Review

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Update on VAP, a ubiquitous signpost for the ER

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Abstract: The small protein family of VAMP-associated proteins (VAPs) have the unique position in cell biology as intracellular signposts for the Endoplasmic Reticulum (ER). VAP is recognised by a wide range of other proteins that use it to target the ER, either simply being recruited from the cytoplasm, or being recruited from separate organelles. The latter process makes VAP a component of many bridges between the ER and other compartments at membrane contact sites. The fundamental observations that identify VAP as the ER signpost have largely remained unchanged for over two decades. This review will describe how increased understanding of the special role of VAP in recent years has led to new discoveries: what constitutes the VAP family, how proteins bind to VAP, and which cellular functions connect to the ER using VAP. It will also describe the pitfalls that have led to difficulties determining how some proteins bind VAP and suggest some possibilities for future research.

Keywords: cyclic AMP (cAMP); FFAT motif; lipid transfer protein (LTP); non-vesicular transport; nucleolus; short linear motif (SLiM)

1 Introduction

Since 2010 there has been an explosion of interest in membrane contact sites, as this three word phrase has become the accepted way to describe places where intracellular compartments contact each other (Figure 1). The clearest cellular function for these sites is direct inter-organelle transfer, both of material, mainly ions such as calcium and iron or lipids, and of information, for example by post-translational modifications (Scorrano et al. 2019, Cali et al. 2025). VAP (standing for VAMP-associated protein, where VAMP is vesicle-associated membrane protein (Skehel et al. 1995)) decorates the cytoplasmic face of the endoplasmic reticulum (ER) (Kagiwada et al. 1998). Given that many of its interactors are located in compartments outside the ER, VAP is a major scaffold of complexes that bridge across cytoplasmic gaps (Olkkonen and Levine 2004).

Why VAP, why now? The 20,000 human protein coding genes create approximately one million protein-protein interactions (Wan et al. 2015), and many proteins, including VAP, participate in this scaffolding and do nothing more. So why devote a whole article to the interactions of VAP? The reason is that VAP is in a unique category: it is the only organelle signpost for the ER. On that basis, if a protein that performs a specific function binds VAP, that is a sure sign that the ER is directly involved in that function. Finding VAP interactors in compartments outside the ER therefore reveals new aspects of how the ER connects with other compartments. This makes studying VAP a major way to learn fundamental cell biology. Indeed, the time-line of publications suggests that the surge of research in membrane contact sites originated from prior studies of VAP over the preceding 5–10 years (Figure 1).

The largest single set of interactors of VAP bind its N-terminal motile sperm protein (MSP) domain using a short linear motif (SLiM) called a 'FFAT' motif, standing for two phenylalanines (FF) in an acidic tract, which is described in detail in Section B. SLiMs are numerous (Tompa et al. 2014), but because their binding affinities are low, xtheir interactions have a low discovery rate (approximately 5 %) (Davey et al. 2017). This explains how, after an initial phase of research into SLiMs that interact strongly with VAP, the cutting edge of VAP research has gradually moved on to studying increasingly weak interactions.

Since our last review of VAP in 2016, several highly informative reviews of VAP have been published (Kamemura and Chihara 2019, Dudas et al. 2021, James and Kehlenbach 2021, Kors et al. 2022a, Subra et al. 2023a, Blair et al. 2025). This article builds on these examples to examine developments since 2016 in three main sections: firstly, about VAP itself, secondly about the group of $FFAT^{+ve}$ proteins that bind VAP, and finally addressing functions that involve VAP.

2 The many faces of VAP

While many aspects of the biology of VAP are understudied, for example little is known about its transcriptional and

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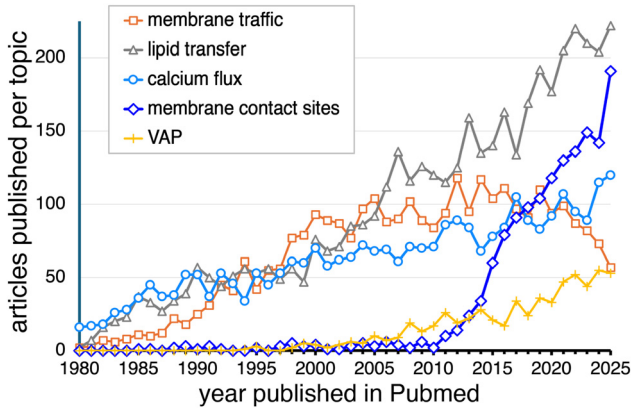

Figure 1: Publications about membrane contact sites and related terms. Number of publications in PubMed by year positive for the search terms membrane contact sites and VAP, compared with three phrases relating in general to the topic of this review: membrane traffic, lipid transfer, calcium flux. Searches were carried out in mid-July 2025, with the 2025 figure extrapolated to cover 365 days. To improve coverage, additional phrases were included with each of the four phrases: membrane contact site, intracellular traffic, lipid traffic and calcium transfer, respectively. VAP searches covered all species and the final list of 618 results was curated by hand to exclude where the same acronym applies to different topics.

post-translational regulation, its biochemistry and structure are well known. VAP is a tail-anchored protein (~250 aa) with three main regions: an N-terminal β-sandwich MSP domain (~130 aa); a linker (~100 aa) that contains an intrinsically disordered region (IDR), sometimes separated into two segments by a helix that dimerises as a coiled-coil; and a C-terminal single transmembrane helix (TMH) with often less than five residues on the lumenal side of the ER membrane (Figure 2A). Peripheral membrane proteins are typically recruited to organelles by binding so-called organelle signposts (Behnia and Munro 2005). VAP is the only organelle signpost in the secretory pathway with a TMH. Other compartments use a combination of Rabs and phosphoinositide lipids as their signposts, which are recruited directly from the cytoplasm or synthesised *in situ*, avoiding the issue of trafficking a signpost with a TMH for these compartments through the ER and other early compartments, which would thus be mislabelled. One other ER signpost utilised much less often than VAP is the presence of phosphatidic acid-rich patches, which are recognised either by amphipathic helices (Olmos et al. 2016, Hofbauer et al. 2018, Thaller et al. 2021) or by a phosphatidic acid-specific globular domain (Ferreira et al. 2025, House et al. 2025). The dominance of VAP as the ER signpost is shown by the GTPase exchange factor for Rab18, a master regulator of both the ER's structure and its links to lipid droplets, being recruited by VAP (Gerondopoulos et al. 2014).

2.1 One more VAP

Most model organisms have several VAP isoforms: human 3, fly 2, yeast 2, *Arabidopsis* 10. In humans, the two most studied VAP isoforms are VAPA and VAPB, which are closely related (75 % overall homology), particularly in the MSP domains (82 % identical, Figure 2A). While a tissue culture cell line without VAPA/B is viable (Dong et al. 2016), making a useful experimental tool (Anwar et al. 2022), in vertebrate embryogenesis VAPA, but not VAPB, is essential for life (McCune et al. 2017). This may be because VAPA is more abundant across the whole human organism (see PaxDb: Protein Abundance Database; https://pax-db.org/protein). Rare familial mutations in VAPB (but not in VAPA) are associated with human neuronal diseases, particularly motor neurone disease (aka Amyotrophic Lateral Sclerosis, ALS) (Chen et al. 2010), this being linked to VAPB's greater propensity to form aggregates, which is aggravated by the missense mutations in its MSP domain (Nakamichi et al. 2011, Subra et al. 2023c). Apart from pathology, efforts to distinguish between the physiological of functions of VAPA and VAPB are still inconclusive.

