Published in final edited form as:
Biochem Biophys Res Commun. 2011 September 9; 412(4): 728–731. doi:10.1016/j.bbrc.2011.08.041.

Decreased Expression of *ATP6V1H* in Type 2 Diabetes: a Pilot Report on the Diabetes Risk Study in Mexican Americans

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Abstract

Objective—Previous studies in mice and humans observed down-regulation of the gene expression of *ATP6V1H* associated with type 2 diabetes. This study identified prospectively changes in *ATP6V1H* expression before and after overt diabetes.

Methods—Expression of *ATP6V1H* in peripheral blood was compared pre and post development of diabetes in nine individuals.

Results—Considerable variation of *ATP6V1H* mRNA levels was observed between different individuals. However, within each individual the decrease in expression of *ATP6V1H* with the development of diabetes was highly statistically significant.

Conclusions—*ATP6V1H* may represent a critical molecular mechanism involved in the development of type 2 diabetes and its compilations through its important regulatory effect on vacuolar-ATPase activity.

Keywords

ATP6V1H; diabetic complications; longitudinal study; type 2 diabetes; vacuolar ATPase

Introduction

The ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H gene (*ATP6V1H*) at Chr8q11.2 encodes for the V1H subunit of vacuolar ATPase (V-ATPase). The ATPase

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Conflict of Interest statement: None declared.

family consists of multi-subunit enzymes that hydrolyze ATP and use the energy to catalyze a variety of critical cellular processes [1]. V-ATPase was originally discovered in plant and fungal vacuoles, and subsequently found widely in a variety of eukaryotic endomembrane organelles [1]. V-ATPase belongs to the rotary ATPase family, membrane complexes that use rotary motor mechanisms to translocate ions across membranes [2]. The major function of V-ATPase is to acidify intracellular compartments by using the energy gathered from ATP hydrolysis to pump protons [3]. This function gives V-ATPase important roles in receptor-mediated endocytosis, intracellular trafficking processes and protein degradation [4].

V-ATPase has two functional domains V_0 and V_1, which are involved in proton translocation and ATP hydrolysis respectively [3,4,5]. The V_0 domain consists of five distinct subunits: a, c, c′, c″, and d, with a stoichiometry of $a_1d_1c_{4-5}c'_1c''_1$ and the function to conduct protons [1]. The V_1 domain contains eight subunits (A-H, with stoichiometry $A_3B_3C_1D_1E_3F_1G_3H_1$), which form a peripheral complex responsible for the hydrolysis of ATP[6,7]. Of particular interest in the V_1 domain is the regulatory subunit H (V1H)[4], owing to the fact that the down-regulation of its gene expression correlates with the presence of type 2 diabetes [8]. Olsson et al. observed down-regulation of *ATP6V1H* in human pancreatic islets from patients with type 2 diabetes[9]. The expression of *ATP6V1H* correlated negatively with HbA1c levels, and positively with glucose-stimulated insulin secretion [9]. To establish the dynamics of gene expression coincident with the development of diabetes, we created a nested cohort with impaired fasting glucose (the Diabetes Risk Study (DRS) cohort: fasting blood glucose ≥100 mg/dl and <126 mg/dl). This DRS cohort was selected from our larger community-recruited Cameron County Hispanic Cohort (CCHC n=2500) consisting of Mexican Americans resident on the Texas Mexico border in whom we document elevated prevalence of diabetes[10].

Methods

1. Ethics Statement

Written informed consent was obtained from each participant, and the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston (UTHealth) approved this study.

2. Subjects

To determine the proximal changes accompanying transition to overt type 2 diabetes we followed 297 DRS participants recruited over 3 years and examined quarterly. During this period 9 participants transitioned from pre-diabetes to overt diabetes (2006 American Diabetes Association). Three were male and 6 female; age range from 32 to 74 years of age (median 57 years). Each participant had 2 to 5 clinic visits (median 4 visits). At each visit a questionnaire and clinical examination were administered and whole blood was collected and stabilized in PAXgene Blood RNA Tubes (Qiagen) for isolation and purification of intracellular RNA.

3. Gene expression profiling

Transcriptome profiling of RNA samples from the 9 participants was performed using the Illumina HumanHT-12 Beachip (*Illumina*, San Diego) at the McGill University and Génome Québec Innovation Centre.

