Hypothesis

# VHS domain marks a group of proteins involved in endocytosis and vesicular trafficking

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**Abstract Endocytosis is driven by a mechanism which is characterized by an orderly congregation of a large number of proteins which effectuate, first, formation of a coated vesicles, second, pinching off the vesicle and, third, regulated transport. True to the nature of many other proteins involved in multimolecular complexes, also endocytosis-associated proteins, such as Eps15, clathrin and AP-2, are characterized by distinct domains which mediate the protein-protein interactions. We now report that a group of well-established endocytosis and/or vesicular trafficking proteins possess a VHS domain, a recently described domain with an unknown function. We suggest that in these proteins VHS serves as a membrane targeting domain which by its specific features together with FYVE, SH3 and/or TAM domains, which are also present in some VHS-containing proteins, is involved in the stage-specific assembly of the endocytic machinery.**



*Key words:* Endocytosis; Signaling; VHS domain

## 1. Introduction

The endocytic process, triggered by ligand-induced clustering of the receptors, initiates a stepwise assembly of a dynamic multimolecular complex which contributes, first, to the formation of clathrin-coated vesicles and, then, to the formation of endosomes [1]. Critical in this process, which leads to down-regulation of activated receptors, are protein-protein interactions mediated by specific domains.

An increasing number of proteins have been associated with the endocytic machinery, reflecting its complexity. One of them is Eps15, a substrate for epidermal growth factor receptor (EGFR) that is indispensable for endocytosis [2]. It contains three Eps15 homology (EH) domains which are also present in yeast proteins involved in endocytosis [3]. Via its EH domain, Eps15 binds to epsin, another protein implicated in clathrin-mediated endocytosis [4]. The EH domain-recognizing site in epsin, as well as in a number of other EH-binding proteins, is a tripeptide Asn-Pro-Phe (NPF motif). In yeast, the NPF motif is found in at least two proteins that are involved in endocytosis [3].

An entire family of proteins came under the spotlight as putative players in endocytosis with the revelation that FYVE, a so far functionally poorly characterized domain, binds inositol lipid PtdIns(3)P [5–8]. It probably serves as a membrane targeting domain of such endocytosis or vesicular trafficking-associated proteins as EEA1, Hrs, Vac1p and VPS27.

## 2. VHS domain

We have recently characterized a new endocytosis-associated protein, EAST. It contains an SH3 domain, a well-known protein-protein interaction domain and a TAM motif, a tyrosine-based activation motif which in immunoreceptors serve as a docking site for SH2 domain-containing proteins [9]. Now we have found, in sequence database comparisons, several proteins with a high degree of similarity with the N-terminus of EAST (Fig. 1A). Comparison with the domain profiles in the SMART database showed that the region corresponds to the VHS domain which was originally discovered in a database screen based on the multiple occurrence of stretches of sequences in signal transduction proteins [10]. The name VHS derives from its occurrence in VPS-27, Hrs and STAM. As originally defined, it is ~140 residues long. As to its role, no tell-tale features have been recognized in VHS. Consequently, it is a domain in search of a function.

## 3. VHS domain and endocytosis

In the sequences in Fig. 1A, VHS invariably resides in the N-terminus. A predominantly α-helical secondary structure (~30%) is predicted for it with a good conservation of hydrophobic residues. The most striking feature is its occurrence in proteins which are associated with endocytosis or vesicular trafficking. VPS27 controls membrane trafficking through the prevacuolar/endosomal compartment in *Saccharomyces cerevisiae* [11]. Hrs, a substrate of HGF (hepatocyte growth factor) receptor, is found on the surface of early endosomes, and is suggested to play a role in vesicular transport [12]. Hrs-2 is closely related to Hrs and it modulates vesicle trafficking in neurotransmission [13]. STAM is involved in cytokine-mediated intracellular signal transduction [14]. It interacts with Hrs and may therefore be involved in endocytosis/vesicular transport as well [15]. Our own studies with EAST further corroborate the association with endocytosis; EAST associates with Eps15 and colocalizes with clathrin in vesicular structures [9].

Tom-1b, YHA2, SPAC19A8.05C and C34G6.7 are less well-characterized. KIAA0154, an as yet unknown protein, is similar to mouse γ-adaptin which, as a part of the AP-1 complex, associates with clathrin-coated vesicles that bud from the *trans*-Golgi [16].