The new, third VAP is MOSPD2, standing for motile sperm domain-containing protein–2. This is larger than the others because of an N-terminal cellular-retinaldehyde binding protein (CRAL) domain that likely transfers phospholipids, though its specific cargo is unknown. The CRAL domain targets MOSPD2 to the surface of lipid droplets (Zouiouich et al. 2022). The C-terminus of MOSPD2 resembles VAPA/VAPB except that its linker is much shorter (Figure 2A), so its interactors must approach nearer to the ER surface than those of VAPA/B. MOSPD2 is included as a genuine VAP because it binds FFAT motifs *in vitro* and *in vivo*, even though its MSP domain shows only 28 % sequence identity to VAPA (Figure 2A) (Di Mattia et al. 2018, Di Mattia et al. 2020). Homologues containing the combination of CRAL and MSP domains are mainly found in animals (Di Mattia et al. 2020). Among the few non-animal sequences (UniProt has seven), the plant proteins contain one domain closely related to plant CRAL domains and one domain related to plant MSPs, ruling out recent horizontal gene transfer from animals. This suggests strong evolutionary pressure to co-express the two domains in one protein through convergent evolution.

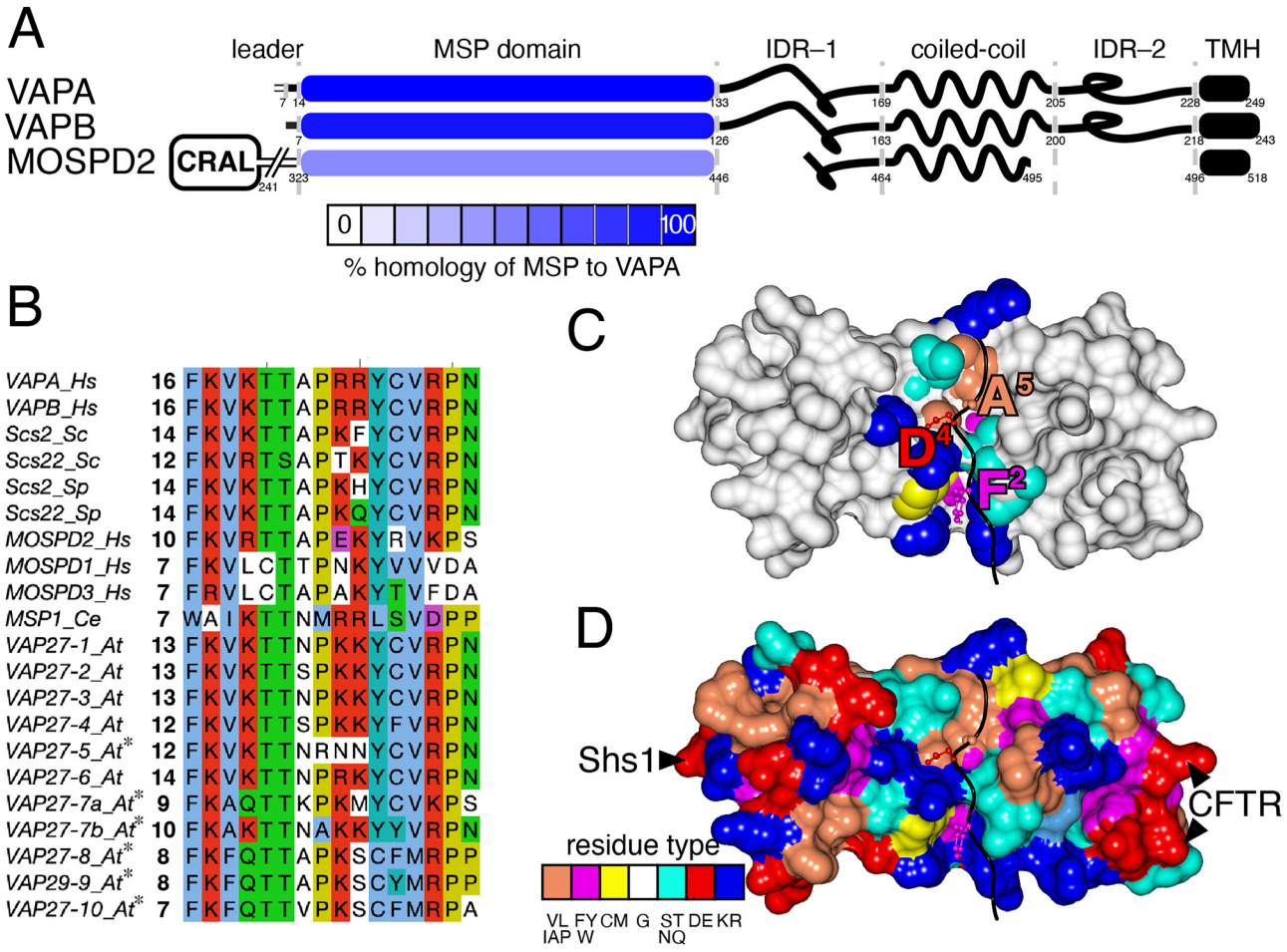

Figure 2: Conservation of key elements in VAP. (A) Alignment of three human VAPs: VAPA, VAPB and MOSPD2. The six main regions are: leader, motile sperm protein (MSP) domain, intrinsically disordered region (IDR)-1, coiled coil, IDR–2 and the transmembrane helix (TMH). The MSP domains of VAPB and MOSPD2 have been coloured to indicate the degree of homology (identity and similarity) to their counterparts in VAPA (scale as indicated). MOSPD2 starts with a cellular-retinaldehyde binding protein (CRAL) domain followed by an IDR linker (not shown). The C-terminus of MOSPD2, as visualised in the AlphaFold database, is a helix that runs directly into the TMH without break, indicating lack of IDR-2. Note that VAPA has a seven residue disordered extension at its N-terminus with two documented phospho-sites that may have a regulatory function. (B) Alignment of 19 VAP consensus sequences from model organisms (human *Hs*, budding yeast *Sc*, fission yeast *Sp* and *A. thaliana At*, as numbered previously (Wang et al. 2016)) along with human non-VAP proteins MOSPD1/3, and MSP1 in *C. elegans*. The number of residues identical to VAPA/VAPB is given for each sequence; asterisks indicate *At* VAPs without TMHs. Three plant MSP proteins with low numbers of identities form a single clade and they diverge not only in sequence but also in localising to the plasma membrane (Wang et al. 2016). Residues are coloured according to the CLUSTAL scheme. (C) Surface of the MSP domain of human VAPA from crystal structure 1z9o; only the sidechains that interact with FFAT are coloured (Neefjes and Cabukusta 2021). The key residues of the interacting FFAT motif from ORP1 are represented as balls/sticks: F2 in a large pocket, A5 in a small pocket, and D4 interacting with a conserved lysine (Kaiser et al. 2005). (D) same view as (C), but with the whole surface coloured, and arrowheads indicating residues that interact with non-FFAT partners (see text). Key indicates colour scheme of sidechains in (C) and (D).

Humans express two other MSP domain proteins: MOSPD1 and MOSPD3. These paralogues lack the majority of the basic residues conserved in VAPs, and correspondingly the 'FFNT' motif, standing for two 'phenylalanines [FF] in a neutral tract', which is common to their interactors, lacks many of the negative charges characteristic of FFAT motifs (Cabukusta et al. 2020). Because MOSPD1 and MOSPD3 do not bind FFAT motifs, they are excluded from the definition of VAP used here. Among features that distinguish VAPs from other MSP domain proteins such as MOSPD1/3, one possibility is the previously identified so-called 'VAP consensus sequence', 16 contiguous residues that are identical in VAPA and VAPB which interact with the most conserved residues of FFAT motifs (Loewen and Levine 2005, Lev et al. 2008,

Johnson et al. 2019, Dudas et al. 2021). Some VAPs in both yeast and plants have as many as 14 identities, showing conservation of the consensus across eukaryote evolution. While MOSPD2 has 10 identities, MOSPD1/3 have seven identities, the same number as nematode MSP1, which is the archetypal MSP protein that has no VAP-like role, as previously explained (Figure 2B) (Loewen and Levine 2005). The protists *Trichomonas* and *Giardia*, have MSP proteins with fewer than seven identities (six and two respectively), supporting the idea that these organisms have no VAP (Kodama et al. 2025). The consensus is a feature by which MSP domains can be assessed. For example, among the 10 VAPs in *Arabidopsis*, seven (VAP27-1 to 7) have between 10 and 14 identities, so are likely to be true VAPs, while three (VAP27-8, VAP27-10 and VAP29-9) have only seven or eight identities, so they may not function as VAPs, having a different role (Figure 2B).