4. Data anlaysis

The Flexarray software[11] was used to analyze the microarray data. The data was normalized using the Lumi Bioconductor package[12] implemented in Flexarray. 34,694

genes were assayed on the HT-12 Beachip, and the expression of 22,251 genes were detectable. 12443 genes were removed from further analysis because of undetectable expression. As shown by the principle component analysis, the transcriptome profiles of the RNA samples were mainly clustered by each individual participant, instead of clustered by disease status. The transcriptome profile of each individual participant was obviously different. Based on this phenomenon, pairwise Z test was selected to compare the differences in gene expression within each individual. Statistical significance was corrected for multiple comparisons by Benjamini and Hochberg False Discovery Rate (FDR) using the QVALUE software[13].

Results

The transcriptome profiles of the RNA samples clustered by each individual participant highlighted the robust longitudinal design of our prospective study. Alterations in gene expression coincident with development of diabetes could not be properly assessed using a case-control design, because of significant variations between individual subjects. However, within serial specimens from each subject we were able to document decreased expression of *ATP6V1H* in peripheral blood with development of diabetes. Among all the genes assayed in our study, the difference of *ATP6V1H* expression between pre-diabetes and overt diabetes was the most significant with uncorrected $P=7.18\times10^{-11}$, and FDR corrected q-value=1.14×10^{-6}. Fig. 1 shows the considerable variations observed for each of the individuals with development of diabetes as the expression of *ATP6V1H* decreased in each individual without exception.

Discussion

Using a unique cohort of extensively documented individuals with pre-diabetes we observed decreased expression of *ATP6V1H* in peripheral white blood cells with the development of clinical diabetes with a high degree of statistical significance. This novel observation casts new light on the mechanisms involved in development of frank diabetes and clues to the processes associated with onset and progress of the disease state and its complications. This study is novel in two ways, one it is a prospective longitudinal study that provides comparison of gene expression in each individual over time associated with actually progression from pre-diabetes to diabetes. Secondly it examines the changes in peripheral blood white cell expression associated with development of diabetes, which provides a highly accessible source of potential markers for development of diabetes. Because these participants were newly diagnosed with diabetes they were not under treatment for diabetes at the time of their conversion.

The V1H encoded by *ATP6V1H* has been shown to exercise an important regulatory effect on V-ATPase activity [14]. V1H has a highly helical structure consisting of two domains: the N-domain with seventeen consecutive α-helices (amino acids 2–352) and the C-domain with eight α-helices (amino acids 353–478) [15]. The α-helical motifs in Subunit H show structural similarity to the armadillo or HEAT motifs (an arrangement of three α-helices, each about 42 amino acids in length) found in the importin family of proteins [15]. The importin proteins bind to nuclear localization signals (NLS) on other proteins via a hydrophobic, shallow groove, facilitating protein transport from the cytosol into the nucleus [16,17]. Further studies show that the N-terminal domain is important for the ATP hydrolysis function of the V_1 domain of V-ATPase, and the C-terminal domain is needed for proper communication between the V_1 and V_0 domains of V-ATPase [18,19,20]. In addition, it has also been shown that the C-terminal domain of V1H is necessary in inhibiting ATPase activity of free V_1 domain complexes by interacting with subunit F via a

cysteine residue at position 381[21]. These studies demonstrated the critical role of V1H in maintaining proper V-ATPase function.

Impaired V1H expression in diabetes may be important in the development of diabetes and its complications. The principle function of V-ATPase is to acidify intracellular compartments [3], allowing it to play an important role in receptor-mediated endocytosis, intracellular trafficking processes and protein degradation (Fig. 2)[4]. The V-ATPase enzyme is ubiquitous, and enzyme malfunction may play important roles in diabetes and its complications, e.g. increased risk of intracellular infections such as tuberculosis.

