

Arv1; a “Mover and Shaker” of Subcellular Lipids

J. Jose Corbalan¹, Karla K. Frieze¹ , Joseph Nickels^{1,2}, and Stephen L. Sturley³ 

Contact
Volume 8: 1–11
© The Author(s) 2025
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/25152564251314601
journals.sagepub.com/home/ctc



Abstract

The composition of eukaryotic membranes reflects a varied but precise amalgam of lipids. The genetic underpinning of how such diversity is achieved or maintained is surprisingly obscure, despite its clear metabolic and pathophysiological impact. The Arv1 protein is represented in all eukaryotes and was initially identified in the model eukaryote *Saccharomyces cerevisiae* as a candidate transporter of lipids from the endoplasmic reticulum. Human Arv1 has been shown to directly bind cholesterol and fatty acid affinity probes. Murine *in vivo* studies point to a role for ARV1 in regulating obesity, glucose tolerance, insulin sensitivity and brain function. Multiple human ARV1 variants have been associated with epileptic encephalopathy, cerebellar ataxia, and severe intellectual deficits. We hypothesize that Arv1 acts as an energy independent, lipid scramblase at the endoplasmic reticulum thereby modulating membrane lipid asymmetry and thus the trafficking of sterols and the substituents of glycosyl-phosphatidylinositol and sphingolipid biosynthesis.

Keywords

cholesterol, sphingolipids, glycosyl-phosphatidylinositol, lipid flippase, encephalopathy

Introduction

The movement of diverse lipids between organelles is a major component of membrane homeostasis and establishes the long known heterogeneous distribution of lipids within eukaryotic cells. For example, the eukaryotic plasma membrane (PM) contains the vast majority of cellular sterols while the endoplasmic reticulum (ER) is sterol-poor, despite being the primary site of sterol biosynthesis as well as the sensing organelle for sterol homeostasis. Similarly the sphingolipid composition of organelle membranes varies but is precise; a case in point, the ganglioside GM1 is often used as a specific marker of the Golgi in many subcellular fractionation experiments. Lipid heterogeneity further persists within the membranes of organelles; sterols and sphingolipids readily form a complex that is unevenly distributed thereby creating membrane microdomains. This asymmetric dissemination of lipids between and within cellular membranes is consequential, facilitating a vast array of cellular processes such as protein folding and trafficking, osmotic integrity, and signal transduction (see review, (Gulati et al., 2010)).

In mammalian cells, lipids such as nascent cholesterol are rapidly transported by an ATP-dependent mechanism (Liscum and Underwood, 1995), a process only partially accounted for by the secretory pathway as treatment with Brefeldin A to collapse the Golgi does not abrogate lipid

movement (Liscum and Underwood, 1995; Hao et al., 2002). Membrane juxtapositions or contact sites (MCS) may be sufficient for diffusion or protein-mediated lipid transport, however until recently, the overall molecular mechanisms in this process have remained obscure. Several candidate subcellular cholesterol or sphingolipid carriers, either as components of human pathophysiology or in model organisms such as *Saccharomyces cerevisiae*, have been identified. For example, the proteins defective in human Niemann Pick disease (NPC1 & 2), a fatal neurodegenerative lysosomal lipidosis bind cholesterol and other substrates. Deprivation of these conduits impacts the distribution of a host of lipids such that exogenous sterols and

¹Institute of Metabolic Disorders, Genesis Biotechnology Group, 1000 Waterview Drive, Hamilton, NJ 08691, USA

²Rutgers Center for Lipid Research, New Jersey Institute for Food, Nutrition, and Health, Rutgers University, New Brunswick, New Jersey 08901, USA

³Department of Biology, Barnard College at Columbia University, 3009 Broadway, New York, NY 10023, USA

Received January 5, 2025. Accepted January 6, 2025.

Corresponding Author:

Stephen L. Sturley, Joseph Nickels, Department of Biology, Barnard College at Columbia University, 3009 Broadway, New York, NY 10023, USA.
Emails: sls37@columbia.edu; jnickels@mdlab.com



plasma-membrane derived sphingolipids accumulate in the lysosome. The process is an ancient one; the NPC channels are impressively well conserved throughout eukaryotic evolution (Higaki et al., 2004) to the extent that expression of the yeast NP-C-related gene 1 (*NCR1*), or yeast *NPC2*, restores lipid transport to Chinese Hamster Ovary mutant cells lacking the corresponding orthologous proteins (Malathi et al., 2004). In this short review we will describe the role of another pivotal lipid transfer protein encoded by the *ARVI* gene. Initially identified in a yeast genetic screen, this protein is also functionally and structurally conserved throughout eukaryotic evolution to the extent that variation in human *ARVI* is strongly associated with neuronal degeneration and encephalopathy.

ARVI, a Historical Perspective

The sterol pool of the plasma membrane is mobile, and continually recycles with intracellular reservoirs. This distribution is maintained by the orchestrated equilibration of sterol biosynthesis, uptake, utilization (as lipoproteins, steroid hormones or bile acids), intracellular transport, and storage as steryl ester in cytoplasmic neutral lipid droplets. This latter process is mediated by the acyl-CoA cholesterol acyltransferases (ACATs). In a search for novel lipid transporters, we reasoned that the loss of any one of these homeostatic pathways (e.g., sterol esterification), particularly in model organisms such as yeast, might be tolerated, however removal of multiple membrane “detoxifying” events would be lethal. In *S. cerevisiae*, the ACAT-related enzymes (Are1 and Are2) are 49 percent identical to each other and exhibit 23 percent identity to human ACAT1 (Yang et al., 1996; Yu et al., 1996). These enzymes represent the principal pathway for esterification and thus storage of sterols. Accordingly, deletion of *ARE1* and *ARE2* produces a viable yeast cell with no detectable esterification activity (Arthington-Skaggs et al., 1996; Yang et al., 1996). We created mutations that confer viability dependence upon sterol esterification to identify the *ARVI* (*ARE2* Required for Viability 1) gene with the concept that such pathways control the flow of sterol from the ER to the plasma membrane (Tinkelenberg et al., 2000; Swain et al., 2002). The deletion of *ARVI* in the context of deficient sterol esterification, is inviable, possibly due to accumulation of unesterified sterols in ER membranes at the expense of plasma membrane sterol pools (Tinkelenberg et al., 2000). Mutations in *ARVI* were also isolated independently in a genetic screen for mutants defective in sphingolipid biosynthesis (J. Nickels, unpublished results). Lower steady state levels of complex sphingolipids, with an accumulation of hydroxylated ceramides and many other defects in lipid metabolism were indicative of a generalized defect in lipid export from the ER in *arv1Δ* mutant cells (Swain et al., 2002). Subsequently, the *ARVI* gene product was hypothesized to act as a glycosylphosphatidylinositol (GPI) flippase (Figure 1) (Kajiwara

et al., 2008; Okai et al., 2020), although since it lacks demonstrable ATPase activity, it might be more accurately considered a scramblase. Interestingly, unlike many lipid homeostats, *ARVI* is unique (with the exception of plants); it is not duplicated, nor does it share any domains with other proteins. Thus if alternate “bypass” pathways exist, they are truly *Arv1* independent, although they likely intersect and interact.