2.2 Full-length VAP is on the ER

VAP localises to the ER, as shown by both N-terminal (cytoplasmic) and C-terminal (lumenal) tagging with GFP in different species, including animals (Kaiser et al. 2005, Gkogkas et al. 2008), fungi (Loewen et al. 2003, Zhang et al. 2012, Chao et al. 2014) and plants (Wang et al. 2014). Antibody staining of endogenous or tagged VAP agrees with this (Kagiwada et al. 1998, Foster et al. 2000, Wyles et al. 2002, Teuling et al. 2007, Prosser et al. 2008, Gonzalez Bolivar et al. 2024). One might expect enrichment of VAP at sites of contact with other organelles, and this has now been demonstrated in living cells. However, the effect is quite subtle, and it can only be seen with advanced single molecule super-resolution microscopy (Obara et al. 2024). Binding of a VAP molecule to a partner on another organelle *in trans* confines it in a single zone of contact for half a second on average, which reduces its diffusion rate.

Despite predominant targeting of VAP to the ER, occasional studies suggest other locations. One early paper showed VAP leaving the ER to reach the ER-Golgi intermediate compartment (Soussan et al. 1999). This might now be reinterpreted on the basis that VAP binds Golgi proteins *in trans*, and is thus enriched on ER elements interacting with Golgi membranes, especially the *trans*-ER cisterna that interdigitates into the Golgi stack (Mogelsvang et al. 2004, Wakana et al. 2015). Other observations of VAP outside the ER might have originated from unappreciated ER adhering to the organelle in question. Examples include VAP found in neuromuscular junctions (Pennetta et al. 2002) or in membranes immunopurified by anti-VAMP2 (Foster et al. 2000) or close to tight junctions in epithelial cells (Lapierre et al. 1999). All these might derive from ER contacting precisely those sites (Fowler and O'Sullivan 2016, Lucken-Ardjomande Hasler et al. 2020, Chung et al. 2022).

Even if these multiple non-ER locations for VAP were revised, there remains one process by which VAP leaves the ER. In flies and worms, VAP is secreted in a cleaved, extracellular form that has distal targets (Tsuda et al. 2008, Zein-Sabatto et al. 2021). Paralleling this, human VAP is processed for atypical secretion, with cleaved VAP present in both serum (Tsuda et al. 2008) and cerebrospinal fluid (Deidda et al. 2014). The role of extracellular VAP is reviewed in detail elsewhere (Kamemura and Chihara 2019, Kamemura et al. 2024).

2.3 MSP domain interactions go beyond FFAT

The MSP domain is the most conserved part of VAP, and its most highly conserved side-chains form two adjacent pockets in an electro-positive face of VAP. These accommodate side-chains of two of the three conserved residues in FFAT motifs (Figure 2C) (Kaiser et al. 2005). The FFAT motif has an extended conformation crossing over the MSP domain, so it can only bind VAP when not folded into a helix or β-strand. Prior to the final, fully docked conformation of a productive VAP-FFAT interaction there is a non-specific charge interaction between the electro-positive face of VAP and the anionic atoms that flank the motif. This early non-specific stage allows time for the specific interactions to fall into place in a process likened to "fly casting" (Furuita et al. 2010).

The MSP domain has other conserved regions, so it may have other, non-FFAT interactions. Evidence exists for two such interactions: Shs1 in yeast requires an acidic residue at one end of the domain; the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) interacts with VAP using charged residues at the other end of the domain (Figure 2D) (Chao et al. 2014, Ernst et al. 2016). On the question of VAP-CFTR, the interaction is very well documented, but without specific molecular detail (Lim et al. 2022, Iazzi et al. 2022, Iazzi et al. 2023). A different new development is that the atypical secretory pathway for mutant CFTR (F508Δ) includes Rab11^{+ve} endosomal tubules are labelled with the FFAT^{+ve} Rab11 binding protein WDR44 (also called Rab11 bp). A general role for the ER in atypical secretion is suggested by

these endosomes running adjacent to ER tubules (Lucken-Ardjomande Hasler et al. 2020, Parolek and Burd 2024).

MSP domains have also been suggested to interact with themselves. The strongest mode of VAP dimerisation involves the coiled-coil present in the C-terminus (Di Mattia et al. 2018), and the TMHs can also dimerise (Murphy and Levine 2016). However, new results revive an old idea that MSP domains dimerise on their own (Johnson et al. 2019). This is supported by new VAP crystal structures in which MSP domains dimerise using an interface that contains residues that also have specific roles binding FFAT motifs. This suggests the possibility that the MSP domain binds either another MSP domain or a FFAT+ve partner, but not both (Shi et al. 2010, Di Mattia et al. 2020, Kodama et al. 2025).

A further set of interactions proposed for the MSP domain are a range of non-specific charge interactions between its electro-positive face and anionic molecules. One weak charge-based interaction proposed for VAP is with the anionic tail of fission yeast Pil1, which is highly concentrated in sensory infoldings on the plasma membrane called eisosomes (Ng et al. 2020). Similarly, VAP has a weak affinity for the inner leaflet of the plasma membrane (Kagiwada and Hashimoto 2007, Manford et al. 2012), which is enriched for phospholipids with anionic headgroups: phosphatidylserine, phosphatidylinositol and small pools of phosphoinositides. It is unclear whether such weak interactions are functionally relevant *in vivo*. For example, yeast VAP mutants losing multiple positive charges show a large functional defect only when a specific lysine that binds FFAT motifs (K94 in VAPA numbering) is affected (Kagiwada and Hashimoto 2007). Likewise, the ability of VAP to create extensive ER-plasma membrane contact in yeast (aka cortical ER) correlates with FFAT binding, not with charge (Manford et al. 2012). Nevertheless, positively charged VAP that cannot bind FFAT weakly generates some cortical ER in both budding yeast (Loewen et al. 2007) and fission yeast (Hoh et al. 2024), which might be explained by weak interactions, such as with anionic phospholipids.

2.4 Cytoplasmic linker

A key new development in the study of VAP is the finding that the linker determines where VAP can act (Subra et al. 2023b). The IDRs of VAPA/B add up to 16 nm to the linkers. Flexibility of the linker allows VAP to adapt to a variety of contact site configurations, as shown for contact sites recreated *in vitro* by cryo-electron tomography (de la Mora et al. 2021). This has been extended to compare two membrane contact sites *in vivo* with different longevity: the ER-mitochondrial interface, which is slow to turn over compared to less stable ER-Golgi contact sites (Mesmin et al. 2017). While wildtype VAPA functions at both locations, a version lacking the linker IDR cannot access the ER-Golgi interface (Subra et al. 2023b). The size of the inter-organelle gap is unlikely to be the issue, since increasing the linker's length but keeping it rigid does not rescue function. This implies that IDR flexibility is a key property, allowing the MSP to move away or towards the membranes, creating flexibility within mobile inter-organelle interfaces, particularly if the intermembrane gap oscillates (Subra et al. 2023b).

