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Review

Vacuolar ATPase in phago(lyso)some biology

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ABSTRACT

Many eukaryotic cells ingest extracellular particles in a process termed phagocytosis which entails the generation of a new intracellular compartment, the phagosome. Phagosomes change their composition over time and this maturation process culminates in their fusion with acidic, hydrolase-rich lysosomes. During the maturation process, degradation and, when applicable, killing of the cargo may ensue. Many of the events that are pathologically relevant depend on strong acidification of phagosomes by the 'vacuolar' ATPase (V-ATPase). This protein complex acidifies the lumen of some intracellular compartments at the expense of ATP hydrolysis. We discuss here the roles and importance of V-ATPase in intracellular trafficking, its distribution, inhibition and activities, its role in the defense against microorganisms and the counteractivities of pathogens.

1. Role of intracellular pH regulation

Eukaryotic cells are highly compartmentalized to provide optimized environments for distinct cellular functions. A critical factor determining the functions of organelles is the local pH, *i.e.*, the amount of available protons. Protonation and deprotonation alter the net charges of biological surfaces and are important for protein structure and function. Whereas the pH is near neutral in the cytosol and ER (endoplasmic reticulum, pH 7.2), it is alkaline within the mitochondrial matrix (pH ca. 8.0) (Casey et al., 2010). In contrast, compartments of the secretory and endocytic pathways progressively acidify along the *cis-trans* axis and from early endosomes to lysosomes. In both pathways, luminal pH may reach values as low as 4.5, providing optimal conditions for enzymes that mediate either post-translational modifications and processing of proteins in the secretory pathway or degradation within endolysosomes. Acidification is central for protein sorting in both pathways, *e.g.*, by mediating receptor-ligand dissociation and, hence, receptor recycling. Acidification also provides the basis for membrane fusion and fission events (Aniento et al., 1996; Gu and Gruenberg, 2000; Ungermann et al., 1999; Wu et al., 2001).

During phagocytosis, microorganisms are captured by an invagination of the plasma membrane resulting in the formation of a phagosome. This compartment has initially a near-neutral pH and it lacks the machinery to clear the enclosed microorganisms. During the further maturation process, the lumen of phagosomes is rapidly and strongly acidified and phagosomes fuse with endosomes and eventually lysosomes generating a hydrolytically competent phagolysosome. The low pH activates enzymes which mediate some of the killing and degradation of microbes (Haas, 2007; Levin et al., 2016). Acidic conditions may also directly affect the growth of bacteria and promote the generation of hydrogen peroxide (detailed below). The main driving force for strong acidification is a large multiprotein complex, the vacuolar ATPase (V-ATPase) (Cotter et al., 2015) which is enriched on lysosome and phago(lyso)some membranes.

2. V-ATPase as a driving force of intra-organelle acidification

V-ATPase belongs to a family of ATP-dependent proton pumps. It consists of 14 different subunits (Cotter et al., 2015) (Fig. 1). Similar as with F-type and A-type ATPases, a rotary mechanism couples ATP hydrolysis within a peripheral V_1 sector to the transport of protons, against the electrochemical gradient, through a membrane-integral V_O sector. At the molecular level, two protons are pumped from the cytosol for each hydrolyzed ATP (Johnson et al., 1982) allowing, in theory, to decrease luminal pH values to below 3.0 (Grabe et al., 2000; Kettner et al., 2003).

Such low pH values are never reached in living cells because several cellular factors regulate the actual extent and velocity of acidification: Proton pumping generates an electrochemical potential difference across the membrane (positive charge on the luminal side) which, if continued permanently, would first decelerate and eventually inhibit further acidification. To prevent this inhibition the generated voltage is

Abbreviations: DAMP, 3 (2,4-dinitroanilino)-30-amino-*N*-methyldipropylamine; mTORC1, mechanistic target of rapamycin complex 1; SNARE, soluble *N*-ethylmaleimide sensitive factor attachment protein receptors; V-ATPase, vacuolar ATPase