Evolutionary Conservation of ARVI

The yeast *ARVI* gene predicts a 322 residue transmembrane protein that is conserved in every eukaryotic genome currently sequenced (Figure 2). Human *ARVI* (*hARVI*) encodes a 271 amino acid protein, the expression of which complements all known phenotypes associated with loss of *ARVI* in yeast (*Scarv1Δ* cells), including the GPI, sterol and sphingolipid transport deficiencies (Tinkelenberg et al., 2000; Swain et al., 2002; Ikeda et al., 2016). Human cell lines from individuals carrying an *Arv1* Gly189Arg variant-associated with neurological deficiency, have reduced levels of GPI proteins. Interestingly, *Scarv1Δ* cells overexpressing the human *Arv1* Gly189Arg variant showed only partial recovery of GPI synthesis and GPI-anchored protein maturation defects (Okai et al., 2020; Segel et al., 2020). A restoration in sphingolipid biosynthesis was suggested, but more detailed analyses need to be performed to resolve this issue. Nevertheless, the functional role of *Arv1* seems likely to have been conserved throughout eukaryotic evolution.

Structural information can be highly informative and guide well-defined biochemical studies designed to elucidate function. However, *Arv1*, a transmembrane-associated protein, is recalcitrant to traditional biochemical structure/function analyses, in part due to limited homology at the prokaryotic species level, the lack of structural homology within the human genome, and absence of crystallographic or cryo-EM data. The primary sequence of *Arv1* (Figure 2) from all eukaryotes indicates a cluster of cysteine residues, the spacing of which suggests a zinc ribbon, which may act to coordinate ligand complexing or protein-protein interactions. The predicted topology of *Arv1p* suggests this *Arv1* homology domain (so termed because this 60 residue span displays the strongest conservation across organisms in the *Arv1* molecule) faces the cytosol.

The AlphaFold Protein Structure Database is a repository of AI-generated 3D protein structures that contains the human proteome and is an invaluable source for uncovering protein function through identification and interpretation of predicted tertiary structure (Jumper et al., 2021; Varadi et al., 2022). AlphaFold predictions have human and yeast *ARVI* structurally closely related although they share only 30% amino acid identity, making results gleaned from both species highly relevant for postulating the mechanistic function of human *ARVI* based on structural predictions (Figure 3A).

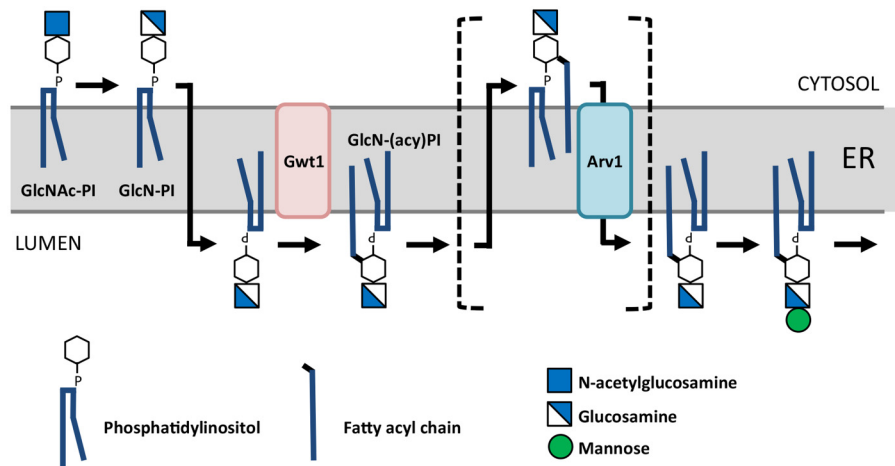


Figure 1. The Role of Arv1 in Glycosyl-Phosphatidylinositol (GPI) Synthesis. Reproduced with Permission (Okai et al., 2020). Arv1 is proposed to transfer (flip) glycosylamino-acyl-phosphatidylinositol (GlcN-PI) from the cytoplasmic side of the ER to the lumen side where it is used as a substrate for the further synthesis of GlcN-(acyl)PI and GPI anchors. It is plausible that as a consequence, sterols are rendered available to the membrane associated acylCoA cholesterol acyltransferases (ACATs) and intermediates in sphingolipid biosynthesis are further metabolized. Alternatively, ARV1 may encode a multi-substrate scramblase.

Human ARV1 predicts a five transmembrane domain protein using Transmembrane DeepTMHMM (<https://dtu.biolib.com/DeepTMHMM>) (Hallgren et al., 2022) (Figure 3B). Amino acid substitutions of relevance to human disease, are shown in the ribbon diagram (Figure 3C). The neurological 101G>A nucleotide variant results in replacement of Cys34 with a tyrosine (Salian et al., 2021) within the zinc-binding domain motif of the AHD. Cys34 corresponds to Cys3 in *S. cerevisiae* and many pathogenic fungi that include *C. albicans* and *C. glabrata*, which are responsible for most fungal infections in individuals living with HIV (Perfect, 2017). Cys3 has been studied in detail in *S. cerevisiae* and *C. albicans*. Studies have shown its requirement for fungal virulence (Villasmil et al., 2020), proper sterol distribution and localization (Gallo-Ebert et al., 2012), polarized cell growth during hyphal invasion (Gallo-Ebert et al., 2012), and normal sensitivity to sterol-targeting azole antifungal drugs (Villasmil et al., 2020). Similarly, the replacement of Gly189 with Arg within the third transmembrane domain and its placement based on ribbon diagrams may suggest a role in oligomerization of human Arv1 in cells (Figure 3B). Accordingly, fibroblasts from individuals expressing Arv1 (Gly189Arg) showed a significantly reduced level of the monomeric protein (Segel et al., 2020).

We have consistently found that *E. coli* expressed human Arv1-HIS recombinant protein purifies as a monomer, dimeric, and trimeric species, with enrichment in the dimeric and trimeric states even under denaturing conditions, suggesting Arv1 may exist as an oligomer whose subunit number is tied to its function. Dimeric and trimeric docking structures were predicted using the GalaxyWEB homomer predictive algorithm with the trimeric structure

shown in Figure 4A and the hexameric shown in Figure 4B (Ko et al., 2012). The hexameric predictions show the presence of a tunnel within the Arv1 structure (Figure 4C) (Latterich and Schekman, 1994). Kajiwara et al., (Kajiwara et al., 2008) have suggested that ScArv1 is required to translocate glucosylamino-acyl-phosphatidylinositol (GlcN-acylPI) from the cytoplasmic side of the ER to the luminal side, possibly acting as a lipid scramblase (Figure 1), a process likely to be energy independent. It has been found that ScArv1 formed ER-associated, clustered foci in yeast cells that also contained Kar2 that is required for ER-membrane fusion (Latterich and Schekman, 1994; Kajiwara et al., 2008). These foci may represent an oligomeric form of ScArv1 or an oligomeric complex binding at specific membrane contact sites where it may shuttle lipids between organelles (K. Funato, personal communication). The proposed “Arv1 lipid tunnel” is made up of the transmembrane segments of the protein and could act as a conduit for lipid translocation by an Arv1 lipid scramblase activity (Hankins et al., 2015; Lyons et al., 2020; Giacometti et al., 2022). Determining whether Arv1 acts to either shuttle or flip lipids within membranes will require rigorous biochemical and biophysical analyses.