In addition to their physical role, cytoplasmic IDR linkers are typically expected to be sites of post-translational modifications (Davey et al. 2017). A single modification in this region of VAP has been studied: yeast VAP (standard name Scs2) is a major SUMOylated protein, and sumoylation of the IDR alters VAP function in terms of recruiting $FFAT^{+ve}$ targets (Felberbaum et al. 2012). It is now known that Siz2, a yeast SUMO ligase, re-initiates DNA-nuclear envelope binding late in mitosis by binding VAP on the inner nuclear envelope in two ways: (i) Siz2 has a FFAT motif; (ii) Siz2 has two SUMO-interaction motifs that reinforce binding after sumoylation (Ptak et al. 2021). VAP linkers are also the sites of many phosphorylations; determining their significance will be an interesting avenue for future research (Kodama et al. 2025).

2.5 Transmembrane helix (TMH)

Many proteins that bind VAP are also in the ER, and the TMH is their site of interaction. This applies in particular to the conserved interaction of VAP with the PI 4-phosphatase Sac1 (Forrest et al. 2013, Wakana et al. 2015). Other interactors with the TMH include the antiviral proteins IFITM1-3 (Amini-Bavil-Olyaee et al. 2013) and viperin (Ghosh et al. 2020). The antiviral effect of IFITMs, which are endosomal residents, derives in part from them inhibiting the VAP-OSBP interaction, which in turn alters cholesterol distribution and thus inhibits viral entry. A future development will be to explore where VAP encounters IFITMs, since one partner must relocalise for the TMHs to interact. Possibly similar to this is the interaction of VAP with the plasma membrane channel potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel-2 (HCN2) (Silbernagel et al. 2018), which is mediated by the TMH. One possible general mechanism might be that VAP interacts with TMHs when they are first translocated into the ER, which may affect both VAP and the partner in ways not yet studied.

The TMH is one possible source of a functional difference between VAPA and VAPB that goes beyond differential expression (Subra et al. 2023b,c). TMH partners differ markedly, with VAPA binding more strongly to both Sac1 and IFITM3 in the examples above. This might derive from protein interaction specificity, but another possibility is that the TMHs interact with membrane lipids differently. This has been proposed to explain how VAPA functions at both ER-Golgi and ER-mitochondrial interfaces, while VAPB only functions at the ER-Golgi in parallel experiments (Subra et al. 2023b). The two VAPs have marginally different hydrophobic segments (15 and 16 residues in VAPA/VAPB respectively), but the helix that includes the hydrophobic segment (as predicted by AlphaFold) has a whole extra turn (+4 residues) in VAPB compared to VAPA (Subra et al. 2023c). This might allow differential access to ER subdomains of specific membrane thickness, similar to TMHs being sorted to the membrane with a specific thickness in the secretory pathway (Sharpe et al. 2010). The concentration of cholesterol, which is high where ER contacts mitochondria, is one possible factor here (Hayashi et al. 2009).

3 The interactions of VAP

3.1 The VAPome

Both VAPA and VAPB have many protein interaction partners, and they are among the proteins with the highest number of physical interactions documented in high-throughput experiments (Huttlin et al. 2015). Among this so-called 'VAPome', interactors detected by co-immunoprecipitation tend to have FFAT motifs; in contrast, interactors detected by proximity ligation tend to be ER residents that mostly interact via their TMH (James and Kehlenbach 2021). Most of the experiments do not specify whether binding is direct, so excluding indirect binding is a major question. The breadth of indirect binding is illustrated by new results showing that VAP's interactions with VAMP and other SNAREs, the interaction for which VAP is named (Skehel et al. 1995, Weir et al. 2001), depend on the presence of third components such as OSBP-related proteins (ORPs), which bring VAP and SNAREs together (Weber-Boyvat et al. 2021).

3.2 FFAT motifs, residue by residue

Approximately 50 % of VAPs interactors either have a sequence resembling a FFAT motif or form a tight complex with such a protein (Huttlin et al. 2015, Murphy and Levine 2016). This variable resemblance to the initially defined FFAT motif means that now there is no precise definition of FFAT motifs or FFAT-like motifs. Instead there are experimental demonstrations that sequences form functionally relevant interactions paralleling the initially defined VAP-ORP interaction. Finding unifying rules to predict new motifs remains a significant topic: one can predict that the definition will improve still further as increasingly divergent FFAT-like sequences are shown to bind VAP in the future.

The best-known sequence features of the motif focus on the three positions 2/4/5 (Figure 2C). This is supported by crystallographic and NMR studies that focus on the same residues. All flanking residues are enriched for acidic side-chains (Figure 3A). New analysis shows that this preference extends not only to upstream residues but also downstream residues, particularly for binding to MOSPD2 (Di Mattia et al. 2020).

The following paragraphs describe current ideas for the key residues across the motif. A common theme is that each residue provides a site at which affinity for VAP can be modulated up and down.

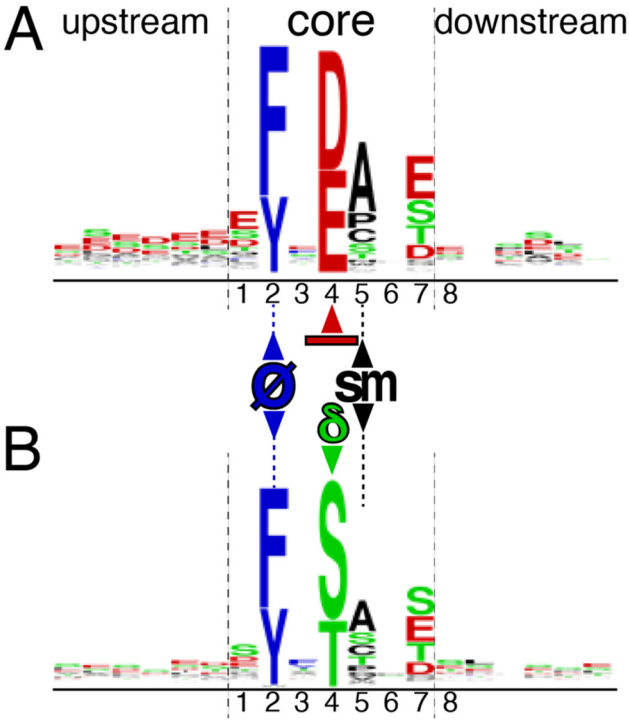

Figure 3: FFAT motifs up close. (A) Logo of 94 FFAT motifs compared to the originally defined EFFDA-E sequence and indicating the core of seven residues with flanks upstream and downstream. (B) Logo of 110 phospho-FFAT motifs, with S and T replacing D and E at position 4. The most highly conserved residues are indicated: Ø is a bulky hydrophobic at position 2, – is a negatively charged residue 4, *δ* is a polar residue four and sm is a small side chain at position 5). Logos reproduced from Di Mattia et al. 2020.

Residue 2: The original definition of FFAT motifs emphasised that residue two is a phenylalanine (F2), or the conservative substitution tyrosine (Y2), for example in ORP4 the paralogue of OSBP (Loewen et al. 2003, Wyles et al. 2007, Mikitova and Levine 2012). Now tryptophan (W2) has been identified as another semi-conservative substitution in fungal ORPs (Slee and Levine 2019) and in human LSG1 (Di Mattia et al. 2020, Sutjita et al. 2024). A question that remains unaddressed is whether Y2 motifs use tyrosine phosphorylation to switch off ER targeting.

Residue 3: The initial finding of F and Y at this position led the initial definition of the motif as having '2 phenylalanines…', but this was shown to be wrong very early on, with histidine (H3) in the OSBP homologue in *C. elegans* (Loewen et al. 2003) followed by bases and acids (E3/K3 respectively) (Saita et al. 2009, Baron et al. 2014). While large alignments show little specificity at this position (Figure 3) (Di Mattia et al. 2020), F3A substitution reduces ER targeting substantially (Loewen et al. 2003). Although the effect on affinity has still not been defined, this may explain how small residues such as glycine (G) and alanine (A) are uncommon or associated with weak motifs, for example the weaker of two in Snx2 (Dong et al. 2016).