The overall domain architecture of VPS27, Hrs and Hrs-2 is intriguing in that the VHS domain is flanked by a FYVE

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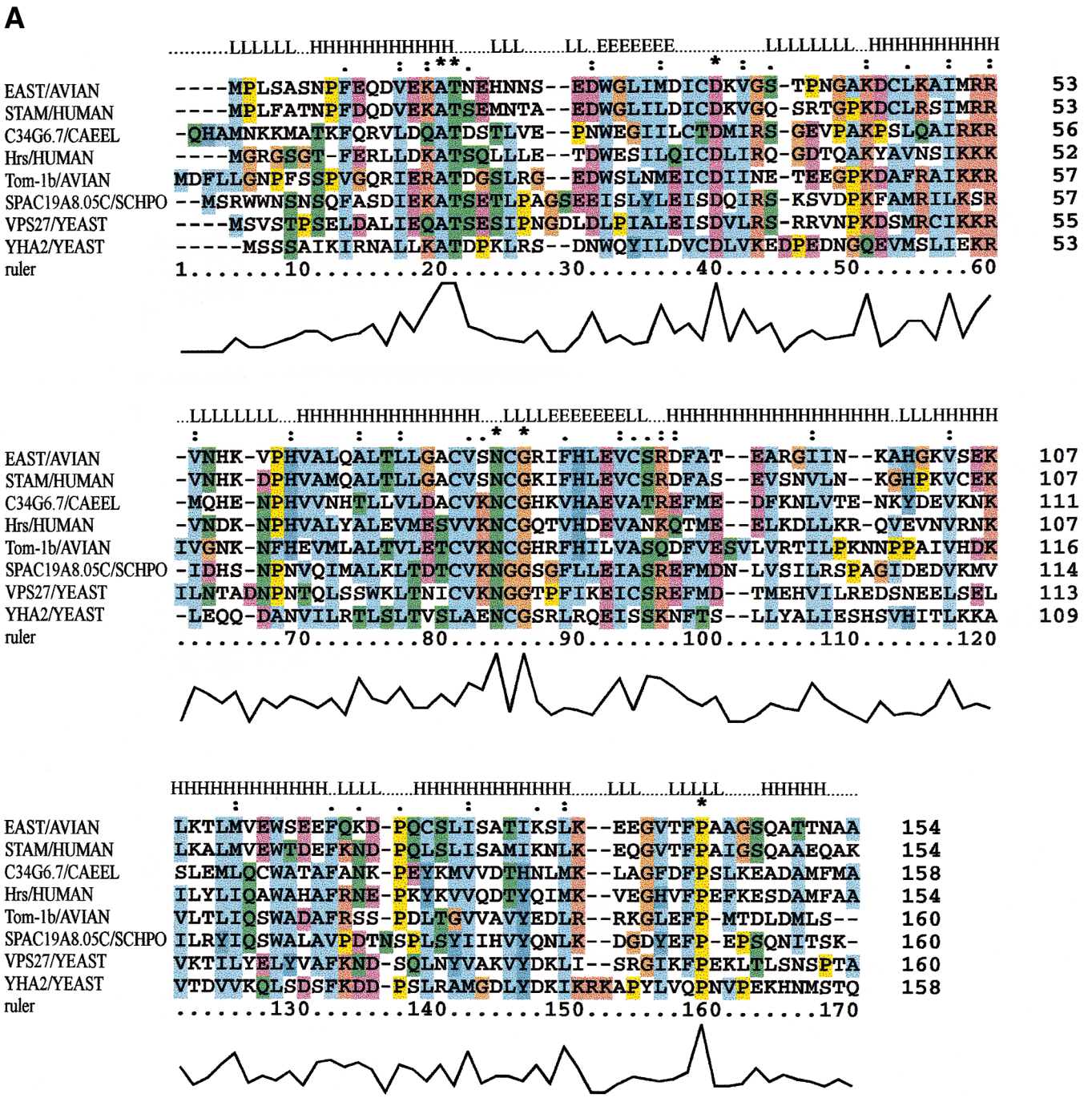