Purified human Arv1 protein has been shown to directly bind to cholesterol and fatty acid affinity probes using proteome-wide lipid binding methods. Along with severe defects in maintaining proper lipid distribution and localization (Swain et al., 2002; Villasmil et al., 2011; Gallo-Ebert et al., 2012; McCourt et al., 2016), *Scarv1Δ* cells show a fatty acid induced inviability phenotype (Ruggles et al., 2014). Therefore, the challenge remains how to discern the primary vs secondary defect in lipid metabolism caused by loss of ARV1.

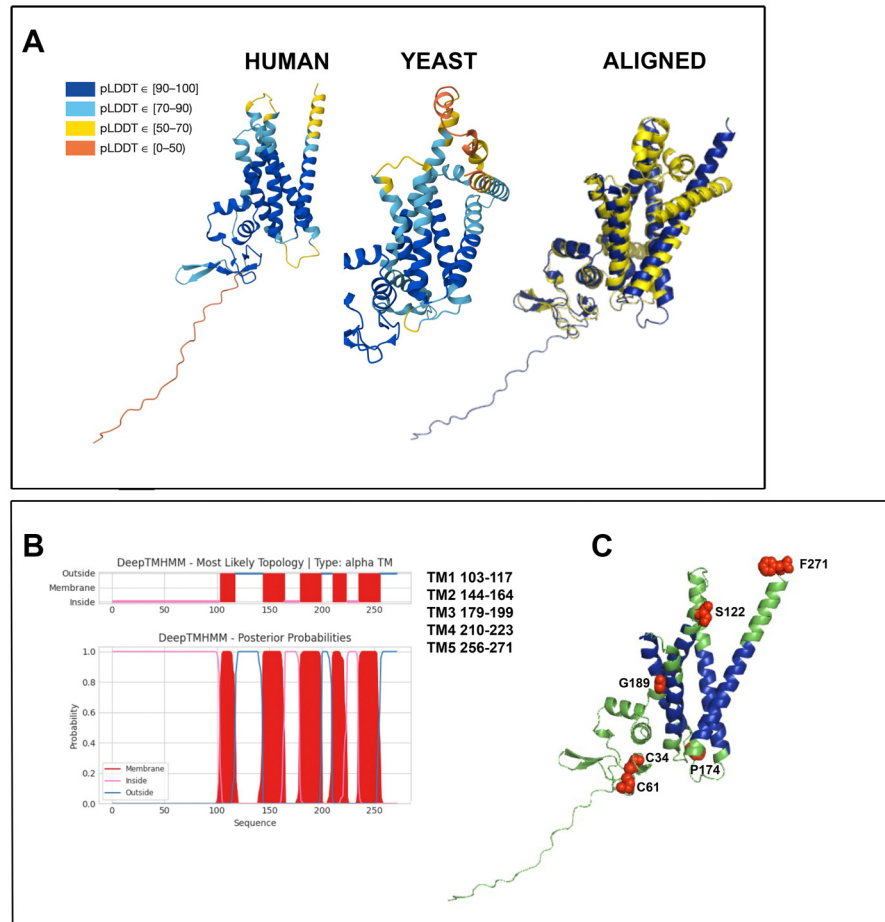


Figure 3. Human ARV1 Topology. A, ribbon diagrams of human and yeast Arv1 proteins. B, transmembrane prediction profile. C, ribbon diagram showing human Arv1 amino acid substitutions found in individuals with epileptic encephalitis. The dashed faint horizontal lines define the approximate extent of the predicted bilayer.

transcription responses reflect induction of the unfolded protein response (UPR) (Shechtman et al., 2011). Moreover, accumulation of CD55, a widely expressed GPI-anchored protein is correlated with activation of ER associated degradation (ERAD) and causes the upregulation of ARV1 expression and GPI synthesis in HEK293 cells (Li et al., 2023). We speculate that in the absence of sterol/lipid detoxification (by whatever mechanism), cell death is avoided or delayed by induction of stress responses. One such response is UPR activation, which serves to upregulate a series of ER associated folding chaperones and degradation pathways to deal with the accumulation of misfolded proteins before they aggregate and exacerbate the lipotoxicity (Kajiwara et al., 2012; Hong et al., 2024).

Murine Models of Arv1: A Role in Lipid Homeostasis, Obesity and Cognition

Murine *in vivo* studies point to a role for Arv1 in regulating obesity, glucose tolerance, and insulin sensitivity (Lagor

et al., 2015; Gallo-Ebert et al., 2018) and mice deficient in neuronal ARV1 show multiple neurological defects (Palmer et al., 2016). Initial anti-sense oligonucleotide studies in mice and the use of mouse knockouts have built a fundamental understanding of the role of mammalian Arv1 in lipid biology, while uncovering an unexpected role in cognitive function and neurological development.

Early anti-sense oligonucleotides studies suggested that Arv1 may regulate FXR signaling and *de novo* cholesterol biosynthesis through altering changes in bile acid composition and *SREBF1* gene expression (Tong et al., 2010). Knock down of ARV1 in the livers of mice caused hypercholesterolemia, elevated levels of serum bile acids, and increased gene expression of the FXR target gene, *SHP*. Expression levels of the *ABCB11* efflux pump and taurocholate *NTCP* bile acid influx pump were increased and reduced, respectively, indicating increased bile acid excretion out of the liver. Expression of *CYP7 α 1*, which encodes the rate-limiting enzyme in bile acid synthesis, was reduced, possibly explaining the hypercholesterolemic phenotype.

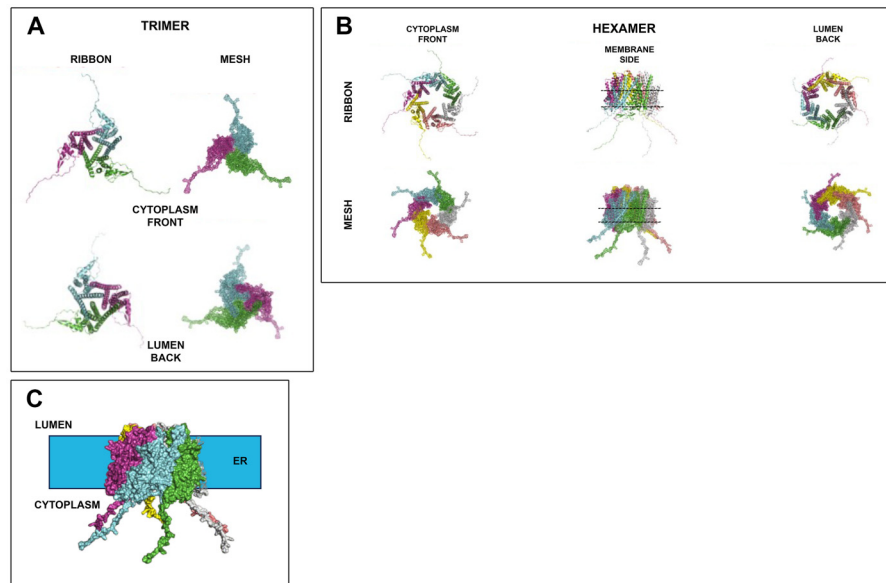


Figure 4. Human ARV1 May Oligomerize. A, ribbon diagram of a human Arv1 trimer. B, ribbon diagram of a human Arv1 hexamer. C, side view of a hexameric human Arv1 embedded in the ER membrane.