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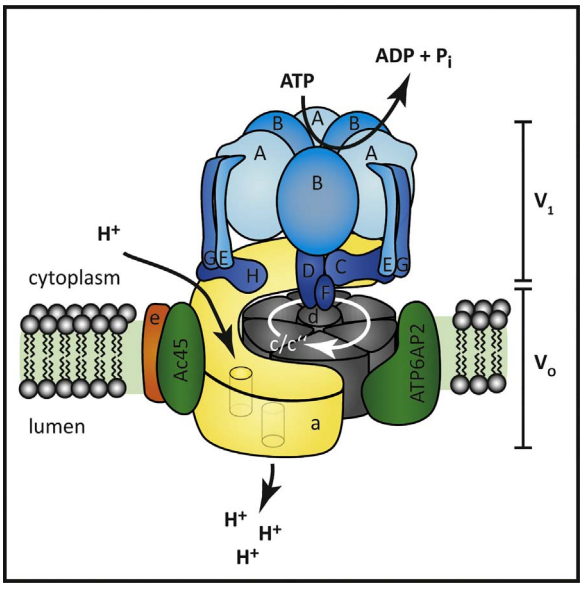

Fig. 1. Model of V-ATPase complex composition. Proton-pumping V-ATPase is organized in two large sectors, named V_1 and V_O. Sector V_1 consists of the subunits A–H and catalyzes ATP hydrolysis. The energy released by this process is transferred into a rotational force which drives proton transport through the membrane-integral V_O sector (subunits a, c, c", d and e). Two accessory subunits, Ac45 and ATP6AP2 (V-ATPase accessory protein 2), act as chaperone for V_O sector assembly.

neutralized by import of anions and/or export of cations. As for regulation by anions an influx of cytosolic chloride is mediated by members of the chloride channel (CLC) family, CLCN3 through CLCN7, which are 2 Cl^--for-1 H^+ antiporters found on endosomes and lysosomes (Mindell, 2012; Stauber and Jentsch, 2013). Furthermore, CFTR (cystic fibrosis transmembrane conductance regulator) is a chloride transporter that may also participate in phagosome acidification (Di et al., 2006; Haggie and Verkman, 2007). Regulation of luminal pH by cations, on the other hand, can be achieved by an efflux of monovalent cations which counteracts the V-ATPase-mediated electrochemical potential (Steinberg et al., 2010). Two members of the TRP (transient receptor potential) channel family, TRPML1 (Bach et al., 1999; Pryor et al., 2006; Soyombo et al., 2006) and TRPM2 (Di et al., 2017), are potential cation exporters but show diverging influence on luminal pH depending on the cell type.

In addition to pH regulation by ion (counter-) fluxes, acidification is regulated by the abundance of V-ATPase on organelles, by the pumping activity of the complex and by proton leakage. In yeast, the V-ATPase V_O subunit a is the only subunit expressed in two isoforms termed Stv1p and Vph1p. V-ATPase complexes containing Stv1p are targeted to the Golgi apparatus, Vph1p-containing V-ATPases to the vacuole (Kawasaki-Nishi et al., 2001a). These two isoforms additionally regulate the efficiency of the proton pump, and therefore luminal pH, by tight (vacuole) or less tight (Golgi) coupling of ATP-hydrolysis on sector V_1 with proton pumping through the V_O sector (Kawasaki-Nishi et al., 2001b). Likewise, mammalian V-ATPases can contain either of four different V_O a-subunits that are specifically localized to certain tissues and organelles (Nishi and Forgac, 2000; Oka et al., 2001). This is exemplified by V_O a-subunit isoform 3 which is highly expressed on the ruffled border in osteoclasts (Toyomura et al., 2000) and it is also the major a-isoform in macrophages (Sun-Wada et al., 2009). However, it is not known whether, like in yeast, mammalian a-subunits can affect coupling efficiency of the proton pump. Temporary regulation of V-ATPase activity can be further achieved by increased association of the proton pump with the target membrane or its disassembly into V_1 and V_O sectors which occurs in baker's yeast and in mammals in response to glucose starvation (Kane, 1995; Parra and Kane, 1998; Sautin et al., 2005).