Fig. 1. A: Multiple alignment of VHS domains constructed using the Clustal X (1.64b) program. The first column gives the name of the protein or the database designation followed by the species (CAEEL, *Caenorhabditis elegans*; SCHPO, *Schizosaccharomyces pombe*). The single letter code is used for amino acids. The residues are colored according to the following scheme: all glycines (G, brown) and prolines (P, yellow) are colored. Other coloring is by recurring feature: hydrophobic residues (A, V, F, M, I, L, W, C) are light blue; tyrosine and histidine (Y, H) are dark blue; asparagines, glutamines, serines and threonines (N, Q, S, T) are green; aspartate and glutamate (D, E) are purple; arginine and lysine (R, K) are orange. More than 50% occurrence of a property results in coloring. A single fully conserved residue is indicated by an asterisk, strong conservation by a colon and weak conservation by a dot. Columns that are left white show poor conservation of a residue or a property. The conservation score for each column is indicated by the plot below the sequences. Secondary structure predictions using the PHD program are shown above the alignment. H denotes sequences with a propensity for α-helix, E sequences with a propensity for β-sheet. L denotes loops. Accession numbers of the sequences are as follows: EAST, AJ224514; STAM, U43899; C34G6.7, U97407; Hrs, U43895; Tom-1b, Y08741; SPAC19A8.05C, Z98974; VPS27, U24218; YHA2, U10555. B: Modular structure of proteins containing the VHS domains. The protein names and abbreviations are as above. The additional domains depicted are: NPF, Asn-Pro-Phe triplet; FYVE, domain present in Fab1, YOTB, Vac1 and EEA1; SH3, Src homology 3 domain; TAM, tyrosine-based activation motif.

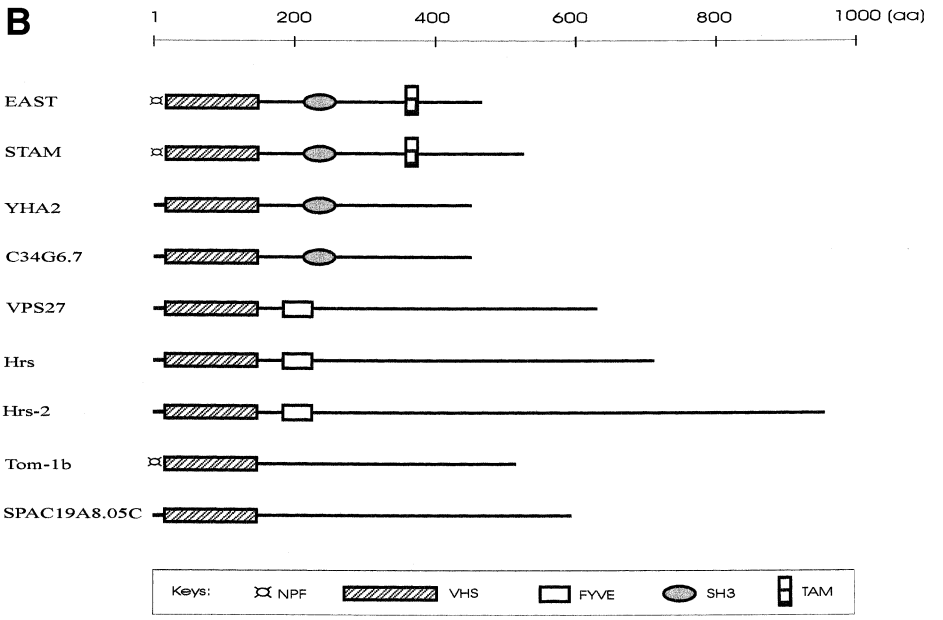


Fig. 1. (*continued*)

domain (Fig. 1B). On the other hand, in EAST, STAM, YHA2 and C34G6.7, the VHS domain is followed by an SH3 domain and a TAM motif (EAST, STAM), or by an SH3 domain alone (YHA2, C34G6.7). Another interesting feature is the presence of the NPF motif in EAST, STAM and Tom-1b. Thus, they all seem to be multidomain proteins possessing sites specialized in lipid binding and targeting (FYVE) or protein-protein interactions (SH3, TAM, NPF).

The presence of VHS in endocytosis-/vesicular traffic-associated proteins suggest that it could be involved in membrane targeting and association. Our own studies support that by showing that the exogenously expressed N-terminus of EAST, which contains the VHS domain, associates with the plasma membrane [9]. A membrane-anchoring function is also suggested by the observation that Hrs retains its membrane association even if its FYVE domain is eliminated [12]. This, together with the data showing an association of EAST with Eps15, suggests that VHS of EAST plays a role in the early stages of endocytosis. Clearly, more functional studies on VHS are warranted.

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