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The Hob Proteins: Putative, Novel Lipid Transfer Proteins at ER-PM Contact Sites

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Abstract

Nonvesicular transfer of lipids at membrane contact sites (MCS) has recently emerged as a critical process for cellular function. Lipid transfer proteins (LTPs) mediate this unique transport mechanism, and although several LTPs are known, the cellular complement of these proteins continues to expand. Our recent work has revealed the highly conserved but poorly characterized Hobbit/Hob proteins as novel, putative LTPs at endoplasmic reticulum-plasma membrane (ER-PM) contact sites. Using both *S. cerevisiae* and *D. melanogaster* model systems, we demonstrated that the Hob proteins localize to ER-PM contact sites via an N-terminal ER membrane anchor and conserved C-terminal sequences. These conserved C-terminal sequences bind to phosphoinositides (PIPs), and the distribution of PIPs is disrupted in *hobbit* mutant cells. Recently released structural models of the Hob proteins exhibit remarkable similarity to other *bona fide* LTPs, like VPS13A and ATG2, that function at MCS. Hobbit is required for viability in *Drosophila*, suggesting that the Hob proteins are essential genes that may mediate lipid transfer at MCS.

Keywords

ER-PM contact sites, phosphoinositides, lipid binding protein, lipid transfer protein, *Drosophila*, *S. cerevisiae*

One of the major cellular roles of membrane contact sites (MCS) is nonvesicular lipid transport between organelle membranes, a process that is mediated by lipid transfer proteins (LTPs). While several families of LTPs have been characterized (Wong et al., 2019), the full complement of LTPs at MCS, and whether their function is essential for animal viability, remains unknown. Our recent work defines the Hobbit/Hob proteins as novel, putative, and essential LTPs that localize to endoplasmic reticulum-plasma membrane (ER-PM) contact sites (Neuman et al., 2022).

The Hob proteins are large (>2000 amino acid) proteins that are conserved throughout eukaryotes, with easily identifiable orthologs in plants, fungi, and animals. The name "*hobbit*" comes from the phenotypic consequences of mutation of the gene in *Drosophila melanogaster*; namely, a dramatic reduction in animal body size resulting in a small pupa phenotype (Neuman & Bashirullah, 2018). *hobbit* mutant animals also arrest development during metamorphosis, indicating that *hobbit* is an essential gene in *Drosophila*; moreover, expression of the human ortholog (KIAA0100) rescues animal viability, demonstrating that Hobbit and KIAA0100 can functionally substitute for one another (Neuman & Bashirullah, 2018). Mutation of *hobbit* orthologs in plants results in a variety of phenotypes, including stunted growth, defects in pollen tube elongation, and aberrant root hair patterning (Cheng & Bezanilla, 2021; Pietra et al., 2015; Procissi et al., 2003); however, the molecular function of the Hob proteins has remained elusive.

In our recent paper (Neuman et al., 2022), we showed that the *S. cerevisiae* orthologs of Hobbit, Fmp27 and Ypr117w (which we propose to name Hobbit homolog 1 and 2 (Hoh1 and Hoh2), respectively), are enriched at cortical ER and co-localize with ER-PM tethers in yeast cells. Protease protection assays demonstrate that the N-terminus of Fmp27 contains a transmembrane domain or hairpin that is anchored in the ER membrane, and the C-terminus of the protein faces the cytosol (Figure 1A). However, deletion of both genes (*fmp27Δ ypr117wΔ*) did not result in any discernible phenotypes under standard growth conditions, nor did it disrupt the structure or abundance of ER-PM contact sites, leaving the function of the Hob proteins at ER-PM contact sites unclear. We therefore turned to *Drosophila*, where *hobbit* is an essential gene, to characterize the function of this protein at MCS. Our

