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VPS13A is a multitasking protein at the crossroads between organelle communication and protein homeostasis
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CHAPTER 1

Introduction and aim of the thesis

INTRODUCTION

Chorea-Acanthocytosis

Chorea-Acanthocytosis (ChAc) (MIM 200150) is a rare autosomal recessive neurodegenerative disorder and member of a family of neurological disorders broadly known as neuroacanthocytosis (NA) syndromes[1–3]. NA involves neurological abnormalities coupled with the presence of abnormally spiked red blood cells (acanthocytes) in the peripheral blood circulation[4]. NA syndromes are broadly classified into two categories; the "core" NA syndromes and NA with lipoprotein disorders[5]. The "core" NA group consists of ChAc, McLeod syndrome (MLS), Huntington's disease-like 2 (HDL-2) and pantothenate kinase associated neurodegeneration (PKAN); all of which display degeneration of basal ganglia and acanthocytosis[5].

ChAc is characterized by progressive adult onset involuntary movements, behavioral and cognitive changes, oral dystonia and occasional seizures[5–7]. Increased creatine kinase levels and a 7-50% acanthocytosis in blood circulation are common features of ChAc[8]. Causative mutations for the onset of ChAc are mapped on the *Vacuolar Protein Sorting 13A* (*VPS13A*) gene[2,8]. In most patients, these mutations lead to reduction or absence of detectable protein levels in red blood cells[10] and hence, Western blotting for VPS13A is used as a diagnostic tool in clinical setups[4,10,11].

The main cause of red blood cell abnormalities and neurodegeneration in ChAc is largely unknown. In this chapter, we will describe a general background of VPS13 family proteins with emphasis on VPS13A. Domain architecture, subcellular localizations and functions of VPS13A will be discussed in the context of various ChAc model organisms.

The human VPS13 family proteins

The human VPS13 family consists of four ubiquitously expressed proteins (VPS13A, VPS13B, VPS13C and VPS13D) that share similarity with yeast Vps13[12]. Mutations in all human *VPS13* genes are associated with the onset of neurological and developmental disorders. *VPS13A*, *VPS13B*, *VPS13C* and *VPS13D* are linked to the onsets of ChAc, Cohen syndrome, Parkinson's disease and septic shock mortality respectively[2,13–15].

The *VP13A* gene spans 73 exons and is located on chromosome 9q21. There are two splicing variants of VPS13A (variant 1a and variant 1b). Variant 1a consists of exons 1-68 and 70-73 whereas variant 1b contains only exons 1-69[12]. Mutations in ChAc patients can be found distributed randomly throughout the *VPS13A* gene and so far there are no potential hotspots identified[4].

VPS13B is mutated in patients with Cohen syndrome[13]. Cohen syndrome is a rare autosomal recessive disorder characterized by obesity, motor clumsiness, microcephaly, mental retardation, neutropenia, facial, oral and ocular abnormalities[16–18]. *VPS13B* is located on chromosome 8q22 and widely expressed in a variety of human tissues and unlike VPS13A, its expression in adult brain is marginally low[13,19]. VPS13B is required to maintain Golgi integrity and proper protein glycosylation[20,21]. Although it was initially predicted to contain 10 transmembrane domains[13], subcellular fractionation study shows that VPS13B is a peripheral

membrane protein localized at the Golgi where it interacts with Rab6 to regulate neurite outgrowth with a mechanism that remains to be determined[20,22].

VPS13C is more similar to VPS13A compared to other VPS13 family proteins[12]. *VPS13C* is located on chromosome 15q22 where truncating mutations and polymorphisms are causally linked to Parkinson's disease[14,23–25]. In addition, mutations and single polynucleotide polymorphisms (SNPs) of *VPS13C* are associated with the risk of type 2 diabetes[26–29]. VPS13C is localized at the mitochondrial membrane and its absence aggravates mitochondrial fragmentation and clearance[14].

