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VAP, a Versatile Access Point for the Endoplasmic Reticulum: Review and analysis of FFAT-like motifs in the VAPome☆

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ABSTRACT

Dysfunction of VAMP-associated protein (VAP) is associated with neurodegeneration, both Amyotrophic Lateral Sclerosis and Parkinson's disease. Here we summarize what is known about the intracellular interactions of VAP in humans and model organisms. VAP is a simple, small and highly conserved protein on the cytoplasmic face of the endoplasmic reticulum (ER). It is the sole protein on that large organelle that acts as a receptor for cytoplasmic proteins. This may explain the extremely wide range of interacting partners of VAP, with components of many cellular pathways binding it to access the ER. Many proteins that bind VAP also target other intracellular membranes, so VAP is a component of multiple molecular bridges at membrane contact sites between the ER and other organelles. So far approximately 100 proteins have been identified in the VAP interactome (VAPome), of which a small minority have a "two phenylalanines in an acidic tract" (FFAT) motif as it was originally defined. We have analyzed the entire VAPome in humans and yeast using a simple algorithm that identifies many more FFAT-like motifs. We show that approximately 50% of the VAPome binds directly or indirectly via the VAP-FFAT interaction. We also review evidence on pathogenesis in genetic disorders of VAP, which appear to arise from reduced overall VAP levels, leading to ER stress. It is not possible to identify one single interaction that underlies disease. This article is part of a Special Issue entitled: The cellular lipid landscape edited by Tim Levine and Anant K. Menon.

The network of the endoplasmic reticulum (ER) permeates the entire cell and plays a central role for biosynthesis of proteins and lipids. Many vital ER processes are carried out by proteins that are either integrated into the membrane or peripherally associated with it. So far, only one mechanism has been discovered by which peripheral proteins target the cytoplasmic face of the ER: they bind to the integral membrane protein Vesicle-associated membrane protein (VAMP)-Associated Protein (VAP), which is conserved in all eukaryotes. Vertebrates have two VAPs (VAP-A and VAP-B), while the major yeast protein is called Scs2p. VAP is clearly important for cellular function, as mutations in VAP-B cause rare forms of late-onset Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis-type 8 (ALS8), rare inherited motor neuron diseases [1–4]. Studies of VAP are clinically important because they may explain the pathogenesis of sporadic (non-familial) ALS, a relatively common and devastating disease, and possibly other aspects of neurodegeneration.

In this review we update the sole previous review on VAP [5]. We include individual VAP interactions, and we also analyze data sets from high-throughput proteomic studies. Proteolytically cleaved, secreted products of VAP that act extracellularly are not discussed [6,7]. Instead we focus on the intracellular interactions of VAP, which is unique because it interacts with many proteins that are attached to, or even anchored in other compartments. This makes VAP a key player for several different membrane contact sites. These nanometer scale zones specialised for intracellular traffic of material and information have moved center stage in recent years, and a greater understanding of VAP is important for their full understanding.

1. VAP links the ER to other organelles

1.1. VAMP-associated by name, but not by nature

VAP consists of a 7-beta strand globular domain in the major sperm protein (MSP) family (120-140 aa), a linker region (≤100 aa) partly forming a coiled coil in some species, and a C-terminal transmembrane helix tail anchor that targets the ER (Fig. 1A). VAP was named for its interaction with the SNARE protein VAMP, but although binding to many SNAREs has been reported [8,9], the molecular basis for any

Abbreviations: AKAP, A-kinase anchor protein; ALS, Amyotrophic Lateral Sclerosis; CERT, ceramide transfer protein; ER, endoplasmic reticulum; ERAD, ER-associated destruction; FFAT, two phenylalanines in an acidic tract; MSP, major sperm protein; ORP, OSBP-related protein; OSBP, oxysterol binding protein; RdgB, retinal degeneration type B; VAMP, Vesicle-associated membrane protein; VAP, VAMP-Associated Protein; VAPome, VAP interactome.