In the same study, it was observed that *ARV1* knockdown in HepG2 cells reduced *CYP7a1* expression, consistent with *in vivo* findings, while also reducing *SREBF1* and *HMGCR* expression. *CYP7a1*-deficient mice fed a western diet, like *ARV1* knockdown mice, display altered bile acid composition and elevated *FXR* and *SHP* gene expression. Based on their data, the authors suggested that Arv1 acts as a central hub for directing cholesterol trafficking from the ER to various other organelles. When Arv1 function is ablated, cholesterol accumulation in the ER will result in increased bile acid synthesis and excretion, and decreased SREBP1c-dependent gene expression. However, eventually cholesterol accumulation would cause dysfunction within the ER.

Based on the metabolic phenotypes seen in *Arv1^{-/-}/Arv1^{-/-}* mice, ARV1 may act as a lipid “rheostat/sensor” that controls general/specific lipid transport in response to nutrient uptake and caloric overload. *Arv1^{-/-}/Arv1^{-/-}* knockout mice generated by Lagor and colleagues (Lagor et al., 2015) were tested in metabolic feeding studies. Fed a chow diet, these mice did not gain weight or increase their white adipose tissue mass when compared to matched wild type littermates. Blood cholesterol and high-density apolipoprotein levels were decreased, whereas energy expenditure rates were increased, and increased fatty acid oxidation was observed. Importantly, no loss of muscle mass was observed, so lack of weight gain due to ablation of *ARV1* was related to altered nutrient utilization.

A second *Arv1^{-/-}/Arv1^{-/-}* mouse was later challenged with a high fat western diet and its metabolic response was characterized (Gallo-Ebert et al., 2018). These mice were resistant to high fat diet-induced obesity. Liver triglyceride levels were normal and there were no signs of hepatic steatosis. They remained insulin sensitive and glucose tolerant throughout

the study. FXR signaling was activated, while *de novo* cholesterol and bile acid syntheses were reduced. Fibroblast growth factor 21 (FGF21) protein levels were elevated, suggesting activated peroxisome proliferator-activating receptor γ (PPAR γ) signaling in the liver. Determining the effect of ablating *ARV1* in genetically obese *ob/ob* and diabetic *db/db* mice on the phenotypes of these mice should help to see if inhibiting ARV1 is an avenue for drug discovery for treating weight loss and type 2 diabetes.

Cholesterol must be synthesized *de novo* in the brain representing ~25% of the body’s cholesterol, thus its proper handling is essential for maintaining brain function (Hussain et al., 2019). Neuronal function has been shown to be impaired due to either loss or aberrant accumulation of cholesterol in neurons (Hussain et al., 2019). At the sub-cellular level, aberrant distribution of cholesterol pools between the ER and mitochondria at membrane contact sites may lead to the neurodegeneration associated with Alzheimer’s and Parkinson diseases (Arenas et al., 2017). Accordingly, Palmer *et al.*, (Palmer et al., 2016) showed that mice ablated for neuronal *ARV1* (ARV1 NKO) displayed frequent circling behavior and were predisposed to severe seizures phenotypes as seen in humans with *ARV1*-variant epileptic encephalopathy (see below). Interestingly, ARV1 NKO mice fed a chow diet gained less weight and had reduced white adipose mass, like *Arv1^{-/-}/Arv1^{-/-}* mice (Lagor et al., 2015; Gallo-Ebert et al., 2018), indicating a function for brain ARV1 in regulating whole body lipid metabolism. Challenging these mice with a western diet and determining the metabolic responses could have the potential to differentiate what specific aspects of lipid metabolism are impacted by the activity of ARV1 in the brain.

Arv1 and Human Pathophysiology: A Role in Neuronal Function?

Given the marked hepatic and neuronal pathology associated with deprivation of Arv1p in murine models, we strongly anticipated a disease state to be associated with variation in this gene in humans. To date, this appears to predominantly manifest as neurological impairment; ten human *ARV1* variants have been linked to epileptic encephalopathy, cerebellar ataxia, and severe intellectual deficits (Figure 5 and Table 1). A comprehensive review of each case report has been published elsewhere (Kamate and Basavanagowda, 2023). Below, we summarize data supporting a role for Arv1 in GPI-anchor biosynthesis that is consistent with the Arv1-variant phenotypes, particularly considering the pivotal role of neuronal GP I-anchored proteins in central nervous system development (Um and Ko, 2017).

When overexpressed, human *ARV1* suppresses the GPI biosynthesis defects of *Scarv1Δ* cells, indicating conservation in function (Ikeda et al., 2016). Subsequent complementation analyses suggest that mutations in human *ARV1* linked to certain infantile seizure disorders are caused by defects in GPI biosynthesis. Further supporting evidence is the fact that cells from afflicted patients have reduced maturation of GPI-anchored proteins (Davids et al., 2020; Segel et al., 2020). Even with this mounting evidence, however, the question remains whether loss of human ARV1 causes a direct or indirect effect on GPI-anchor biosynthesis (Liu et al., 2023). It must be pointed out that in only one case has a human *ARV1* mutant been tested for the ability to suppress the sphingolipid biosynthesis defects of *Scarv1Δ* cells (Segel et al., 2020). None were tested for the ability to restore normal sterol trafficking.

A homozygous *ARV1* variant (565G > A:p.Gly189Arg), was first identified in children with neurogenetic disorders born of consanguineous parents (Alazami et al., 2015). Subsequently, two homozygous *ARV1* mutant variants (294 + 1G > A:p.Lys59_Asn98del; 674-2A > T:p.Thr266_Phe271del) were linked to infantile seizure disorders including epileptic encephalopathy 38, which is an autosomal neurological disease linked to defects in GPI biosynthesis (Davids et al., 2020). While the Lys59_Asn98del was unable to complement the temperature sensitivity phenotype of *Scarv1Δ* cells when overexpressed, the Gly189Arg mutant could partially restore growth (Palmer et al., 2016; Segel et al., 2020). The Gly189Arg could not, however, suppress the GPI biosynthesis defects, as there remained the sustained decrease in the maturation of the GPI-anchored Gas1 protein (Segel et al., 2020). The Gly189Arg may have suppressed the sphingolipid biosynthesis defects observed in *Scarv1Δ* cells (Swain et al., 2002). Whether this allele restored normal sterol trafficking to mutant cells was not tested.

Cell culture experiments further tied ARV1 function to GPI-anchor protein trafficking, seen as reduced expression of GPI-anchor protein biomarkers at the plasma membrane.

Neutrophils from patients harboring the *ARV1* Thr266_Phe271del allele had reduced levels of the GPI-anchored proteins CD16, CD66b, CD55 & 59, and FLAER (Davids et al., 2020). Fibroblasts from a patient harboring the *ARV1* Lys59_Asn98del allele had decreased expression of CD59 and CD87 (Palmer et al., 2016). Fibroblasts from a patient with a homozygous c.363_364del, p:Ser122Glnfs*7 frameshift allele had reduced levels of CD73 and CD109 (Salian et al., 2021). Lentiviral expression of human *ARV1* in these cells fully rescued the reduced expression levels of GPI-anchor defects. Fibroblasts from patients carrying the p.Gly189Arg allele had decreased levels of FLAER, CD73, and CD59. Lentivirus expression of *ARV1* partially rescued the decrease in FLAER expression while completely rescuing CD73 and CD59 cell surface levels. As with yeast complementation assays, cells were not tested for restoring normal cholesterol trafficking in HepG2 cells (Tong et al., 2010). Overall, results do suggest that ARV1 regulates some aspect of GPI-anchor biosynthesis. Only when the molecular function of ARV1 has been uncovered will we resolve how it does so at the molecular level and how this accounts for the striking metabolic consequences of variation in this lipid transporter.