VPS13D is a ubiquitin binding protein that regulates mitochondrial size and clearance both in *Drosophila* and human cultured cells[30]. Furthermore, a *VPS13D* gene variant is associated with increased septic shock mortality and overproduction of interleukin-6 (IL-6) in patients' plasma and cultured cells[31]. Recent molecular autopsy analysis identified *VPS13D* gene mutation as one of the genes linked to early embryonic mortality[15].

All of the human VPS13 family proteins share conserved N- and C-terminal domains[12]. Nonetheless, the diversity of diseases associated with different human VPS13 family proteins predict that each protein may function in different cellular pathways. Indeed, not all human VPS13 family proteins have similar subcellular localization patterns. VPS13B is localized to the Golgi complex while VPS13C is localized to mitochondria and lipid droplets (LDs)[14,20,22,32]. VPS13D, on the other hand, colocalizes with the lysosomal protein, LAMP1[30]. The biggest issues to be solved in VPS13A research are to define the localization of the protein in mammalian cells[33] and to identify functional domains of the VPS13A protein.

Domains of VPS13A

Sequence alignment studies identify multiple domains of VPS13A. The known domains of VPS13A include Chorein, two phenylalanines in an acidic tract (FFAT), short root transcription factor-binding domain (SHR-BD), aberrant pollen transcription 1 (APT1), ATG-C terminal domain (ATG-C) and pleckstrin homology (PH) domain (Figure 1)[12,34,35].
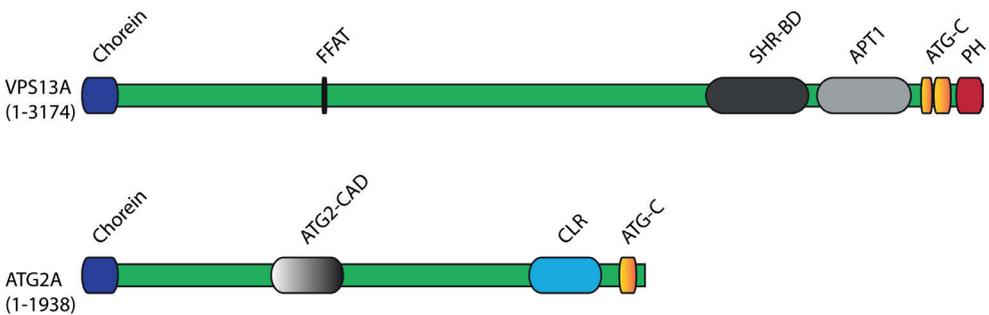
Figure 1. Schematic representations of VPS13A and ATG2. Known domains of both proteins are labelled and similar domains are color-coded. FFAT (two phenylalanines in acidic tract), SHR-BD (short root transcription factor-binding domain), APT1 (aberrant pollen transcription 1), ATG-C (ATG-C terminal domain) and PH (pleckstrin homology), ATG2-CAD (cysteine-alanine-aspartic acid triad). CLR (C-terminal localization region)[34,35,37–39].

The Chorein domain is an evolutionarily conserved domain with an unknown function[12]. The FFAT is a short stretch of amino acids commonly present in lipid transfer proteins with properties of building membrane contact sites with ER[34]. The APT1 domain was first identified in maize APT1 protein. APT1 colocalizes with a Golgi marker protein in tobacco pollen tubes and mutations in APT1 protein lead to defective pollen tube germination and transmission[36]. *In vitro*, Vps13 APT1 fragments bind specifically to PtdIns3p[35]. In the primary structure of VPS13A, the APT1 domain is located between SHR-BD and ATG-C domains[35,37].

SHR-BD is a highly conserved domain that was previously known as domain of unknown function 1162 (DUF1162)[35]. SHR-BD is present in vacuolar protein sorting (At5g24740) of *A. thaliana*. At5g24740 is also known as SHRUBBY and mutation in this gene leads to an aberrant root growth in *Arabidopsis*[40]. The SHR-BD fragment of Vps13 binds to a variety of phosphoinositides as well as to lysophosphatidic acid and phosphatidic acid[35]. Interestingly, the SHR-BD-APT1 fragment binds specifically to PtdIns3p unlike the SHR-BD alone, indicating that APT1 determines the specificity of lipid binding[35]. VPS13 also contains a PH domain and two ATG-C domains that are conserved in both yeast and human[35,37,41,42].