Residue 4: While the anionic side-chain aspartate dominates here (D4), many confirmed VAP interactors have serine (S4) or threonine (T4). Phospho-S and phospho-T resemble acidic residues at many positions across a FFAT motif, with phosphorylation of position four being studied most, which has led to these being the only motifs with the name phospho-FFAT (Alpy et al. 2013, McCune et al. 2017, Johnson et al. 2018). In general, S4 is accompanied by S (or T) at most other positions in place of D/E throughout the core and flanks, which indicates that active motifs are created by multiple phosphorylations (Figure 3B). A good example of this is the motif in Kv2.1 (aka KCNB1), the motif of which contains only one acidic residue, one threonine and six serines: three of these are essential phospho-S sites (Lim et al. 2000). Detailed structural studies of a phospho-FFAT showed that the phospho-S4 side-chain, being longer than D4, makes a charge interaction with a conserved lysine (K50 in VAPA) that is not possible for D4 (Di Mattia et al. 2020). The resulting shift of the Cα backbone for phospho-S4 towards K50 changes the mode of binding of phospho-FFATs compared to conventional motifs, affecting residue 5. This can be compensated by proline replacing alanine in that position, because the rigidity of P5 re-positions phospho-S4 closer to K50 (Di Mattia et al. 2020). The structural effect of a long side chain at position 4 may explain the relative exclusion of glutamate (with its even longer acidic side-chain) from this position (Mikitova and Levine 2012, Hantan et al. 2014).

Residue 5: This position is dominated by alanine (A), the small side-chain of which fits into a small hydrophobic pocket on VAP (Kaiser et al. 2005). Other residues with small side-chains found in this position include serine, threonine, cysteine (C) and proline (P). Regarding P5: in conventional FFATs, this partially inhibits the interaction with VAP (Loewen et al. 2003), but as explained above, P5 aids binding for phospho-FFATs.

Phosphorylable residues (S or T) at position five are significant because acidic substitutions here, which presumably mimic phospho-S and phospho-T, strongly inhibit the VAP-FFAT interaction (Mikitova and Levine 2012, Ptak et al. 2021, Kors et al. 2022b). Cysteine is a significant common variant at this position (Figure 3), strongly hinting that C5 is reversibly modified by reactive oxygen species (ROS) to become the larger, more polar sulphenic acid (Cremers and Jakob 2013). It remains to be tested if this reduces the affinity for VAP, but if it is the case C5 motifs in proteins such as Kv2.1, LSG1 and Rab3GAP1 may be inhibited by redox signalling at multiple membrane contact sites (Booth et al. 2016, Kors et al. 2022a).

3.3 Mutagenesis to test VAP-FFAT motifs

The general approach to obtain experimental evidence for VAP-FFAT interactions is to specifically mutate each partner (Loewen et al. 2003, Kawano et al. 2006). On the VAP side, usually residues that engage F2 are mutated, either K94D and M96D (VAPA numbering), or T54A (Chao et al. 2014, Brinkmann et al. 2024), sometimes combined with T53A (Suzuki et al. 2009, Manford et al. 2012, Willet et al. 2024). While mutating VAP confirms the involvement of a FFAT motif, it is necessary to identify the correct functional $FFAT^{+ve}$ partner. This is achieved by mutating the proposed FFAT motif. Most subtly, alanine is substituted at position 2 (sometimes also position 3) or position 4, or an acidic side-chain is substituted into position 5 (Ptak et al. 2021, Kors et al. 2022b).

3.4 Predicting the full extent of VAP-FFAT interactions

The foremost criterion for predicting a FFAT motif is its sequence. Considering which sequences might bind VAP, we

developed a position weighted matrix (PWM) that assigns a positive score for suboptimal elements, with the canonical motif EFFDAxE scoring zero, and we suggested that a sequence with up to 2.5 suboptimal elements could bind VAP (Murphy and Levine 2016). We also suggested three further criteria for FFAT motifs: the motif cannot function if it is translocated across a membrane out of the cytoplasm; sequences should have no secondary structure, forming IDRs but not helices or β-strands; and the motif should show conservation across orthologues that focusses on residues 2/4/5 (Murphy and Levine 2016). When motifs with a large number of suboptimal elements in the PWM, *i.e.* weak motifs, interact with VAP they are often are accompanied by a strong motif in the same protein. Examples of this include ORP3 (Weber-Boyvat et al. 2015) and Num1 (Chao et al. 2014, Casler et al. 2024). While it is possible that a second motif acts as a back-up to support 1:1 binding of VAP, an alternative is 2:1 binding with one protein using two motifs to bind each member of a VAP dimer simultaneously (Furuita et al. 2021, Kors et al. 2022a). Understanding this in detail will be a fruitful area for future research.

The PWM and three accompanying criteria have since been applied across whole proteomes, with partial analysis of yeast hits (Slee and Levine 2019). Motifs scoring up to one suboptimal element are rare, but the number of motifs identified rises rapidly as an increasing number of suboptimal elements is tolerated, with 3 % of all proteins in humans and yeast containing a sequence with ≤2.5 suboptimal elements. Based on statistical and biological controls, ~25 % of these are likely to be true positives (~0.8 % of the proteome), with the remainder being false positives (Slee and Levine 2019). A pilot set of yeast proteins was examined for the three other criteria, with new FFAT motifs suggested for 1/3 of them (18 motifs in 56 proteins). Only one of these has been tested experimentally: Ypr097w/Lec1 showed no obvious role for its motif or functional link to VAP, even though it is a lipid transfer protein (Castro et al. 2022).

Another observation from analysing FFAT motifs in protein databases is that the presence of motifs in any one protein family is variable. For example, across the whole ORP family approximately half are FFAT^{+ve} (Olkkonen 2015, Ye et al. 2020), even though 99 % of orthologues of OSBP are FFAT^{+ve} (Slee and Levine 2019). Similarly, motif variability in peroxisomal ACBD proteins indicates selective pressure for ER targeting across the family (Kors et al. 2024). This indicates that potential VAP interactors, including paralogues diverging from a single ancestral protein, are under selective pressure to modulate how much they target the ER.

3.5 The structural criterion may need revision

The current literature has accumulated a reasonable body of work examining VAP-FFAT interactions in depth, so it is now possible to determine if the four criteria we put forward in 2016 are still viable (Murphy and Levine 2016). Twenty five different purported FFAT motifs, especially those that diverge from the canonical motifs identified 20 years ago, can be categorised by examining their supporting evidence (Table 1). The evidence positively demonstrates a motif in less than 50 % of these instances, with two other outcomes applying to the remainder: either negative, *i.e.* unlikely to be a FFAT motif; or unclear – more evidence needed.

Where there are grey areas, questions still remain about a motif because there is a mixture of positive and negative evidence. The most interesting cases are the motifs that fold as helices and nevertheless bind VAP; these challenge the structural criterion. This particularly applies to Pma1 in fission yeast: it has a motif that relocates VAP in a knock-sideways assay, but not with a single point mutation representing F2A (Hoh et al. 2024). A second example is the human neuronal protein secernin-1 (SCRN1), the C-terminus of which co-precipitates with VAP but not the K94D/M96D mutant, and not with a point mutation representing F2A (Lindhout et al. 2019). However, the whole Pma1 motif is in a helix found in crystal structures of closely homologous budding yeast Pma1. Similarly, the whole SCRN1 motif is in a helix strongly predicted by AlphaFold2 (pLDDT>95 %). A third similar example has been suggested for the family of rRNA-methyltransferases orthologous to NSun6 in humans (Nop2 in yeast), in which a helix present in crystal structures also has statistical evidence for FFAT-like sequences ($F^2/E^4/A^5$, in animals $p = 10^{-10}$, in fungi $p = 10^{-27}$) but absent in bacterial homologues ($L^2/A^4/A^5$) (Slee and Levine 2019).

