures and are also named as multivesicular bodies (MVBs). The MVBs comprise inward invaginations of late endosome membranes and may contain certain receptors. In general, late endosomes are closer to nucleus than early endosomes, and they have extensive exchange with trans-Golgi-network (TGN). Lysosomal hydrolases are carried to late endosomes from TGN through mannose-6-phosphate receptors. Once the hydrolases are released, the receptor is retrieved to the Golgi by retromer and Rab9 [13]. Along with further drop of luminal pH, late endosomes mature into lysosomes. Lysosomes appear as electron dense bodies when viewed under electron microscopy. They are highly heterogeneous in terms of composition, morphology and density mainly due to the diversity of cargos and their degradation levels.

Rab GTPases, a family of small GTPases that regulate membrane trafficking, have been found to be the most important biomarkers for organelle identity and regulators of the endocytic pathway [13]. Rab GTPases are associated with cell/organelle membranes when they are in the guanine triphosphate (GTP)-binding 'ON' form and they will be removed when in the guanine diphosphate (GDP)-binding 'OFF' form. Rab5 is a biomarker for early endosomes, because normally Rab5-GDP resides in the cytosol, and its guanine-nucleotide exchange factors (GEFs) activate it to GTP-binding form on early endosome membranes. Rab4 and Rab11 are also early endosome markers, but Rab11 is mainly located on recycling endosome membranes. Maturation of early endosomes to late endosomes involves a switch from Rab5 to Rab7 that also mediates the fusion with lysosomes [3]. Another late endosome biomarker, Rab9, mediates the trafficking between late endosomes and TGN. Lysosomes are enriched in a family of glycosylated lysosome associated membrane proteins (LAMPs), so Rab7 and LAMPs are both markers for them. Although these membrane proteins are useful markers of organelle identity, since endocytic organelles are highly dynamic, and there is extensive traffic among them, it is possible that none of these proteins are exclusively associated with any of these components. Multiple characterizations may be needed in some cases.

The machinery of lysosomal biogenesis was unclear for a long time until recently a specific gene network called coordinated lysosomal expression and regulation (CLEAR) was identified. Most lysosomal genes share a common CLEAR sequence (GTCACGTGAC) close to the transcriptional start site, which mediates transcriptional activation [14]. The transcriptional factor EB (TFEB) can bind to this sequence and induce the transcription of lysosomal genes. When lysosomes are under stress, TFEB translocates from cytoplasm to nucleus and activates its target genes. Thus, TFEB is considered as the master regulator of lysosomal biogenesis and function, which coordinates the transcription of lysosomal genes. Recently, it has also been found to regulate starvation-induced autophagy and lipid metabolism [15], placing the lysosomal network in the center of cell metabolism and homeostasis.

1.2. The physiological importance of pH in endo/lysosomal maturation and function

Acidification is substantial to endosome maturation. Endocytic vesicles are more acidic than a lot of other organelles, and lysosomal pH values can be as low as 4.0–4.5 [4]. The low pH not only offered an optimal environment for hydrolase activation, it is also essential for uncoupling ligands from receptors, inactivation of microbicidal factors, membrane trafficking, cargo transportation and other important reactions. The primary way of delivering protons into the organelle lumen is through proton-pumping vacuolar-ATPase (v-ATPase) [4]. V-ATPase are composed of two distinct subunits: a transmembrane V_0 complex that transfers protons across membranes and a V_1 cytosolic complex that hydrolyzes ATP and converts chemical energy into mechanical force required for proton displacement. Na^+/K^+ ATPase, Cl^- and Ca^{2+} channels that influx counter ions or efflux cations are also in place to maintain the balance of membrane potential and pH [16–18].

Cargo release, hydrolase maturation, degradation, autophagy and intracellular trafficking are all dependent on pH gradients [17]. Bafilomycin A1 (baf A1) [19] and concanamycins [20] are specific inhibitors of v-ATPase and can efficiently dissipate the proton gradient across organelle membranes of the whole endocytic pathway. Baf A1 is sufficient to block the transportation of cargos from early endosomes to late endosomes in most cases indicating a defect of endosome maturation [21,22]. It has also been used to block the fusion of late endosomes [23] or autophagosomes [24] with lysosomes indicating an important role that luminal pH plays in cargo transportation.

In the therapeutics of diseases with disturbed lysosomal pH, restoring the acidity of endo/lysosomes could be an efficient way to promote the degradation and clearance of accumulated substances. Presenelin-1 is an important protein in Alzheimer's disease, and it has also been shown to regulate the trafficking of v-ATPase to lysosomal membranes [25]. Mutated presenelin-1 is one of the major causes of familial Alzheimer's disease, which results in disrupted lysosomal pH, defective hydrolase maturation and activity that may be attributed to abnormal v-ATPase trafficking [25].

Defective or blocked endocytic pathways and/or global disturbance of intracellular pH have been associated with a variety of pathological conditions [4,26], such as oncogenic transformation [27], autoimmune disease [28] and neurodegenerative diseases [29,30]. However, specific knowledge is still lacking on how local (i.e., organelle-specific) pH variations may affect cell physiology. The biochemical functions of many membrane-bound proteins are highly sensitive to minor pH