Conclusions and Alternate Models: is ARV1 a Lipid Scramblase?

The endoplasmic reticulum represents a protected, sequestered environment for protein folding and lipid homeostasis. As such it is subjected to numerous levels of quality control that are pivotal to cell function. The discovery and characterization of Arv1 mediated pathways suggests a role for lipid metabolism, particularly lipid efflux, in the homeostasis of this key compartment of the cell. Arv1 appears to check all the boxes for a lipid transporter; it is clearly an ER resident, it binds lipids, it interacts with multiple lipid regulatory proteins and its deficiency results in numerous lipid related consequences. That said, an impartial reader might question the precise biological role of Arv1p, particularly in terms of primary vs. secondary roles. For example, what is the connection between Arv1's apparent role in lipid trafficking and GPI biosynthesis and regulation of membrane contact sites that links lipid homeostasis and cognitive function? The mouse and human phenotypic data implicating Arv1 in cognitive function has become well established, but cell culture studies are needed to uncover the causative mechanism. Very little is known about how Arv1 functions to regulate lipid metabolism and GPI synthesis, even though it was identified over twenty years ago.

Preliminary two-hybrid screening in our laboratory using the hAHD identified several proteins involved in endoplasmic reticulum/Golgi transport (Liu and Nickels, unpublished data). One such Arv1 ligand; the RING finger protein 139, also known as TRC8, is an E3 ubiquitin ligase known to

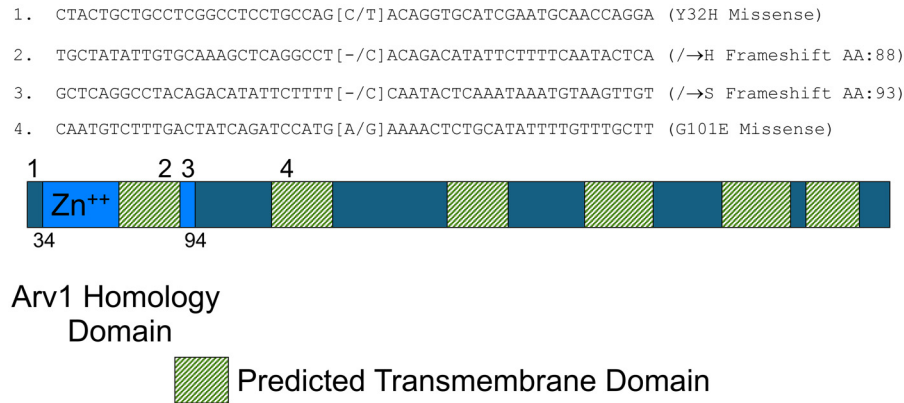


Figure 5. Single Nucleotide Polymorphisms in Human ARV1. Note these variants are non-synonymous, 3 of which are in the ARV1 homology domain (AHD) and thus likely to impact activity of Arv1 (see table 1 for more details).

Table 1. Human ARV1 Variants and Associated Consequences.

ARV1 variant	genotype	reference	GPI-anchor Defects	Pathology
c.565G>A, p.Gly189Arg	missense homozygous	(Alazami et al., 2015; Segel et al., 2020)	+	Epileptic encephalopathy
c.294 + 1G>A, p.Lys59_Asn98del	splicing homozygous	(Palmer et al., 2016; Davids et al., 2020)	+	Infantile epileptic encephalopathy
c.674-2A>T, p.Thr266_Phe271del	splicing homozygous	(Davids et al., 2020)	+	Infantile epileptic encephalopathy
c.363_364del, p.Ser122Glnfs*7; c.489G>A, p.Trp163*	compound heterozygous	(Darra et al., 2021)	NS	Epileptic encephalopathy
c.363_364del, pSer122Gln, fs*7	homozygous frameshift	(Salian et al., 2021)	+	Epileptic encephalopathy
c.518dupA, p.Pro174Alafs*14; c.101G>A, p.Cys34Tyr	compound heterozygous	(Salian et al., 2021)	NS	Epileptic encephalopathy
c.182G>A, p.Cys61Tyr	missense homozygous	(Salian et al., 2021)	NS	Epileptic encephalopathy
c.674-1G>A	splicing homozygous	(Salian et al., 2021)	NS	Epileptic encephalopathy
c.518dupA, p.Pro174Alafs*14	homozygous	(Salian et al., 2021)	NS	Epileptic encephalopathy
c.554_556delTAT, p.L185del	-	(Karabinos et al., 2022)	NS	Seizures; intellectual disabilities

interact with the sterol cleavage activating protein (SCAP)-SREBP-2 complex and hinder its translocation from the endoplasmic reticulum to the Golgi. A recent report has suggested that SCAP regulates an oxysterol binding protein complex involved in cholesterol transport at ER-Golgi membrane contact sites (Apodaca and Brown, 2014; Wakana et al., 2021). Fitting in with this result is the fact that cholesterol has been shown to regulate multiple different organelle membrane contact sites (Ridgway and Zhao, 2018). Perhaps TRC8, by causing the rapid degradation of the SCAP-SREBP-2 complex, decreases cholesterol biosynthesis. If ARV1 is involved in human sterol transport, as in both yeast (ergosterol) (Villasmil et al., 2011; Gallo-Ebert et al., 2012; McCourt et al., 2016) and mice (Tong et al.,

2010), there may exist an ARV1-TRC8-SREBP2 axis regulating sterol levels to sustain proper membrane contact site fusion. Arv1's role would be cholesterol transport, and possibly GPI synthesis that would be required for modification of proteins necessary for contact site biogenesis/function.

Is Arv1a lipid scramblase whose function is essential for maintaining metabolic and neurologic health? It is tempting to speculate that by being localized within plasma membrane-associated tubules of the cortical ER and Golgi-associated ER (Swain et al., 2002; Tong et al., 2010), Arv1 acts as a lipid "sensor" and/or lipid scramblase necessary for maintaining proper membrane contact site interactions. The lipid-specific Arv1-dependent activity would depend on the lipid to which it is bound ("sensing"),

which would then trigger its lipid transport activity that is not only involved in ensuring proper lipid symmetry between organelles but lipid shuttling through specific pathways for sphingolipid and/or GPI synthesis (Devaux et al., 2008; Ristovski et al., 2021). *Saccharomyces cerevisiae* cells lacking *ScARV1* have abnormal ER and PM morphologies (Georgiev et al., 2013). It is known that ER-mitochondrial membrane contact sites are enriched in ceramides and sterol (Scorrano et al., 2019). Loss of *ARV1* in yeast results in accumulation of ceramides and defects in sterol trafficking (Swain et al., 2002; Villasmil et al., 2011), suggesting the possibility that the putative transport activity of Arv1 may include sterols and sphingolipids. Human Arv1 contains several cholesterol binding motifs (CRAC/CARC) (Fantini and Barrantes, 2013), some within the AHD, consistent with an interaction with cholesterol or other sterol intermediates at membrane contact sites (Muallem et al., 2017).