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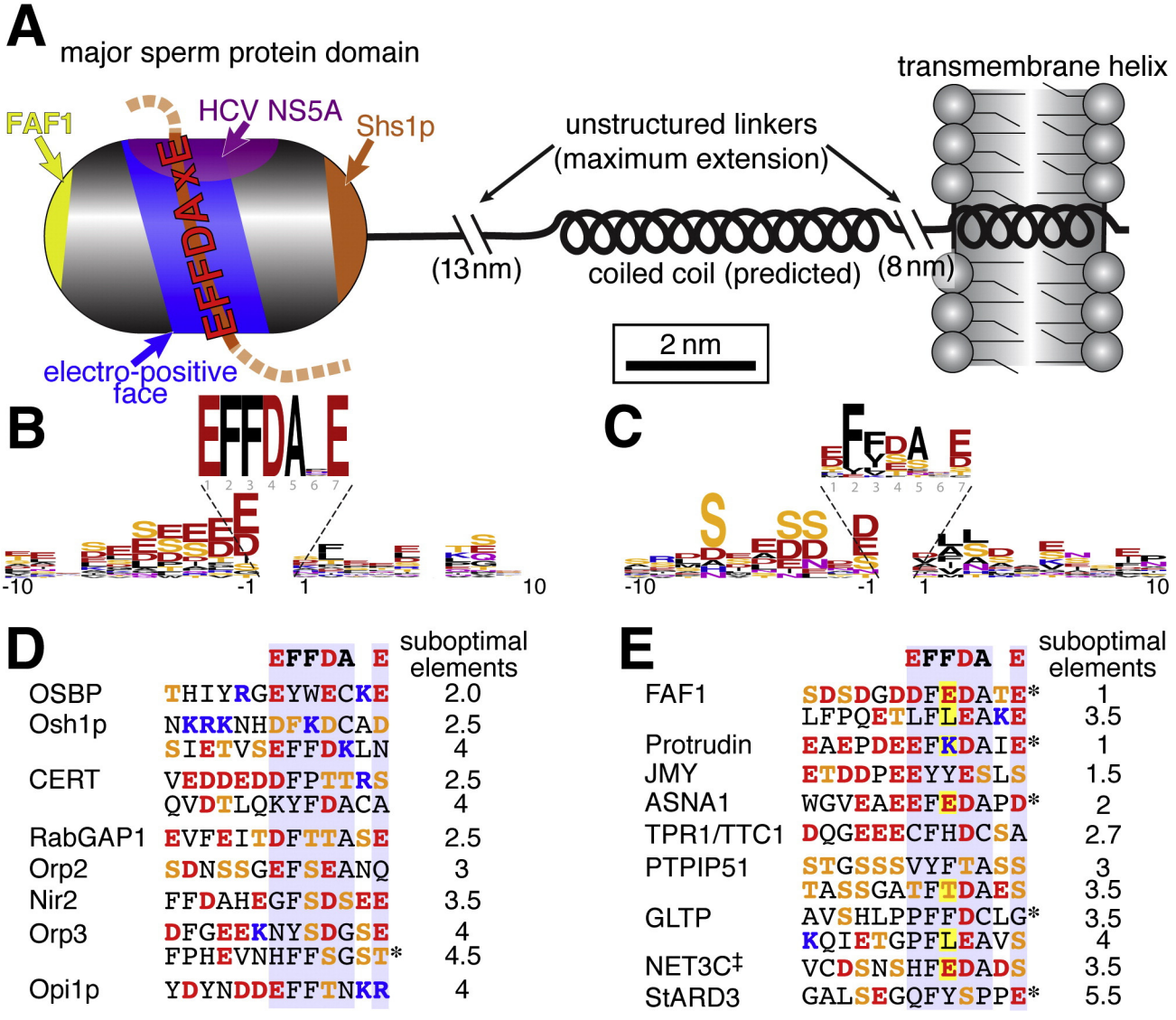