V-ATPase plays an important role in the development of insulin resistance and diabetes. The V-ATPase pumps protons into the endosome lumen, creating an acidic environment that not only enables ligand-receptor complexes (brought in from the plasma membrane) to dissociate [22], but also allows for the vesicular trafficking of released ligands from early endosomes to late endosomes[23]. These processes are important in the dissociation of insulin and its receptor [24,25]. A study conducted by Benzi et al. in 1997 compromised endosomal acidification by using an ionophore known as monensin and effectively mimicked the abnormal processing of insulin in patients with type 2 diabetes [26]. V-ATPase is also involved in the increased risk of tuberculosis in patients with diabetes. V-ATPase is involved in endocytosis through the acidification of the phagosome [27]—a critical process in intracellular microbial killing [28], especially in specialized phagocytic cells such as macrophages. Bidani et al. showed that the inhibition of V-ATPase in alveolar macrophages led to a rise in phagosomal pH and a decrease in reactive oxygen species (ROS) production, both effects compromise substantially intracellular microbial killing [29]. *Mycobacterium tuberculosis* (MTB) has already evolved a mechanism to resist acidification by excluding V-ATPase from the phagosome [27]. Impaired function of V-ATPase in diabetes may deteriorate the susceptibility to MTB.

This is a unique study in that we were able to follow individuals with pre-diabetes prospectively and document gene expression as they developed diabetes. Though the small numbers of individuals transitioning to diabetes limited our study, the consistency of our observations is striking and gives confidence that these are real events in the evolution of the disease. This new knowledge throws fresh light on an important molecular mechanism involved in development of diabetes, and importantly, it is consistent with a newly recognized major risk of diabetes, specifically increased susceptibility to tuberculosis.

Acknowledgments

We thank our cohort recruitment team, particularly Rocio Uribe, Elizabeth Braunstein and Julie Ramirez. We also thank Marcela Montemayor and other laboratory staff for their contribution, and Christina Villarreal for administrative support. We thank Valley Baptist Medical Center, Brownsville for providing us space for our Center for Clinical and Translational Science Clinical Research Unit. We also thank the community of Brownsville and the participants who so willingly participated in this study in their city.

This work was supported by MD000170 P20 funded from the National Center on Minority Health and Health Disparities (NCMHD), and the Centers for Translational Science Award 1U54RR023417-01 from the National Center for Research Resources (NCRR). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. M.F.M. is supported by a summer student fellowship from the University of Texas at Austin. H.Q.Q is supported by intramural funding from the University of Texas School of Public Health.

References

1. Futai, M.; Wada, Y.; Kaplan, JH. Handbook of ATPases: Biochemistry, Cell Biology, Pathophysiology. Wiley-VCH; Weinheim: 2004.