Finally, the extent of acidification is limited by leakage of protons from the organelle, for example by spontaneous flux directly through the hydrophobic membrane core. In studies using macrolid inhibitors of V-ATPase, the proton leakage was followed as a gradual alkalinization of the lumen and calculated to be in the order of 0.1 ΔpH/min for lysosomes and phagosomes (Lukacs et al., 1991; Steinberg et al., 2007).

Attempts to knockout essential subunits of the proton-pumping ATPase (*e.g.*, V_Oc) result in early lethality in higher eukaryotes (Dow et al., 1997; Oka and Futai, 2000; Sun-Wada et al., 2000) yet depletion of some subunit isoforms yields viable organisms likely because it does not lead to complete loss of the complex. Such knockout systems helped to understand subunit functions and roles of the V-ATPase that exceed

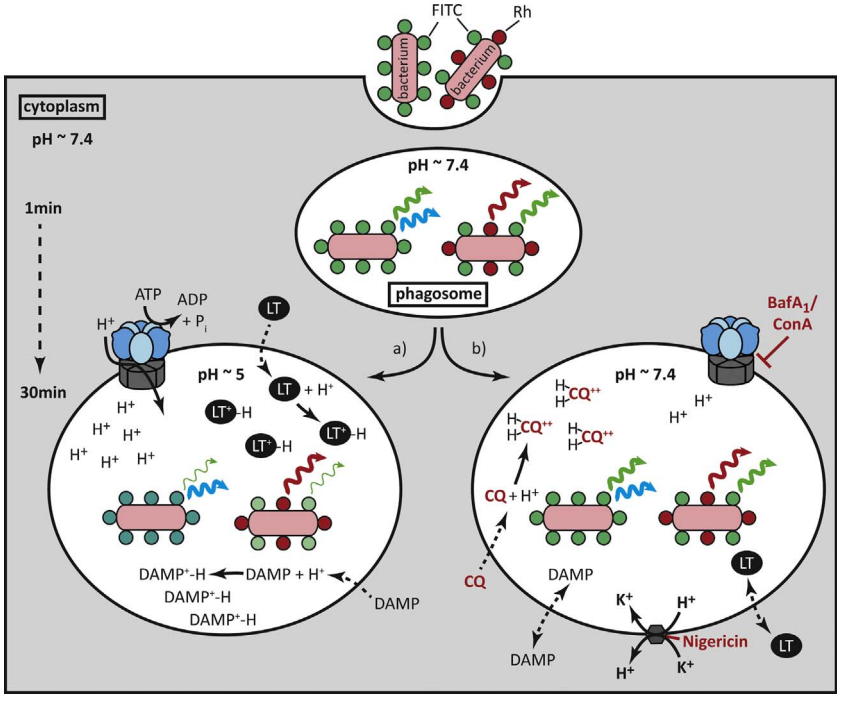

Fig. 2. Analysis of phagosome pH. Bacteria that were chemically surface-labeled with fluorescein isothiocyanate (FITC) or a combination of FITC and Rhodamine (Rh) are phagocytosed by a macrophage. At neutral pH, FITC yields high fluorescence intensity when excited at 490 nm (green arrows). a) This fluorescence decreases at acidic pH, whereas fluorescence emission is constant upon excitation at 440 nm (blue arrows, isosbestic point). The ratio between pH-sensitive and pH-insensitive signal intensities in phagosomes or whole cells is used to assess phagosome acidification. Alternatively, pH-independent fluorescence emission of Rh (red arrows) can be used for pH determination in combination with FITC (490 nm) signals. Acidification can be visualized with LysoTracker™ (LT) or DAMP as explained in the main text. b) Various drugs interfere with lysosome acidification. Bafilomycin A_1 ($BafA_1$) and concanamycin A (ConA) bind to and inhibit V-ATPases. Nigericin, an ionophore, dissipates the proton gradient by exchanging H^+ against K^+. Finally, phagosome pH can be raised by extracellular addition of membrane-permeable weak bases, *e.g.*, chloroquine (CQ), which are retained in the phagosome lumen following protonation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)