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Short communication

The single epsin homolog in *Giardia lamblia* localizes to the ventral disk of trophozoites and is not associated with clathrin membrane coats

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ABSTRACT

Epsins serve as recruitment platforms for clathrin membrane coat protein components and induce membrane curvature via their N-terminal homology (ENTH) domain. Unexpectedly, the single ENTH domain protein, a putative epsinR homolog (Glepsin), in the diverged protozoan parasite *Giardia lamblia*, localizes exclusively to the specialized attachment organelle, the ventral disk (VD). Glepsin binds both to phosphatidylinositol (3,4,5)-trisphosphate phospholipids and the VD cytoskeleton, but lacks canonical domains for interaction with clathrin coat components. This suggests reassignment of giardial epsin function from membrane trafficking to a structural role in linking the plasma membrane to the highly specialized VD during evolution of this genus.

To prepare for survival in the environment, the protozoan parasite *Giardia lamblia* transforms from a flagellated form into a cyst before being shed by its mammalian host. Encystation entails synthesis and regulated secretion of a composite biopolymer matrix composed of three cyst wall proteins (CWPs) and glycan [14]. Cyst wall material (CWM) is selectively accumulated and matured in encystation-specific vesicles (ESVs) [10]. Even though ESVs along with all other giardial organelles appear to be fixed in position in trophozoites [4,10,16], free exchange of fluid phase material containing cyst wall proteins between organelles is ensured by highly dynamic tubular membrane structures [16]. In *Giardia*, two genes code for factors that are potentially able to induce formation of membrane tubules and recruit effectors to the cytoplasmic side of membranes [5,6,17]. A dynamin-like protein [4] and an ENTH (epsin N-terminal homology) domain protein [9] (*Giardia* genome database accession number GL50803_3256). The single giardial dynamin may play a general role in organelle morphogenesis, since conditional expression of a dominant-negative variant led to massive enlargement of both ESVs and *Giardia*-specific endosomal-lysosomal compartments termed peripheral vesicles (PVs) [4]. However, formation of membrane tubules allowing inter-organelle protein exchange does not seem to depend on dynamin function (Hehl, Stefanic and Gächter, unpublished). Here, we set out to test whether the only ENTH domain containing protein, which is predicted to bind to lipid membranes and induce membrane curvature, localized to ESVs and could play a role in formation of membrane tubular inter-organelle connections.

Mammalian epsin proteins contain a highly conserved N-terminal ENTH domain and function as adaptors providing interaction platforms for various components of clathrin-dependent endocytosis such as recognition and selection of cargo [6]. Four epsin isoforms have been characterized in mammals. Their ENTH domains are characterized by seven alpha helices, one of which, the alpha-0 helix, can insert into the cytoplasmic leaflet of the lipid bilayer and is capable of inducing membrane curvature [3]. The three canonical epsins 1,2,3, named after their ability to interact with epidermal growth factor receptor substrate 15 (Eps15) via their C-terminal domains, bind to adapter protein complex (AP) 2 as well as clathrin and are involved in clathrin-mediated endocytosis [3]. Furthermore, they can bind to ubiquitinated proteins via a conserved ubiquitin interacting domain (UID) (Fig. 1A) A fourth non-canonical epsin, epsinR, lacks an UID and does not bind to Eps15 [11]. Instead, epsinR proteins contain an AP/GGA (Golgi-localized, gamma-ear – containing ADP-ribosylation factor – binding protein) binding motif with a role in AP1 recruitment [11]. Mills et al. [11] suggested that epsinR is involved in