A PH domain is composed of approximately 100 amino acids[43] and is considered as one of the most common domains in the human proteome. PH domain containing proteins are known for their affinities to phosphoinositides; specifically to those with a pair of adjacent phosphate groups such as (PtdIns(4,5) P2 and (PtdIns(3,4,5)P3[44].
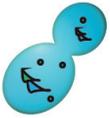
Additionally, VPS13A has two ATG-C domains that show homology with the C-terminal region of ATG2A[35]. There is a 25% identity between the C-terminal regions of VPS13A (aa 2939-3025) and ATG2 (aa 1830-1916)(Figure 1)[39]. ATG2 proteins have a membrane binding ability and are essential for autophagy and LD distribution[39,45]. Similarly, VPS13A is also required to maintain proper autophagic flux [42].

Cellular functions of VPS13A

Most of our current understanding about the cellular functions of Vps13 is derived from studies in yeast[35,46–54]. Vps13 was first identified in a genetic screen for mutants displaying impaired delivery of carboxypeptidase Y (CPY) to the vacuole[55]. Carboxypeptidase Y is a vacuolar protease synthesized in the endoplasmic reticulum (ER) as pro-CPY. Pro-CPY is transported to the Golgi complex where glycosylation occurs and subsequently delivered to the vacuole where modification to the active form takes place[56–58].

Mutants that fail to transport CPY to the vacuolar compartment secrete pro-CPY to the periplasm and ultimately to the extracellular medium[55]. By screening for secretion defects, together with morphological examinations, 41 Vps mutant strains were identified. These mutants are grouped into six classes based on their vacuolar morphology [55,59,60]. The different classes of Vps mutants and the description of their vacuolar morphology is summarized in table 1.

Table 1. Classification of Vps mutants based on their vacuolar morphology. All Vps mutants secrete CPY at various degrees59. Green circles (Vacuoles), small blue circles (fragmented vacuole like structures), orange circles (pre-vacuolar or class E compartment).

Class	Vps mutant	Characteristic	
A	Vps8, Vps10,Vps13,Vps29 Vps30, Vps35,Vps38, Vps44 Vps46	Normal vacuolar morphology with 1-3 large vacuoles per cell.	
B	Vps5, Vps17,Vps39,Vps41 Vps43	Large number (20-40) of small and fragmented vacuolar like compartments.	
C	Vps11, Vps16,Vps18,Vps33	Severe defect of vacuole assembly. These mutants barely show vacuoles, but instead accumulate small fragmented vesicles.	
D	Vps3, Vps6,Vps9,Vps15 Vps19, Vps21, Vps34,Vps45	One large vacuole in the parent cell, which fails to be acidified and to segregate to budding daughter cells.	
E	Vps2, Vps4,Vps20,Vps22 Vps24, Vps25, Vps27,Vps28 Vps31,Vps32, Vps36, Vps37	Possess a different population of vesicles (prevacoular endosome like compartment) that contain proteins from both late Golgi and vacuole.	
F	Vps1, Vps26	Large central vacuole surrounded by small fragments without any observable segregation defects.	
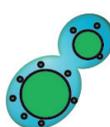
As a member of class-A Vps mutants, Vps13 mutants possess morphologically normal vacuoles[59]. Further characterization revealed that Vps13 is a peripheral membrane protein involved in the transport of membrane bound proteins between the trans-Golgi network (TGN) and pre-vacuolar compartment (PVC)[46,61] or from endosome to vacuole [62]. In vps13 mutant cells, there is an increased secretion of insulin and pro-CPY[46,63]. In control cells pro-CPY is actively sorted from late Golgi to vacuole by the sorting receptor Vps10[64]. In Vps13 mutant strains however, Vps10 is mislocalized and rapidly degraded which accounts for an apparent extracellular secretion of pro-CPY[46]. Severe impairment in the production of viable spores is also an apparent phenotype of Vps13 mutants[46].