The list of lipid transporters within membrane contact sites is ever increasing (Scorrano et al., 2019; Castro et al., 2022). Yeast Csf1 has been proposed to act at membrane contact sites and shuttle phosphatidylethanolamine to Mcd4 to be used as a substrate for GPI synthesis (Toulmay et al., 2022). An ortholog of Csf1 is found in humans and is required for synaptic vesicle recycling (Verstreken et al., 2009). The yeast Lam6/Ltc1 protein (Gatta et al., 2015) that is an ortholog of the StART family of cholesterol binding proteins (Epanand, 2006) transports sterol across ER-mitochondrial membranes (Elbaz-Alon et al., 2015) and acts as a tether for stabilizing ER-mitochondrial contact sites (Kormmann et al., 2009; Murley et al., 2015).

If Arv1 is a *bona fide* lipid scramblase, its function must be critical for the exchange of lipids back and forth between adjacent membranes to maintain proper MCS lipid composition under different physiological conditions (Montigny et al., 2016; Hammond and Burke, 2020; Lopez-Marques, 2021). The activity of Arv1 may translocate lipids with diverse structures based on the pleiotropic defects seen with its loss in yeast and possible propensity for binding several lipid species in human cells. The profound consequences of loss of Arv1 to any cell, from the unicellular context of a yeast to the multicellular complex environment of the human brain strongly suggests a key role of the MCS in pathophysiology and merits further pursuit.



Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Supported in part by awards from the Ara Parseghian Medical Research Foundation and Dana's Angels Research Trust, NIH, (grant number DK54320).

ORCID iDs

Karla K. Frieze  <https://orcid.org/0000-0002-4708-8009>
 Stephen L. Sturley  <https://orcid.org/0000-0001-6805-3270>

References

- Alazami AM, Patel N, Shamseldin HE, Anazi S, Al-Dosari MS, Alzahrani F, Hijazi H, Alshammari M, Aldahmesh MA, Salih MA, et al. (2015). Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of pre-screened multiplex consanguineous families. *Cell Rep 10*, 148–161. <https://doi.org/10.1016/j.celrep.2014.12.015>.
- Apodaca G, Brown WJ (2014). Membrane traffic research: Challenges for the next decade. *Front Cell Dev Biol 2*, 52. <https://doi.org/10.3389/fcell.2014.00052>.
- Arenas F, Garcia-Ruiz C, Fernandez-Checa JC (2017). Intracellular cholesterol trafficking and impact in neurodegeneration. *Front Mol Neurosci 10*, 382. <https://doi.org/10.3389/fnmol.2017.00382>.
- Arthington-Skaggs BA, Crowell DN, Yang H, Sturley SL, Bard M (1996). Positive and negative regulation of a sterol biosynthetic gene (*ERG3*) in the post-squalene portion of the yeast ergosterol pathway. *FEBS Lett 392*, 161–165. [https://doi.org/10.1016/0014-5793\(96\)00807-1](https://doi.org/10.1016/0014-5793(96)00807-1).
- Castro IG, Shortill SP, Dziurdzik SK, Cadou A, Ganesan S, Valenti R, David Y, Davey M, Mattes C, Thomas FB, et al. (2022). Systematic analysis of membrane contact sites in *Saccharomyces cerevisiae* uncovers modulators of cellular lipid distribution. *Elife 11*. <https://doi.org/10.7554/eLife.74602>.
- Darra F, Lo Barco T, Opri R, Parrini E, Bianchini C, Fiorini E, Simonati A, Dalla Bernardina B, Cantalupo G, Guerrini R (2021). Migrating focal seizures and myoclonic status in *ARV1*-related encephalopathy. *Neurol Genet 7*, e593. <https://doi.org/10.1212/NXG.0000000000000593>.
- Davids M, Menezes M, Guo Y, McLean SD, Hakonarson H, Collins F, Worgan L, Billington CJ Jr., Maric I, Littlejohn RO, et al. (2020). Homozygous splice-variants in human *ARV1* cause GPI-anchor synthesis deficiency. *Mol Genet Metab 130*, 49–57. <https://doi.org/10.1016/j.ymgme.2020.02.005>.
- Devaux PF, Herrmann A, Ohlwein N, Kozlov MM (2008). How lipid flippases can modulate membrane structure. *Biochim Biophys Acta 1778*, 1591–1600. <https://doi.org/10.1016/j.bbame.2008.03.007>.
- Elbaz-Alon Y, Eisenberg-Bord M, Shinder V, Stiller SB, Shimoni E, Wiedemann N, Geiger T, Schuldiner M (2015). Lam6 regulates the extent of contacts between organelles. *Cell Rep 12*, 7–14. <https://doi.org/10.1016/j.celrep.2015.06.022>.
- Epanand RM (2006). Cholesterol and the interaction of proteins with membrane domains. *Prog Lipid Res 45*, 279–294. <https://doi.org/10.1016/j.plipres.2006.02.001>.
- Fantini J, Barrantes FJ (2013). How cholesterol interacts with membrane proteins: An exploration of cholesterol-binding sites including CRAC, CARC, and tilted domains. *Front Physiol 4*, 31. <https://doi.org/10.3389/fphys.2013.00031>.
- Gallo-Ebert C, Francisco J, Liu HY, Draper R, Modi K, Hayward MD, Jones BK, Buiakova O, McDonough V, Nickels JT Jr (2018). Mice lacking *ARV1* have reduced signs of metabolic syndrome and non-alcoholic fatty liver disease. *J Biol Chem 293*, 5956–5974. <https://doi.org/10.1074/jbc.RA117.000800>.
- Gallo-Ebert C, McCourt PC, Donigan M, Villasmil ML, Chen W, Pandya D, Franco J, Romano D, Chadwick SG, Gyax SE,