Fig. 1. FFAT-like motifs in proteins that bind VAP **A:** Diagram of VAP, a C-terminally membrane anchored protein with three conserved and partly overlapping binding sites on the N-terminal globular MSP domain: (i) electro-positive face (blue) binds EFFDAxE and FFAT-like motifs (red) [15–17] as well as anionic lipids [63]; (ii) base (brown = Scs2p residues equivalent to VAP-A S36 and D37) binds a helix in Shs1p [47]; and (iii) side (magenta = Q6, M89, V90, Q91, D116, L119, and V122 in VAP-A) binds the fuzzy domain of NS5A and the C-terminus of NS5B, both from hepatitis C virus (HCV) [51,52]. In addition, acidic residues in a loop near the tip (yellow = D77, D79 in VAP-A) contribute to binding by FAF1, a FFAT-positive protein [10,43]. Scale bar (2 nm) indicates the relative size of the different domains, except two unstructured segments of the linker (≤8 and ≤13 nm) that are not shown. **B:** Consensus of the flanking regions (10 amino acids both sides) in 14 eukaryotic sequences that contain EFFDAxE. The amino terminal flank is enriched for acidic residues. **C:** Consensus of the core and flanking regions of 21 predicted FFAT-like motifs [30]. Serines replace acidic residues in multiple positions in the amino-terminal flank, allowing activation by phosphorylation [65,91]. **D:** Example of second FFAT-like sequences in proteins with originally defined FFAT motifs; *only the sequence scoring 4.5 in Orp3 has so far been tested [34]. Other motifs fit the criteria F/Y^2, $D/E/S/T^4$, and not D/E in position 5; all need verification. Note that the additional FFAT-like motifs in OSBP/Osh1p occur at the extreme C-terminus of their lipid transfer domains. Other second motifs shown here are all outside known domains. **E:** FFAT-like motifs identified in VAP interactors, all human [10,31,33,36–38,40] except NET3C‡ from *Arabidopsis* [39]. Non-conservative substitutions for F/Y^3 that were previously excluded [30] are highlighted in yellow. * indicates motifs studied before in molecular detail. In D and E the number of suboptimal elements was calculated using the algorithm in Box 1, and amino acids are colored by (possible) charge: D/E = red, S/T = orange, K/R = blue.

such interaction has not been established [10]. Only a minority of protein-protein interaction studies have identified any directly interacting SNARE [11,12], indicating that VAP-VAMP binding may only occur under certain lysis conditions. Among SNAREs that bind VAP is the ER SNARE Sec22b [12], which has significant functions at membrane contacts with the plasma membrane [13], so it is important to determine if and how VAP binds Sec22b.

1.2. FFAT, a short motif for proteins to bind VAP

The major set of VAP interactors identified to date are cytoplasmic proteins containing two phenylalanines (FF) in an acidic tract (the FFAT motif). The motif has a core with six defined elements across a stretch of seven residues: E^1-F^2-F^3-D^4-A^5-x-E^7 (using the single letter amino acid code, where x is any amino acid). This core is supplemented by a seventh, less well defined element: the flanking regions. The immediately adjacent residues (especially upstream) contain multiple acids, but very few basic residues (Fig. 1B) [14]. Highly conserved residues in VAP contribute to FFAT binding with a micromolar dissociation constant [15–17]. The FFAT binding site forms an electro-positive face on the major sperm protein globular domain of VAP (Fig. 1A). The critical residues in this binding site have been identified from alanine substitutions as K45, T47 and K118 [15]. Another way to inhibit FFAT binding is to introduce a double charge substitution in residues involved in binding F^2: K87D/M89D [16], although alanine mutations show that these two residues are not individually critical for binding [18]. By X-ray crystallography the FFAT motif forms an extended loop that lies across an electro-positive face of the globular domain of VAP, the side-chains of

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