At the earliest phase of sporulation, Vps13 is diffusely distributed throughout the cytoplasm. Whereas later in meiosis, it is localized at the prospore membrane[48]. Compared to wild type strains, Vps13 mutants have a few very small prospores that often fail to encapsulate nuclei[47].

The cellular functions of VPS13 proteins are intricately broad. Studies in several model organisms revealed that VPS13A plays an array of conserved roles to maintain protein homeostasis, phosphoinositide metabolism, actin cytoskeleton, membrane contact sites and LD homeostasis.

Knock-out of one of the six *Dictyostelium* VPS13 genes (*VPS13F*) delays intracellular destruction of phagocytic cargo attributed to failure in sensing bacterial folate without affecting phagosome maturation[65]. Similarly, *Tetrahymena* VPS13A (TtVPS13A) decorates the phagosome membrane and is required for efficient clearance of phagocytic cargo[66]. In cultured insect cells, Vps13 depletion delays endocytic processing[67]. Another *Dictyostelium* VPS13 (TipC), was identified in a screen for mutations affecting tip formation[68], similar to a phenotype that is observed in autophagy mutants[69]. *tipc*^{-/-} cells accumulate ubiquitinated protein aggregates accompanied by a decreased number of GFP-LC3 and GFP-ATG18 puncta. In mammalian cells, depletion of VPS13A raises the number of GFP-LC3 puncta but decreases liberation of free GFP indicative for a slow autophagic flux[42].

Both endocytic and autophagic degradation pathways are highly regulated by phosphoinositides[70–72]. Interestingly, synthetic genetic screens revealed that Vps13 mutants show similar sets of genetic interactions with Vps30 and Vps38[73,74]. Vps30 and Vps38 are the components of yeast complex I and complex II phosphatidylinositol 3-phosphate kinase (PI3K) complexes, respectively[75,76]. A plausible importance of Vps13 in phosphoinositide metabolism is further established as Vps13 directly binds to an array of phosphoinositides[35,50]. In addition, lipids -such as phosphatidic acid (PA), phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2 and phosphatidylinositol 4-phosphate (PtdIns4p) are reduced at the prospore membrane of Vps13 mutants[48]. Phosphoinositides regulate a multiplicity of cellular processes including actin polymerization and their mis-regulation is linked to a variety of human diseases[71,77,78]. Of importance, impaired actin polymerization is apparent in ChAc patient cells, VPS13A depleted cultured cells and Vps13 mutant yeast cells. In different organisms, VPS13A forms a complex with actin[35,79].

Vps13 is localized at multiple membrane contact sites (MCS)[49,51,54]. MCSs regulate lipid distribution and maintain proper lipid gradients across membranes of different organelles[80,81]. The type and abundance of lipid species determines the subcellular localization of MCS proteins[82]. A number of proteins involved in MCSs are identified through bioinformatics, imaging, biochemical and synthetic biology screens[83–87]. ER occupies the largest intracellular space in eukaryotic cells and is essential for the biosynthesis of proteins and lipids and the ER regulates cellular calcium homeostasis. It is therefore not surprising that most organelles communicate with the ER[81,82,88–95]. Organelle communication is not limited to the ER and it is now clear that MCSs are established between LDs and mitochondria[96], peroxisomes and mitochondria[97,98], lysosomes and peroxisomes[99], LDs and endosomes[100], nucleus and vacuoles[101]and mitochondria and vacuoles[86,87].

Vps13 is recruited to ER-Mitochondrial Encounter Structure (ERMES), vacuole and mitochondria patch (vCLAMP) and NVJ (nuclear vacuole junction) depending on metabolic growth conditions[49,54]. ERMES mutants are synthetically lethal when combined with Vps13 loss of function[49,54]. When harboring a dominant point mutation (Vps13-D716H), Vps13 is able to restore growth defects of ERMES mutants suggesting that Vps13 and ERMES are functionally redundant[49,51,54]. The mammalian ERMES counterpart has yet to be identified and the role of VPS13A in organelle communication is unknown.