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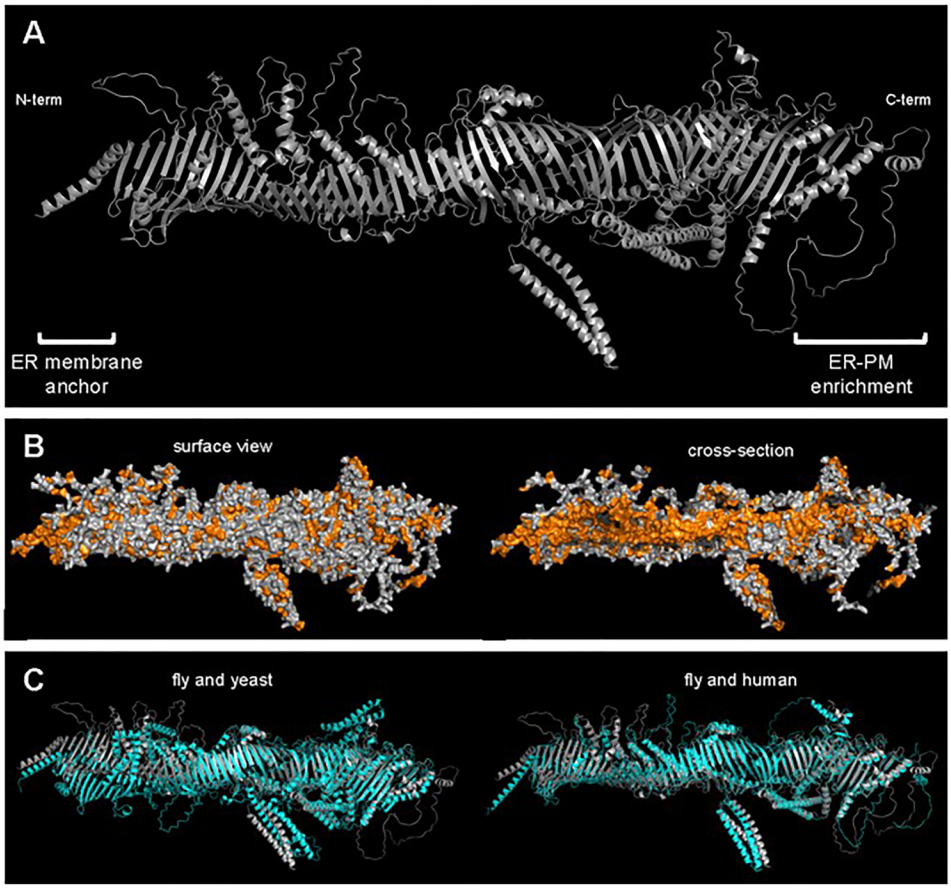

Figure 1. Predicted structure of the Hob proteins. A: Ribbon model of *Drosophila* Hobbit shows that the protein is predicted to fold to form a long tube. Experimentally determined N-terminal endoplasmic reticulum (ER) membrane anchor and C-terminal sequences required for enrichment at ER-plasma membrane (ER-PM) contact sites are labeled. B: Space-filling model labeling hydrophobic (orange) and all other amino acid residues (gray) in *Drosophila* Hobbit. Left image shows a surface view of the protein; right image shows a cross-section of the protein and highlights a hydrophobic channel running down the length of the tube. C: Pairwise comparisons showing ribbon models of the predicted structures of *Drosophila* Hobbit (gray) with *S. cerevisiae* Fmp27 (cyan) (left), and *Drosophila* Hobbit (gray) with human KIAA0100 (cyan) (right). PDB files for all proteins were downloaded from AlphaFold (Jumper et al., 2021) and visualized in PyMOL version 2.4.