- Nickels JT Jr (2012). Arv1 lipid transporter function is conserved between pathogenic and nonpathogenic fungi. *Fungal Genet Biol* 49, 101–113. <https://doi.org/10.1016/j.fgb.2011.11.006>.
- Gatta AT, Wong LH, Sere YY, Calderon-Norena DM, Cockcroft S, Menon AK, Levine TP (2015). A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. *Elife* 4. <https://doi.org/10.7554/eLife.07253>.
- Georgiev AG, Johansen J, Ramanathan VD, Sere YY, Beh CT, Menon AK (2013). Arv1 regulates PM and ER membrane structure and homeostasis but is dispensable for intracellular sterol transport. *Traffic (Copenhagen, Denmark)* 14, 912–921. <https://doi.org/10.1111/tra.12082>.
- Giacometti SI, MacRae MR, Dancel-Manning K, Bhabha G, Ekiert DC (2022). Lipid transport across bacterial membranes. *Annu Rev Cell Dev Biol* 38, 125–153. <https://doi.org/10.1146/annurev-cellbio-120420-022914>.
- Gulati S, Liu Y, Munkacsı AB, Wilcox L, Sturley SL (2010). Sterols and sphingolipids: Dynamic duo or partners in crime? *Prog Lipid Res* 49, 353–365. <https://doi.org/10.1016/j.plipres.2010.03.003>.
- Hammond GRV, Burke JE (2020). Novel roles of phosphoinositides in signaling, lipid transport, and disease. *Curr Opin Cell Biol* 63, 57–67. <https://doi.org/10.1016/j.ceb.2019.12.007>.
- Hankins HM, Baldrige RD, Xu P, Graham TR (2015). Role of flippases, scramblases and transfer proteins in phosphatidylserine subcellular distribution. *Traffic* 16, 35–47. <https://doi.org/10.1111/tra.12233>.
- Hao M, Lin SX, Karylowski OJ, Wustner D, McGraw TE, Maxfield FR (2002). Vesicular and non-vesicular sterol transport in living cells. The endocytic recycling compartment is a major sterol storage organelle. *J Biol Chem* 277, 609–617. <https://doi.org/10.1074/jbc.M108861200>.
- Higaki K, Almanzar-Paramio D, Sturley SL (2004). Metazoan and microbial models of Niemann-Pick Type C disease. *Biochim Biophys Acta* 1685, 38–47. <https://doi.org/10.1016/j.bbali.2004.08.010>.
- Hong S, Lee HG, Huh WK (2024). ARV1 Deficiency induces lipid bilayer stress and enhances rDNA stability by activating the unfolded protein response in *Saccharomyces cerevisiae*. *J Biol Chem* 300, 107273. <https://doi.org/10.1016/j.jbc.2024.107273>.
- Humphreys IR, Pei J, Baek M, Krishnakumar A, Anishchenko I, Ovchinnikov S, Zhang J, Ness TJ, Banjade S, Bagde SR, et al. (2021). Computed structures of core eukaryotic protein complexes. *Science* 374, eabm4805. <https://doi.org/10.1126/science.abm4805>.
- Hussain G, Wang J, Rasul A, Anwar H, Imran A, Qasim M, Zafar S, Kamran SKS, Razzaq A, Aziz N, et al. (2019). Role of cholesterol and sphingolipids in brain development and neurological diseases. *Lipids Health Dis* 18, 26. <https://doi.org/10.1186/s12944-019-0965-z>.
- Hallgren J, Tsirigos KT, Pedersen MD, Armenteros JJ, Marcatili P, Nielsen H, Krogh A, Winther O (2022). DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. <https://doi.org/10.1101/2022.04.08.487609>.
- Ikeda A, Kajiwara K, Iwamoto K, Makino A, Kobayashi T, Mizuta K, Funato K (2016). Complementation analysis reveals a potential role of human ARV1 in GPI anchor biosynthesis. *Yeast* 33, 37–42. <https://doi.org/10.1002/yea.3138>.
- Ji Z, Tinti M, Ferguson MAJ (2021). Proteomic identification of the UDP-GlcNAc: PI alpha1-6 GlcNAc-transferase subunits of the glycosylphosphatidylinositol biosynthetic pathway of *trypanosoma brucei*. *PLoS One* 16, e0244699. <https://doi.org/10.1371/journal.pone.0244699>.
- Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A, et al. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589. <https://doi.org/10.1038/s41586-021-03819-2>.
- Kajiwara K, Muneoka T, Watanabe Y, Karashima T, Kitagaki H, Funato K (2012). Perturbation of sphingolipid metabolism induces endoplasmic reticulum stress-mediated mitochondrial apoptosis in budding yeast. *Mol Microbiol* 86, 1246–1261. <https://doi.org/10.1111/mmi.12056>.
- Kajiwara K, Watanabe R, Pichler H, Ihara K, Murakami S, Riezman H, Funato K (2008). Yeast ARV1 is required for efficient delivery of an early GPI intermediate to the first mannosyltransferase during GPI assembly and controls lipid flow from the endoplasmic reticulum. *Mol Biol Cell* 19, 2069–2082. <https://doi.org/10.1091/mbc.e07-08-0740>.
- Kamate M, Basavanagowda T (2023). ARV1 gene: A novel cause of autosomal recessive cerebellar ataxia with elevated alpha fetoprotein. *Cerebellum*. <https://doi.org/10.1007/s12311-023-01606-5>.
- Karabinos A, Hyblova M, Eckertova M, Tomkova E, Schwartzova D, Luckanovicova N, Magyarova G, Minarik G (2022). Dilated cardiomyopathy is a part of the ARV1-associated phenotype: A case report. *J Med Case Rep* 16, 98. <https://doi.org/10.1186/s13256-022-03291-0>.
- Ko J, Park H, Heo L, Seok C (2012). GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Res* 40, W294–W297. <https://doi.org/10.1093/nar/gks493>.
- Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS, Walter P (2009). An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 325, 477–481. <https://doi.org/10.1126/science.1175088>.
- Lagor WR, Tong F, Jarrett KE, Lin W, Conlon DM, Smith M, Wang MY, Yenilmez BO, McCoy MG, Fields DW, et al. (2015). Deletion of murine Arv1 results in a lean phenotype with increased energy expenditure. *Nutr Diabetes* 5, e181. <https://doi.org/10.1038/nutd.2015.32>.
- Latterich M, Schekman R (1994). The karyogamy gene KAR2 and novel proteins are required for ER-membrane fusion. *Cell* 78, 87–98. [https://doi.org/10.1016/0092-8674\(94\)90575-4](https://doi.org/10.1016/0092-8674(94)90575-4).
- Liscum L, Underwood KW (1995). Intracellular cholesterol transport and compartmentation. *J Biol Chem* 270, 15443–15446. <https://doi.org/10.1074/jbc.270.26.15443>.
- Liu YS, Wang Y, Zhou X, Zhang L, Yang G, Gao XD, Murakami Y, Fujita M, Kinoshita T (2023). Accumulated precursors of specific GPI-anchored proteins upregulate GPI biosynthesis with ARV1. *J Cell Biol* 222. <https://doi.org/10.1083/jcb.202208159>.
- Lopez-Marques RL (2021). Lipid flippases in polarized growth. *Curr Genet* 67, 255–262. <https://doi.org/10.1007/s00294-020-01145-0>.
- Lyons JA, Timcenko M, Dieudonne T, Lenoir G, Nissen P (2020). P4-ATPases: How an old dog learnt new tricks - structure and mechanism of lipid flippases. *Curr Opin Struct Biol* 63, 65–73. <https://doi.org/10.1016/j.sbi.2020.04.001>.
- Malathi K, Higaki K, Tinkelenberg AH, Balderes DA, Almanzar-Paramio D, Wilcox LJ, Erdeniz N, Redican F, Padamsee M, Liu Y, et al. (2004). Mutagenesis of the putative sterol-sensing domain of yeast niemann pick C-related protein reveals a primordial role in subcellular sphingolipid distribution. *J Cell Biol* 164, 547–556. <https://doi.org/10.1083/jcb.200310046>.