These examples represent an exciting novelty. One possibility is that they bind VAP in the helical forms found in their structural models. In that case, the large and small hydrophobic residues that interact with the large and small hydrophobic pockets on VAP (F^2 and A^5 respectively) must surely be spaced further than three residues apart, so the sequence pattern used is (by definition) wrong, indicating both flawed logic, and a need to define a specific sequence for helical motifs. Another possibility is that secondary structure

Table 1: Verification of 25 purported divergent VAP-FFAT interactions.

Line	Protein	Org.	Aa	Score	Exp	Start	Flank	Core	p4	SS	Evidence	Conclusion	Final	cat.
1	ORP2A	*At*	721	2.0	+	300	SEEDEP	SFHDTKE		c	F2A/F3A prevents Y2H with VAP	Heterologous system (Y2H) & minimal mutations	+	Positive
2	SCAR-2	*At*	2,217	2.0	+	1,757	TESEGD	DFVDALY		c	Deletion of 30 aa prevents Y2H with VAP	Many aa deleted/ mutated → lacks ultimate specificity	+?	
3	Epr1	*Sp*	380	2.5	+	361	VEISDN	EFYTASE	T4	c	F2A prevents Y2H with VAP	Heterologous system (Y2H) & minimal mutations	+	
4	Jjj1	*Sc*	590	2.0	+	478	ETEESE	SFDDTKD		c	No experiments	Untested but homologue of Epr1 (*Sp*); motif in same part	+?	
5	RdRP	CoV	568	2.5	+	114	VVEVVD	KYFDCYD		C	Motif at 555 (EFYEAMY, score 3.5): binds VAP by NMR	*in vitro* binding gold standard	+	
6	ORP3	*Hs*	887	1.0	++	450	ITDSLS	EFFDAQE		c	F2V D4V reduces binding PLUS p-motif at 161 (HFFSGST, score 4.5): HAAAAAA combined with F2V D4V inhibits binding	Examples of suboptimal motifs that interact with VAP that are accompanied by a strong motif in the same protein	+ (x2)	
7	Num1	*Sc*	2,748	2.5	+	2,134	STTTSS	MFTDALD		c	F2A inhibits binding; PLUS motif at 316 (DIFDIVI, score 5.5): VAP T54 inhibits binding		+ (x2)	
8	Kv2.1	*Hs*	858	3.5	–	590	SMSSID	SFISCAT	S4	c	*p*-FFAT score 1.0; binds VAP *in vitro* by NMR and co-IP. S Are essential phospho-sites	Examples of phospho-FFATs with promising scores on *p*-FFAT matrix formulated for these sequences, which otherwise score suboptimally	+	
9	StARD3	*Hs*	445	4.0	–	206	GALSEG	QFYSPPE	S4	c	*p*-FFAT score 2.5; binds VAP *in vitro* by co-IP		+	
10	SCRN1	*Hs*	414	3.0	–	401	PAEVGD	LFYDCVD		h	F2A prevents co-IP; other motif at 39 (VYFSAAD, score 2.0): Y2A has no effect	Despite being in helices in crystal structures and/or AlphaFold models, motifs test positive in detailed experiments. Do they unfold?	+?	
11	Pma1	*Sp*	943	2.5	+	610	NSTDIN	EYVTAQE	T4	H	Isolated motif translocates to VAP, F2A does not translocate		+?	
12	SQSTM1	*Hs*	440	5.0	–	167	RGHTKL	AFPSPFG	S4	E	VAP mutated K94D/M96D still interacts	If mutating key VAP residues has no effect, evidence for a motif is weak	–	Negative
13	CDIP1	*Hs*	208	5.0	–	186	IPCLIN	DFKDVTH		c	F2A "retained the ability to interact"	If mutating key motif residues is only partly inhibitory, evidence for a motif is weak	–	
14	PRA1	*Hs*	185	5.5	–	102	LLVALA	VFFGACY		h	Q4 motif at 72 (EYYQSNY, score 6.0): mutagenesis – > partial effects		–	
15	ULK1	*Hs*	1,050	4.0	–	276	DEFFHH	PFLDASP		C	Motif at 93 (EYCNGGD, score 5.5): Y2A inhibits binding BUT in kinase domain and no binding by NMR	Another possible motif exists but is very suboptimal	–?	
16	CaSR	*Hs*	1,078	2.5	+	761	ELEDEI	IFITCHE	T4	C	In lumen; same for motif at 478 (TFDECGD, score 3.0)	Translocated out of the cytoplasm	–	

Table 1: (continued)

Line	Protein	Org.	Aa	Score	Exp	Start	Flank	Core	p4	SS	Evidence	Conclusion	Final	cat.
17	AnxA6	*Hs*	673	3.5	–	94	GLMRPP	AYCDAKE		H	Other motif at 133 (AYKDAYE, score 3.5) also helical	Motifs in middle of helical domains; untested.	–	
18	PITPβ	*Hs*	271	6.0	–	106	NEYMKD	DFFIKIE		E	F2AF3A prevents an interaction with VAPA	Crystal structures place motif in a conserved *β*-strand with many residues inside the LTP, incl. F2.	–	
19	Ano1	*Hs*	986	2.5	+	239	DLSDKD	SFFDSKT		c	Motif tested by S5E reduces FRET with VAPA not VAPB	Doubt when results are mixed	?	Unclear
20	NET3C	*At*	225	3.5	–	112	VCDSNS	HFEDADS		c	Motif at 209 (KFFGKLF) has score 8.5: F2A F3A has no effect	Separate, more plausible motifs (because they have fewer suboptimal	?	
21	SEIPIN2	*At*	526	3.0	+/–	19	TTDEFD	RFLDAPD		c	F2G no effect on Y2H, but multiple a mutation has partial effect; ?uses motif at 27 (FYYDCLP, score 3.5)	elements) have not yet been tested	?	
22	SEIPIN3	*At*	509	3.0	+/–	24	LDAEDE	FFYDSFS		c	Deletion 1–24 aa loses activity; this also contains motif at 16 (RFLDAED, score 4.0)		?	
23	IRBIT	*Hs*	530	3.0	+/–	74	SQSSTD	SYSSAAS	S4	c	Motif and adjacent one at 80 (SYTDSSD, score 3.0): both Y2A reduces functional output	Only indirect outputs assayed	?	
24	Epo1	*Sc*	943	2.5	+	610	NSTDIN	EYVTAQE	T4	c	PLUS second binding site for VAP mapped to C-terminus; no FFAT detail; T54A inhibits binding	Assaying only VAP mutations leaves doubt, especially if multiple sites may bind VAP	?	
25	TRPC3	*Hs*	921	3.5	–	30	LTAEEE	RFLDAAE		h	Other unlikely sequence at 219 (DFYAYDE, score 5.5) has been tested indirectly: F2A/Y3A inhibits function	For a very suboptimal sequence tests need strong evidence *e.g.* direct binding	?	