studies demonstrated that, like yeast, Hobbit is enriched at ER-PM contact sites in the *Drosophila* larval salivary glands and that ER membrane localization and topology are conserved between yeast and flies (Figure 1A). Several loss-of-function *hobbit* mutant alleles were previously reported, and one of these alleles contains a nonsense mutation just 82 amino acids from the C-terminus of the 2300 amino acid protein (Neuman & Bashirullah, 2018). Notably, deletion of these C-terminal 82 amino acids abolished Hobbit enrichment to ER-PM contact sites (Figure 1A), and expression of this truncated protein did not rescue the viability of *hobbit* mutant animals, suggesting that Hobbit's essential function occurs at MCS. We also showed that a highly conserved C-terminal fragment of the fly Hobbit protein bound to phosphatidylinositol (PI) and all phosphoinositide (PIP) moieties, and the subcellular distribution of $PI(4,5)P_2$ was disrupted in *hobbit* mutant cells, suggesting that *hobbit* plays a functional role in regulating the subcellular distribution of these critical lipid moieties (Neuman et al., 2022).

The C-terminus of the Hob proteins exhibits sequence homology to VPS13A/C and ATG2, two other large proteins that are conserved throughout eukaryotes. Both proteins have recently been shown to localize to ER MCS and to exhibit lipid transfer capabilities *in vitro* (Li et al., 2020; Valverde et al., 2019); structural analysis of VPS13A and ATG2 demonstrates that both fold to form a rod-like shape with a hydrophobic internal cavity that facilitates solubilization and transport of lipids (Li et al., 2020; Valverde et al., 2019). Thus, VPS13 and ATG2 have been described as the founding members of a new family of large LTPs that function at ER MCS. Recently released AlphaFold (Jumper et al., 2021) structural predictions of Hob proteins illustrate remarkable similarity to both VPS13A and ATG2, with Hobbit (and its orthologs) predicted to take on a long tube-like shape that is lined with hydrophobic amino acid residues

(Figure 1A–C). Although this structural prediction will need to be experimentally validated and a lipid transfer function directly tested, given that Hob proteins localize to ER-PM contact sites and bind directly to lipids, it seems likely that these proteins may be new members of the VPS13 and ATG2 family of LTPs at MCS.

VPS13 and ATG2 function appears to be required in situations that require rapid remodeling or growth of membranes, including phagophore formation during autophagy (ATG2) and prospore membrane formation during yeast meiosis (Vps13) (Ugur et al., 2020). In *Drosophila*, *hobbit* function is required cell-autonomously for regulated exocytosis, particularly for secretion of *Drosophila* insulin-like peptides (Dilps) from the insulin producing cells (IPCs) and release of massive quantities of mucin proteins from the larval salivary glands (Neuman & Bashirullah, 2018). Interestingly, mutation of the plant orthologs of *hobbit* also results in visible phenotypic defects in cells with high secretory loads, including elongating pollen tubes and expanding root hairs (Pietra et al., 2015; Procissi et al., 2003). The rapid and high-volume secretion of proteins results in a significant amount of organelle and plasma membrane flux; thus, Hob protein function may be required to maintain membrane equilibrium to sustain high secretory rates.

hobbit is an essential gene whose function is required for survival to adulthood (at least in *Drosophila*), while many other LTPs are not essential for viability. For example, mutation of human VPS13A results in the rare neurodegenerative disease chorea-acanthocytosis, a condition that usually manifests in early to mid-adulthood, and *VPS13A* knockout mice exhibit chorea-acanthocytosis-like phenotypes (Ugur et al., 2020). Flies with protein null mutations in *Vps13* (orthologous to mammalian VPS13A/C) survive to adulthood but exhibit neurological deficits (Ugur et al., 2020). In contrast, *hobbit* mutant flies die as pupae and thus fail to reach adulthood (Neuman & Bashirullah, 2018). Although the reason for this discrepancy is not yet clear, it seems likely that the Hob proteins perform an essential lipid transfer function that cannot be circumvented by other pathways. Thus, future analysis of the putative lipid transfer function of the Hob proteins will reveal new insights about the role of lipid trafficking during animal development.

Our work has revealed that the highly conserved but poorly characterized Hob proteins are novel and conserved lipid binding, and putative lipid transfer, proteins at ER-PM contact sites. This highlights the Hob proteins as new players in the rapidly evolving world of LTPs and their functions at MCS.

Declaration of Conflicting Interests

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