- McCourt P, Liu HY, Parker JE, Gallo-Ebert C, Donigan M, Bata A, Giordano C, Kelly SL, Nickels JT Jr (2016). Proper sterol distribution is required for *Candida albicans* hyphal formation and virulence. *G3 (Bethesda)* 6, 3455–3465. <https://doi.org/10.1534/g3.116.033969>.
- Montigny C, Lyons J, Champeil P, Nissen P, Lenoir G (2016). On the molecular mechanism of flippase- and scramblase-mediated phospholipid transport. *Biochim Biophys Acta* 1861, 767–783. <https://doi.org/10.1016/j.bbaliip.2015.12.020>.
- Muallem S, Chung WY, Jha A, Ahuja M (2017). Lipids at membrane contact sites: Cell signaling and ion transport. *EMBO Rep* 18, 1893–1904. <https://doi.org/10.15252/embr.201744331>.
- Murley A, Sarsam RD, Toulmay A, Yamada J, Prinz WA, Nunnari J (2015). Ltc1 is an ER-localized sterol transporter and a component of ER-mitochondria and ER-vacuole contacts. *J Cell Biol* 209, 539–548. <https://doi.org/10.1083/jcb.201502033>.
- Okai H, Ikema R, Nakamura H, Kato M, Araki M, Mizuno A, Ikeda A, Renbaum P, Segel R, Funato K (2020). Cold-sensitive phenotypes of a yeast null mutant of ARV1 support its role as a GPI flippase. *FEBS Lett* 594, 2431–2439. <https://doi.org/10.1002/1873-3468.13843>.
- Palmer EE, Jarrett KE, Sachdev RK, Al Zahrani F, Hashem MO, Ibrahim N, Sampaio H, Kandula T, Macintosh R, Gupta R, et al. (2016). Neuronal deficiency of ARV1 causes an autosomal recessive epileptic encephalopathy. *Hum Mol Genet* 25, 3042–3054. <https://doi.org/10.1093/hmg/ddw157>.
- Perfect JR (2017). The antifungal pipeline: A reality check. *Nat Rev Drug Discov* 16, 603–616. <https://doi.org/10.1038/nrd.2017.46>.
- Ridgway ND, Zhao K (2018). Cholesterol transfer at endosomal-organelle membrane contact sites. *Curr Opin Lipidol* 29, 212–217. <https://doi.org/10.1097/MOL.0000000000000506>.
- Ristovski M, Farhat D, Bancud SEM, Lee JY (2021). Lipid transporters beam signals from cell membranes. *Membranes (Basel)* 11. <https://doi.org/10.3390/membranes11080562>.
- Ruggles KV, Garbarino J, Liu Y, Moon J, Schneider K, Henneberry A, Billheimer J, Millar JS, Marchadier D, Valasek MA, et al. (2014). A functional, genome-wide evaluation of liposensitive yeast identifies the “ARE2 required for viability” (ARV1) gene product as a major component of eukaryotic fatty acid resistance. *J Biol Chem* 289, 4417–4431. <https://doi.org/10.1074/jbc.M113.515197>.
- Salian S, Scala M, Nguyen TTM, Severino M, Accogli A, Amadori E, Torella A, Pinelli M, Hudson B, Boothe M, et al. (2021). Epileptic encephalopathy caused by ARV1 deficiency: Refinement of the genotype-phenotype spectrum and functional impact on GPI-anchored proteins. *Clin Genet* 100, 607–614. <https://doi.org/10.1111/cge.14033>.
- Scorrano L, De Matteis MA, Emr S, Giordano F, Hajnoczky G, Kornmann B, Lackner LL, Levine TP, Pellegrini L, Reinisch K, et al. (2019). Coming together to define membrane contact sites. *Nat Commun* 10, 1287. <https://doi.org/10.1038/s41467-019-09253-3>.
- Segel R, Aran A, Gulsuner S, Nakamura H, Rosen T, Walsh T, Denda H, Zeligson S, Eto K, Beerl R, et al. (2020). A defect in GPI synthesis as a suggested mechanism for the role of ARV1 in intellectual disability and seizures. *Neurogenetics* 21, 259–267. <https://doi.org/10.1007/s10048-020-00615-4>.
- Shechtman CF, Henneberry AL, Seimon TA, Tinkelenberg AH, Wilcox LJ, Lee E, Fazlollahi M, Munkacsı AB, Bussemaker HJ, Tabas I, Sturley SL (2011). Loss of subcellular lipid transport due to ARV1 deficiency disrupts organelle homeostasis and activates the unfolded protein response. *J Biol Chem* 286, 11951–11959. <https://doi.org/10.1074/jbc.M110.215038>.
- Swain E, Stukej J, McDonough V, Germann M, Liu Y, Sturley SL, Nickels JT Jr (2002). Yeast cells lacking the ARV1 gene harbor defects in sphingolipid metabolism. Complementation by human ARV1. *J Biol Chem* 277, 36152–36160. <https://doi.org/10.1074/jbc.M206624200>.
- Tinkelenberg AH, Liu Y, Alcantara F, Khan S, Guo Z, Bard M, Sturley SL (2000). Mutations in yeast ARV1 alter intracellular sterol distribution and are complemented by human ARV1. *J Biol Chem* 275, 40667–40670. <https://doi.org/10.1074/jbc.C000710200>.
- Tong F, Billheimer J, Shechtman CF, Liu Y, Crooke R, Graham M, Cohen DE, Sturley SL, Rader DJ (2010). Decreased expression of ARV1 results in cholesterol retention in the endoplasmic reticulum and abnormal bile acid metabolism. *J Biol Chem* 285, 33632–33641. <https://doi.org/10.1074/jbc.M110.165761>.
- Toulmay A, Whittle FB, Yang J, Bai X, Diarra J, Banerjee S, Levine TP, Golden A, Prinz WA (2022). Vps13-like proteins provide phosphatidylethanolamine for GPI anchor synthesis in the ER. *J Cell Biol* 221. <https://doi.org/10.1083/jcb.202111095>.
- Um JW, Ko J (2017). Neural glycosylphosphatidylinositol-anchored proteins in synaptic specification. *Trends Cell Biol* 27, 931–945. <https://doi.org/10.1016/j.tcb.2017.06.007>.
- Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, Yuan D, Stroe O, Wood G, Laydon A, et al. (2022). AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Res* 50, D439–D444. <https://doi.org/10.1093/nar/gkab1061>.
- Verstreken P, Ohyama T, Haueter C, Habets RL, Lin YQ, Swan LE, Ly CV, Venken KJ, De Camilli P, Bellen HJ (2009). Tweek, an evolutionarily conserved protein, is required for synaptic vesicle recycling. *Neuron* 63, 203–215. <https://doi.org/10.1016/j.neuron.2009.06.017>.
- Villasmil ML, Ansbach A, Nickels JT Jr (2011). The putative lipid transporter, Arv1, is required for activating pheromone-induced MAP kinase signaling in *Saccharomyces cerevisiae*. *Genetics* 187, 455–465. <https://doi.org/10.1534/genetics.110.120725>.
- Villasmil ML, Barbosa AD, Cunningham JL, Siniossoglou S, Nickels JT Jr (2020). An Erg11 lanosterol 14- α -demethylase-Arv1 complex is required for *Candida albicans* virulence. *PLoS One* 15, e0235746. <https://doi.org/10.1371/journal.pone.0235746>.
- Wakana Y, Hayashi K, Nemoto T, Watanabe C, Taoka M, Angulo-Capel J, Garcia-Parajo MF, Kumata H, Umemura T, Inoue H, et al. (2021). The ER cholesterol sensor SCAP promotes CARTS biogenesis at ER-Golgi membrane contact sites. *J Cell Biol* 220. <https://doi.org/10.1083/jcb.202002150>.
- Yang H, Bard M, Bruner DA, Gleeson A, Deckelbaum RJ, Aljinovic G, Pohl TM, Rothstein R, Sturley SL (1996). Sterol esterification in yeast: A two-gene process. *Science* 272, 1353–1356. <https://doi.org/10.1126/science.272.5266.1353>.
- Yu C, Kennedy NJ, Chang CC, Rothblatt JA (1996). Molecular cloning and characterization of two isoforms of *Saccharomyces cerevisiae* acyl-CoA:sterol acyltransferase. *J Biol Chem* 271, 24157–24163. <https://doi.org/10.1074/jbc.271.39.24157>