Proteins with proposed FFAT motifs are listed along with evidence that is positive (lines 1–11), negative (12–18) or not clear (19–25). Also shown: 'org' organism: *Hs* human, *Sp S. pombe*, *Sc Saccharomyces cerevisiae*, *At A. thaliana*; CoV SARS-CoV2; length of protein; 'score': number of suboptimal elements the motif scores in the PWM (Murphy and Levine 2016); 'exp': expected interaction based on score alone; 'start': the starting residue; 'flank': six preceding residues; 'core' seven residues of motif; p4: indicates phospho-FFATs; 'SS': secondary structure of motif: H/E/C if experimentally determined helix/strand/coil (IDR), h/e/c if predicted; 'evidence' summarises the best evidence for a FFAT motif here; 'conclusion' is the general conclusion that this example illustrates; 'final' for final estimation of whether there is a motif, in three categories ('cat.'): positive (+) which includes positive with a caveat (+?), negative (–) or unclear (?). Citations for each line: 1. Yu et al. 2023; 2. Xu et al. 2025; 3. Zhao et al. 2020; 4. Slee and Levine 2019; 5. Furuita et al. 2021; 6. Weber-Boyvat et al. 2015; 7. Chao et al. 2014; Casler et al. 2024; 8/9. Di Mattia et al. 2020; Furuita et al. 2021; 10. Lindhout et al. 2019; 11. Hoh et al. 2024; 12. James et al. 2021; 13. Inukai et al. 2021; 14. Abu Irqeba and Ogilvie 2020; 15. Zhao et al. 2018; Furuita et al. 2021; 16. Gorkhali et al. 2021; 17. Rentero et al. 2018 and Enrich et al. 2021; 18. Park et al. 2025; 19. Lin et al. 2025; 20. Wang et al. 2014; 21/22: Greer et al. 2020; 23. Lin et al. 2025; 24. Chao et al. 2014, Neller et al. 2015 and Wang et al. 2021; 25. Liu et al. 2022.

elements like helices can locally unfold into IDR that binds VAP. A caveat here is that there is no previous example of motifs that unwind from helices in folded domains to become IDR to bind their target. The closest thing is the reverse, with motifs that become more helical upon binding their target (Zavrtanik et al. 2024). Therefore, findings about helical FFAT motifs merit further research so we can determine if revisions are needed for the structural criterion.

If the structural criterion needs nuancing and possibly revision, are the other criteria also uncertain? The PWM used in bioinformatic screening can be updated as the group of FFAT sequences gradually enlarges to include new

developments, such as W2 motifs. However, this approach will always have the fundamental problem that it is designed for known FFAT motifs. Divergent motifs must be missed. This flaw of circular logic could be addressed by experiments to measure the binding affinity of all single and multiple substitutions in the motif.

4 Expanding the map of VAP's interactions and ER functions

Recently discovered VAP-FFAT interactions take part in a wide range of physiological and pathological processes (Table 2 and Figure 4). The value in discovering a new VAP interactor, particularly one outside the ER, is that this points to an unappreciated role for the ER. In addition, if the interaction conforms to the conserved VAP-FFAT type, the role of recruited ER can be probed with easily predictable point mutations.

A notable novelty is the involvement of proteins in the nucleus. The finding that nucleolar proteins across eukaryote evolution have FFAT motifs is unexpected because nucleoli in many species are in the centre of nuclei, several microns distant from the inner nuclear envelope, the nearest part of the ER. This is a puzzle yet to be resolved. A group of three nucleoplasmic proteins with FFAT motifs from widely differing species all act late in the cell cycle when chromatin reconnects with membranes of the nuclear envelope, a specialised ER domain. All three examples share the common feature of allowing chromosomes to attach to the nuclear envelope, either during open mitosis in animal cells or closed mitosis in yeast (Figure 4, bottom). A further role of VAP (and hence the ER) related to the cell-cycle is in plant cytokinesis.

Outside the nucleus, VAP is involved in an ever-expanding set of cell functions (Figure 4). Many involve communication between the ER and other membranes, such as recycling endosomes, phagophores and eisosomes, or they play a central role in zones of three-way contact, which typically involve the ER (Guyard and Giordano 2025). Other functions newly linked to the ER are scaffolding of cAMP nanodomains by recruitment of protein kinase A anchoring proteins (AKAPs) and roles in ribosome function. As well as roles in fundamental intracellular processes, VAP has cell-type-specific roles, which are well-defined in neurons, both in the axon initial segment and in axons. In addition, there are several disease processes that involve VAP interactors, including α-synuclein, the major pathogenic protein in Parkinson's Disease, and proteins produced by pathogenic species (protozoa, bacteria and viruses) that have acquired FFAT motifs to parasitise host cell ER.

5 Future developments

In addition to the many gaps in our knowledge mentioned above, there are two further topics ripe for future development that have yet to be addressed.

5.1 Mapping the VAPome

It may be a useful concept that VAP ubiquitously recruits proteins to ER membranes that communicate with other cellular components. If this view is applied as a null hypothesis, VAP would eventually be allocated a role in all such processes, even where at first no role for it is apparent, for example in the interaction of the ER with membrane-less organelles (Lee et al. 2020).

The VAPome is large: VAPA VAPB and MOSPD2 combined have 2018 interactions with 920 proteins documented in the BioGRID (https://thebiogrid.org). Identifying genuine hits is a major project. Screening sequences for FFAT motifs could be helpful, but this cannot identify non-FFAT interactions, such as with Shs1 and CFTR. A better way forward in bioinformatics may be to use AlphaFold Multimer, either with AlphaFold-2 (Evans et al. 2022) or AlphaFold-3 (Abramson et al. 2024), addressing several criteria (sequence, conservation and structure) together. Thus, bioinformatics is increasingly able to check possible motifs and might predict some of VAP's interactions (Willet et al. 2024, Sutjita et al. 2024, Liu et al. 2025). Another aspect of VAP biology yet to be determined is whether the VAPome saturates the total number of VAP binding sites, and how unbound or total VAP are regulated, presumably by transcription.

5.2 ER nanodomains

As described above, interactions with other compartments in trans are known to create unique biochemical zones within the ER (Obara et al. 2024). At a higher level of detail, multiple ER complexes have now been shown to cooperate in a single overall process (Lin et al. 2025). Thus, VAP and its partners, particularly those in the ER, may create heterogeneity within the ER. A future research goal might be to map the precise environment of different parts of the ER on the scale of 10–30 nm. No ultrastructural features mark VAP-positive regions (Hoffmann et al. 2019, Collado et al. 2019), but there may be specialised bilayer composition because of recruited partners, including lipid transfer proteins and lipid modifying enzymes such as Sac1.

Table 2: A list of the recently discovered VAP interactions.