2. Muench SP, Trinick J, Harrison MA. Structural divergence of the rotary ATPases. Q Rev Biophys. 2011:1–46.
3. Stevens TH, Forgac M. Structure, function and regulation of the vacuolar (H+)-ATPase. Annu Rev Cell Dev Biol. 1997; 13:779–808. [PubMed: 9442887]
4. Geyer M, Fackler OT, Peterlin BM. Subunit H of the V-ATPase involved in endocytosis shows homology to beta-adaptins. Mol Biol Cell. 2002; 13:2045–2056. [PubMed: 12058068]
5. Jefferies KC, Cipriano DJ, Forgac M. Function, structure and regulation of the vacuolar (H+)-ATPases. Arch Biochem Biophys, United States. 2008:33–42.
6. Arai H, Terres G, Pink S, Forgac M. Topography and subunit stoichiometry of the coated vesicle proton pump. J Biol Chem. 1988; 263:8796–8802. [PubMed: 2897963]
7. Kitagawa N, Mazon H, Heck AJ, Wilkens S. Stoichiometry of the peripheral stalk subunits E and G of yeast V1-ATPase determined by mass spectrometry. J Biol Chem, United States. 2008:3329–3337.
8. Lu H, Yang Y, Allister EM, Wijesekara N, Wheeler MB. The identification of potential factors associated with the development of type 2 diabetes: a quantitative proteomics approach. Mol Cell Proteomics, United States. 2008:1434–1451.
9. Olsson AH, Yang BT, Hall E, Taneera J, Salehi A, Dekker Nitert M, Ling C. Decreased expression of genes involved in oxidative phosphorylation in human pancreatic islets from patients with type 2 diabetes. Eur J Endocrinol. 2011
10. Fisher-Hoch SP, Rentfro AR, Salinas JJ, Perez A, Brown HS, Reininger BM, Restrepo BI, Wilson JG, Hossain MM, Rahbar MH, Hanis CM, McCormick JB. Socioeconomic status and prevalence of obesity and diabetes in a Mexican American community, Cameron County, Texas, 2004–2007. Prev Chronic Dis. 2010; 7:A53. [PubMed: 20394692]
11. Blazejczyk, M.; Miron, M.; Nadon, R. FlexArray: A statistical data analysis software for gene expression microarrays. Genome Quebec; Montreal, Canada: 2007.
12. Du P, Kibbe WA, Lin SM. lumi: a pipeline for processing Illumina microarray. Bioinformatics. 2008; 24:1547–1548. [PubMed: 18467348]
13. Storey JD. A direct approach to false discovery rates. Journal of the Royal Statistical Society Series B. 2002; 64:479–498.
14. Parra KJ, Keenan KL, Kane PM. The H subunit (Vma13p) of the yeast V-ATPase inhibits the ATPase activity of cytosolic V1 complexes. J Biol Chem, United States. 2000:21761–21767.
15. Sagermann M, Stevens TH, Matthews BW. Crystal structure of the regulatory subunit H of the V-type ATPase of Saccharomyces cerevisiae. Proc Natl Acad Sci USA, United States. 2001:7134–7139.
16. Peifer M, Berg S, Reynolds AB. A repeating amino acid motif shared by proteins with diverse cellular roles. Cell, United States. 1994:789–791.
17. Kobe B. Autoinhibition by an internal nuclear localization signal revealed by the crystal structure of mammalian importin alpha. Nat Struct Biol. 1999; 6:388–397. [PubMed: 10201409]
18. Lu M, Vergara S, Zhang L, Holliday LS, Aris J, Gluck SL. The amino-terminal domain of the E subunit of vacuolar H(+)-ATPase (V-ATPase) interacts with the H subunit and is required for V-ATPase function. J Biol Chem, United States. 2002:38409–38415.
19. Landolt-Marticorena C, Williams KM, Correa J, Chen W, Manolson MF. Evidence that the NH2 terminus of vph1p, an integral subunit of the V0 sector of the yeast V-ATPase, interacts directly with the Vma1p and Vma13p subunits of the V1 sector. J Biol Chem, United States. 2000:15449–15457.
20. Liu M, Tarsio M, Charsky CM, Kane PM. Structural and functional separation of the N- and C-terminal domains of the yeast V-ATPase subunit H. J Biol Chem, United States. 2005:36978–36985.
21. Jefferies KC, Forgac M. Subunit H of the vacuolar (H+) ATPase inhibits ATP hydrolysis by the free V1 domain by interaction with the rotary subunit F. J Biol Chem. 2008; 283:4512–4519. [PubMed: 18156183]
22. Geuze HJ, Slot JW, Strous GJ, Lodish HF, Schwartz AL. Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis. Cell, United States. 1983:277–287.