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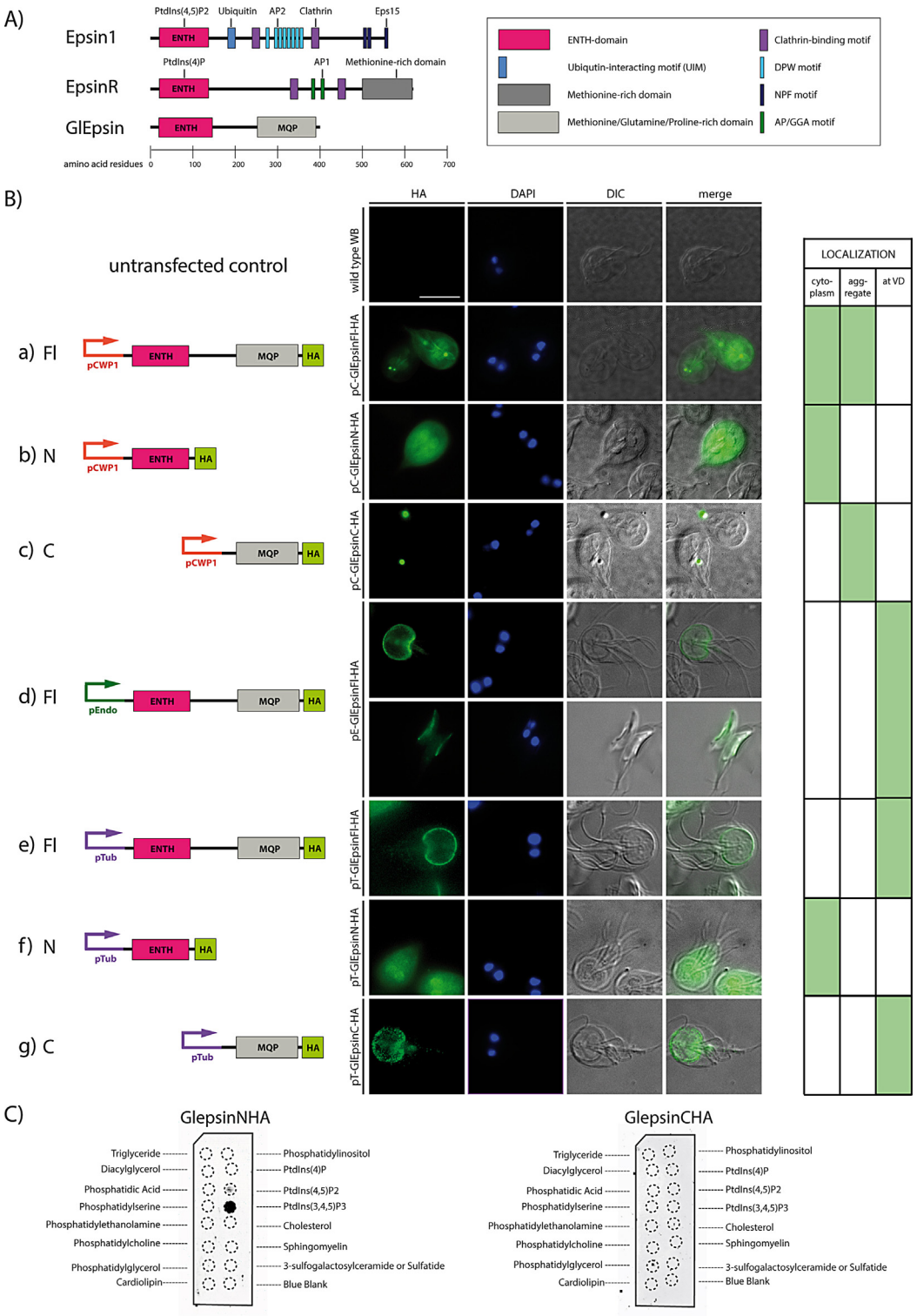

Fig. 1. (A) Domain structures of human epsin1 and epsinR homologues in comparison to Glepsin. The proteins share a highly conserved N-terminal ENTH domain. Epsin1 possesses several additional motifs, such as ubiquitin-interacting motifs, clathrin binding motifs as well as DPW motifs that bind to AP2, and NPFs motifs that bind to Eps15. EpsinR proteins have no ubiquitin-interacting motifs (UIM) or asparagine-proline-phenylalanine (NPF) repeats. However, they possess an additional AP/GGA binding motif as well as a methionine rich C-terminal domain with unknown function. The giardial Glepsin harbors no UIM or NPF domains and is structurally most similar to epsinRs including a low complexity domain enriched in methionine, proline and glutamine residues (MQP). (B) Subcellular localizations of Glepsin variants. Representative localization of C-terminally HA-tagged variants after inducible (8 h) (pCWP1) or constitutive (pEndo, pTub) expression (wide field microscopy). FITC-conjugated rat anti-HA antibody (green) was used to detect HA-tagged Glepsin variants. Nuclear DNA was labeled with DAPI. The table on the right side schematically depicts the localization pattern of Glepsin (abbreviations: pCWP1: inducible CWP1 promoter; pendo: endogenous promoter; DPW, NPF and GGA represent amino acid residues; scale bar: 10 μm). (C) Lipid overlay assay and detection of recombinant, HA-tagged Glepsin fragments. GlepsinN-HA binds to phosphatidylinositol (3,4,5)-trisphosphate. GlepsinN-HA or GlepsinC-HA were expressed in *E. coli* and incubated with membrane lipid strips (Echelon Biosciences, Salt Lake City, Utah, United States). Recombinant protein was detected by luminescence assay using a primary rat-derived anti-HA antibody and HRP-conjugated secondary goat anti-rat antibody. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)