AIM AND OUTLINE OF THE THESIS

The overall aim of our research was to uncover the cellular functions of VPS13A in health and disease. The biggest hurdles to study the biology of VPS13A were the absence of reliable genetic model systems and limited biochemical and labelling tools. This is mainly attributed to the absence of antibodies that would detect endogenous VPS13A protein in immunolabelling experiments and partly because of the inherently big size of the protein which in turn makes cloning and overexpression difficult. Indeed, Vps13 is the fifth largest protein in the yeast proteome[49]. We initially characterized a *Drosophila* model of ChAc with an aim to investigate phenotypic consequences of VPS13A-loss of function at the organismal level. We next aimed to identify the localization and interaction partners of VPS13A in cultured human cell lines. Through combined applications molecular biology, biochemistry and cellular imaging, we uncovered previously unknown functions of VPS13A in membrane contacts and LD homeostasis.

Chapter 2: Drosophila Vps13 is required for protein homeostasis in the brain.

Reports about Vps13 function are mainly derived from studies in unicellular eukaryotes such as *Saccharomyces cerevisiae* and *Tetrahymena thermophile*. The availability of multicellular models to study ChAc is limited and there is an obvious demand to generate and validate genetic ChAc models. Although VPS13A mutant mouse models that recapitulate some of the ChAc phenotype were generated[102], it later became clear that the phenotypes were not merely caused by VPS13A loss of function but rather dependent on genetic backgrounds[103]. In this chapter, we aimed to characterize an isogenic Vps13 mutant *Drosophila* line and showed that Vps13 deficient flies have motor impairments, shorter lifespan, neurodegeneration and accumulation of ubiquitinated protein aggregates. Some of these phenotypes were reverted by ubiquitous expression of human VPS13A in Vps13 deficient lines.

Chapter 3: Drosophila Vps13 mutants show overgrowth of larval neuromuscular junctions.

Impaired synaptic communication and plasticity have been previously implicated in many neurodegenerative diseases[104–106] . The neuromuscular junction (NMJ) is a specialized type of synapse that regulates muscle movement by controlling output of neuronal signals[107]. Cytoskeletal integrity of the NMJ not only determines synaptic architecture but also the quality of neuronal impulses[107–110] . Although, VPS13A depletion leads to mis-stabilized actin cytoskeleton[111–113] and abnormal bleb formation at neurite terminals of cultured cells[114], it is unclear whether defective neuro-synaptic architecture contributes to neurodegeneration in ChAc patients or the *Drosophila* model. In this chapter, we investigated and the NMJ anatomy of Vps13 mutants that were validated in chapter 2. Body wall muscles of Vps13 mutants were equally developed as their wild type counterparts. Nonetheless, Vps13 mutant larvae were highly mobile. Our data also indicate that Vps13 loss of function is associated with a large increase in number of boutons that are smaller in size compared to wild type controls.

Chapter 4: Human VPS13A is associated with multiple organelles and required for lipid droplet homeostasis

In chapter 2, we described that *Drosophila* Vps13 co-fractionates with endosomal proteins. However, the subcellular localization of mammalian VPS13A and interaction partners were unresolved for a long time. In this chapter, we provide evidence that VPS13A is localized at the ER-mitochondria interface and directly binds to the ER resident protein, VAP-A, through a specific motif. We also show that the VPS13 C-terminal part acts as a mitochondrial localization signal. When cellular lipid is surplus, VPS13A shifts from its reticular arrangement to LDs where it halts LD mobility. Moreover, we find that, upon VPS13A loss of function, LDs accumulate in both cultured cells and *Drosophila* brain. We also discuss that improper organelle communication and LD handling could contribute to the onset and progression of ChAc.

Chapter 5: Summarizing discussion

Our research highlights a conserved function of VPS13A to control proper protein homeostasis, neuronal growth, organelle communication and LD dynamics. Initially reported as a class A Vps family in yeast, Vps13 later emerged as a protein with multiplicity of cellular functions ranging from intracellular transport, prospore formation, mitochondrial clearance and MCSs. In this chapter, we summarize and discuss the available literature in the VPS13 field and propose a model in which VPS13A is not limited to a single subcellular compartment but it is associated of with multiple organelles dependent on cellular lipid content.

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1