23. Gu F, Gruenberg J. ARF1 regulates pH-dependent COP functions in the early endocytic pathway. J Biol Chem. 2000; 275:8154–8160. [PubMed: 10713138]
24. Duckworth WC. Insulin degradation: mechanisms, products, and significance. Endocr Rev. 1988; 9:319–345. [PubMed: 3061785]
25. Knutson VP. Cellular trafficking and processing of the insulin receptor. FASEB J. 1991; 5:2130–2138. [PubMed: 2022311]
26. Benzi L, Cecchetti P, Ciccarone AM, Nardone A, Merola E, Maggiorelli R, Campi F, Di Cianni G, Navalesi R. Inhibition of endosomal acidification in normal cells mimics the derangements of cellular insulin and insulin-receptor metabolism observed in non-insulin-dependent diabetes mellitus. Metabolism, United States. 1997:1259–1265.
27. Lee BY, Jethwaney D, Schilling B, Clemens DL, Gibson BW, Horwitz MA. The Mycobacterium bovis bacille Calmette-Guerin phagosome proteome. Mol Cell Proteomics, United States. 2010:32–53.
28. Ip WK, Sokolovska A, Charriere GM, Boyer L, Dejardin S, Cappillino MP, Yantosca LM, Takahashi K, Moore KJ, Lacy-Hulbert A, Stuart LM. Phagocytosis and phagosome acidification are required for pathogen processing and MyD88-dependent responses to Staphylococcus aureus. J Immunol, United States. 2010:7071–7081.
29. Bidani A, Reisner BS, Haque AK, Wen J, Helmer RE, Tuazon DM, Heming TA. Bactericidal activity of alveolar macrophages is suppressed by V-ATPase inhibition. Lung, United States. 2000:91–104.
30. Kakinuma Y, Ohsumi Y, Anraku Y. Properties of H+-translocating adenosine triphosphatase in vacuolar membranes of SAccharomyces cerevisiae. J Biol Chem. 1981; 256:10859–10863. [PubMed: 6116710]
31. Moriyama Y, Maeda M, Futai M. The role of V-ATPase in neuronal and endocrine systems. J Exp Biol. 1992; 172:171–178. [PubMed: 1362770]
32. Sun-Wada GH, Toyomura T, Murata Y, Yamamoto A, Futai M, Wada Y. The a3 isoform of V-ATPase regulates insulin secretion from pancreatic beta-cells. J Cell Sci, England. 2006:4531–4540.
33. Toyomura T, Murata Y, Yamamoto A, Oka T, Sun-Wada GH, Wada Y, Futai M. From lysosomes to the plasma membrane: localization of vacuolar-type H+-ATPase with the a3 isoform during osteoclast differentiation. J Biol Chem, United States. 2003:22023–22030.
34. Pietrement C, Sun-Wada GH, Silva ND, McKee M, Marshansky V, Brown D, Futai M, Breton S. Distinct expression patterns of different subunit isoforms of the V-ATPase in the rat epididymis. Biol Reprod, United States. 2006:185–194.
35. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. Nat Rev Mol Cell Biol, England. 2003:202–212.
36. Klionsky DJ, Nelson H, Nelson N. Compartment acidification is required for efficient sorting of proteins to the vacuole in Saccharomyces cerevisiae. J Biol Chem. 1992; 267:3416–3422. [PubMed: 1531340]

This prospective study investigated gene expression in progress of diabetes in human.

ATP6V1H in peripheral blood decreased with progression from pre-diabetes to diabetes.

This observation casts new light on the mechanisms involved in diabetes and its complications.