Type	Site	#	ER functions involving VAP and FFAT motifs
Normal cells – physiology	Nucleus/cell cycle	1	Nucleoli across eukaryotes contain a statistical excess of proteins with FFAT motifs (Slee and Levine 2019). While fungal nucleoli are attached to the inner nuclear envelope, in most organisms a mechanism for nucleoli to access the ER is unclear.
		2	Progression through anaphase in open mitosis in animal cells involves non-core regions of chromosomes being recruited to reforming nuclear envelope ER membranes by ELYS (aka AHCTF1) on chromosomes (Murphy and Levine 2016, James et al. 2019, James et al. 2024).
		3	NE reassembly in telophase in fly cells requires Ankle2 (Li et al. 2025). By comparison, human Ankle2 (aka LEM4) has FFNTs that bind to MOSPD1/3 (Cabukusta et al. 2020).
		4	Re-attachment of DNA to nuclear envelope in closed mitosis of budding yeast requires Siz2 (aka Nfi1), which sumoylates yeast VAP and then binds it in two different modes simultaneously (Ptak et al. 2021).
		5	Actin is nucleated by SCAR-2 in plant cytokinesis in part through binding to ER-derived vesicles recruited to the cell division plate (Xu et al. 2025).
	Membranes +	6	The mitochondria-ER-Cortex anchor (MECA) in yeast depends on Num1, which binds the ER as well as plasma membrane and mitochondria (Chao et al. 2014, Casler et al. 2024).
		7	Atypical secretion, for example of CFTR Δ508, passes through a tubular endosome network that requires WDR44 (aka Rab11bp), which interacts with ER tubules running close by (Lucken-Ardjomande Hasler et al. 2020, Parolek and Burd 2024)
		8	ER-phagy in fission yeast involves Epr1, recruited to the ER from the cytoplasm (Zhao et al. 2020). The budding yeast homologue Jjj1 has a FFAT motif in a similar location (Slee and Levine 2019), and presumably this also mediates ER recruitment of Jjj1, which is an ER-phagy receptor too (though this has not yet been tested).
		9	Eisosomes interact with cortical ER in fission yeast (Zhang and See 2022), which is consistent with homologues of the eisosomal protein Seg2 containing a statistical excess of FFAT motifs (Slee and Levine 2019).
		10	A range of cAMP-derived signals are structured in nanodomains by the minority of AKAPs with FFAT motifs, which are located at different membrane contact sites, including: ER-plasma membrane in neurons (Vierra et al. 2023); endosome-mitochondria-ER in pancreatic *β*-cells (Austin et al. 2025).
		11	Regulation of ribosomal assembly: LSG1 for ribosome maturation in animals (Sutjita et al. 2024), Rqc1 for ribosomal quality control in yeast (Slee and Levine 2019).
	Neuron	12	Reversible large contacts between plasma membrane in the axon initial segment and ER, often stacked, through multiple phosphorylation of a phospho-FFAT in Kv2.1 (Johnson et al. 2018, Kirmiz et al. 2019).
		13	In axons the cytoplasmic protein secernin1 (SCRN1) is recruited to the ER; this interaction is required for normal axonal ER structure and synaptic vesicle recycling (Lindhout et al. 2019).
Pathology - disease	PD	14	α-synuclein binds VAP: after the original observation (Paillusson et al. 2017), new studies reveal a three way VAP–PTPIP51–α-synuclein complex at ER-mitochondrial junctions (Liu et al. 2025).
	Intracellular infection	15	*Leishmania amazonensis* infection is facilitated by recruiting ER to parasitophorous vacuoles, and is mediated by lipophosphoglycan export from parasite to host, mechanism unknown (Gdovinova and Descoteaux 2025).
		16	*Toxoplasma* infection is facilitated by recruiting ER to inclusions, in part dependent on MOSPD2 (Cygan et al. 2021, Ferrel et al. 2023).
		17	*Coxiella* alter host cell lipid droplet function (they enlarge) through the secreted bacterial effector EPF1 protein, which is located at ER-lipid droplet contacts (Angara et al. 2024).
		18	One of the tomato pathogen *Xanthomonas* type-3 effector proteins is XopM, which localises to the ER in cells in infected leaves and reduces their immune response (Brinkmann et al. 2024).
		19	RNA-dependent RNA polymerase (RdRp) (aka nsp12), which replicates the SARS-CoV-2 genome inside double membrane vesicles derived from the ER, has a FFAT motif of unknown significance (Furuita et al. 2021).

VAP-FFAT interactions grouped by type (normal cell function *vs.* pathological process) and by site, as shown, to accompany the map in Figure 4. Number for each entry (#) cross-refers to the same item in Figure 4. Abbreviation: PD = Parkinson's Disease.

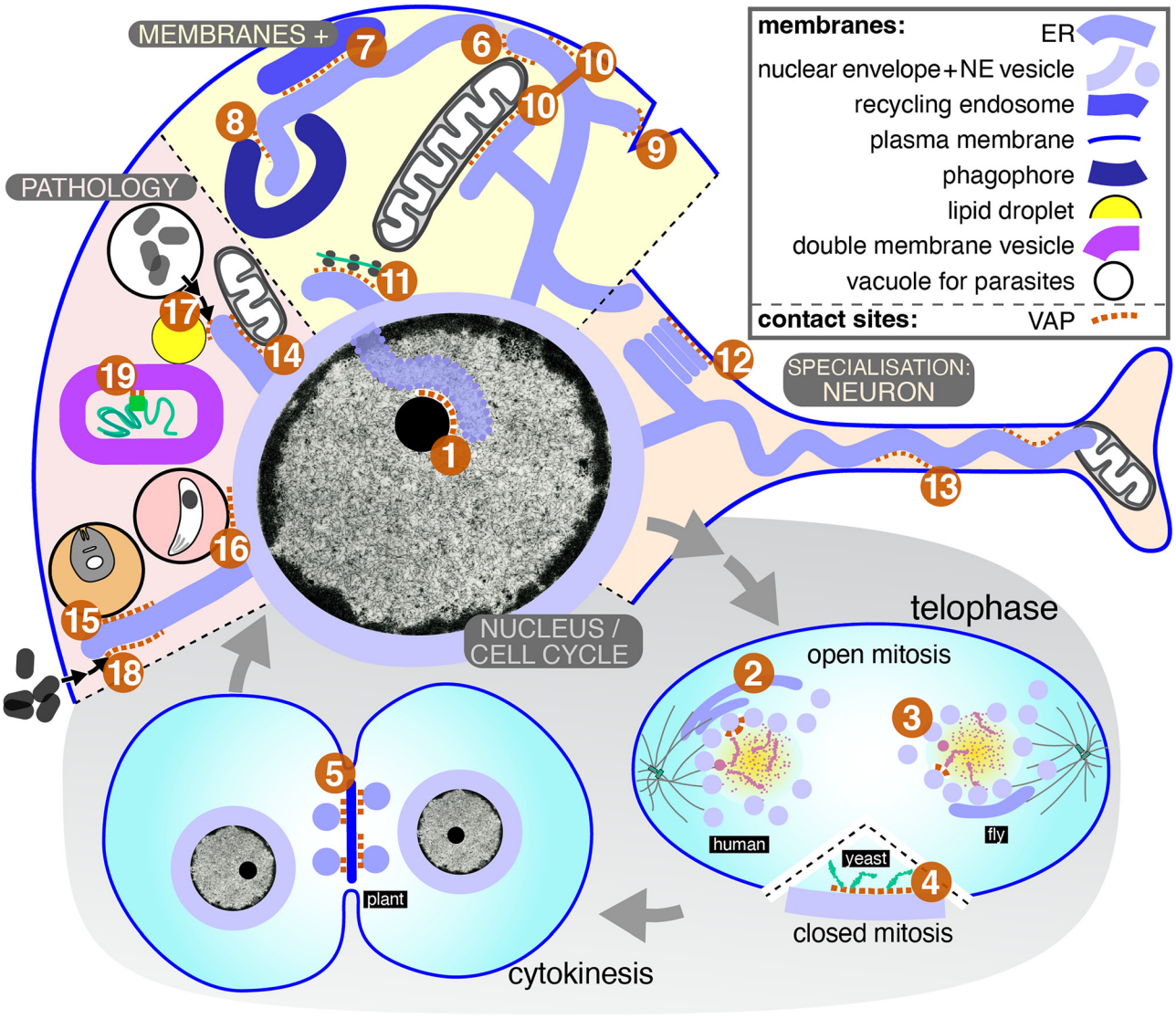

Figure 4: A map of the recently discovered interactions of VAP. See Table 2 for details of each numbered process and relevant citations. The key shows the scheme of cell membranes and of VAP^{+ve} ER and nuclear envelope. Numbers 1–19 cross-refer to the relevant lines in Table 2, which gives citations. Species are indicated for processes related to the cell-cycle (numbers 2–5). Components used from licenced sources: EM of nucleus – Mike Kayser, Wellcome Collection (https://wellcomecollection.org/works/evc5w355); mitochondrion – SwissBioPics (Le Mercier et al. 2022); mitosis sequence – LadyofHats, Wikimedia Commons.

6 Conclusions

VAP is a small, simple protein with a central role both in ER biology and in the growing field of interorganelle communication. While we now have mapped out the full range of VAPs, we are a long way from understanding all their interactions. Future research into VAP will be a gateway to discovering more about the ER. In addition, we should turn our attention to the regulatory pathways that keep VAP at the centre of so many functions of the ER.

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Abbreviations

aka	also known as
CRAL	cellular-retinaldehyde binding protein
ER	endoplasmic reticulum
FFAT	two phenylalanines in an acidic tract
IDR	intrinsically disordered region
MSP	motile sperm protein
ORP	OSBP-related protein
OSBP	oxysterol binding protein
SLiM	short linear motif
TMH	transmembrane helix
VAMP	vesicle-associated membrane protein
VAP	VAMP-associated protein.

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