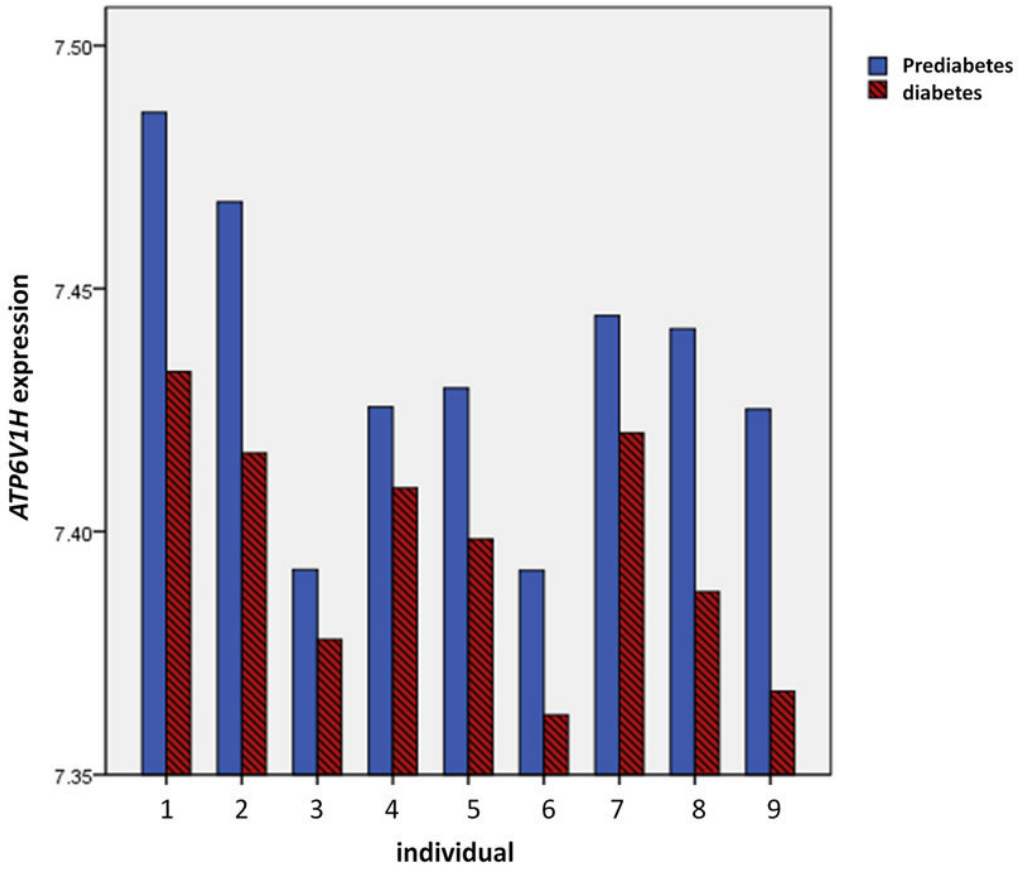

Fig. 1.

The expression of *ATP6V1H* in each participant. Dramatic variations were observed among the different individuals, which was concordant with our PCA finding of the whole transcriptome profiles. However, compared with pre-diabetes status, the expression of *ATP6V1H* in diabetes decreased in each individual without exception, with pairwise Z test $P=7.18\times10^{-11}$. This observation on *ATP6V1H* expression highlighted the robust design of our longitudinal study.

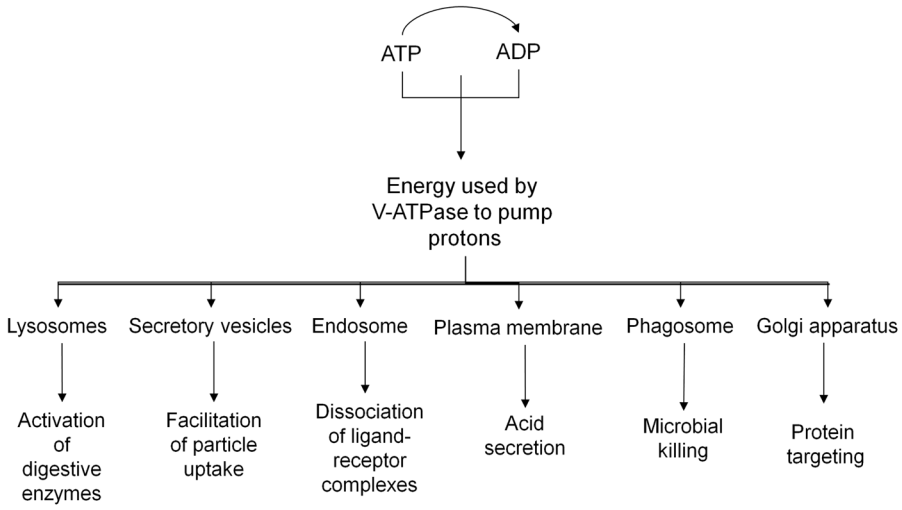

Fig. 2.

Cellular localizations and functions of V-ATPase. V-ATPase is present in a variety of intracellular organelles. In the lysosomes and central vacuoles of eukaryotic organisms, the action of the V-ATPase creates an acidic environment which facilitates the degradation of macromolecules by activating digestive enzymes [30]. In synaptic vesicles and chromaffin granules, the proton gradient generated by V-ATPase acts as a driving force, which promotes the uptake of neurotransmitters and other small molecules destined for secretion [31]. In some cases, a low pH is needed in secretory vesicles to activate enzymes which can convert hormones such as insulin from their precursor to mature form [32]. At the plasma membrane in a variety of highly specialized cells, the V-ATPase is involved in acid secretion—an important process in urine formation[5], bone resorption[33], and sperm maturation and storage [34]. The enzyme also plays an important role in the lysosomal targeting of enzymes in the Golgi apparatus, giving rise to a low pH within late endsomes that allows the enzymes to dissociate from the mannose-6-phosphate receptor [35]. Finally, in the Golgi apparatus, the acidification provided by V-ATPase ensures proper sorting of proteins to their destined location within the cell [36].