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Article Addendum

AtEHDs in endocytosis

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Endocytosis regulates many important and diverse processes in eukaryotic life. EH domain containing proteins function as regulators of endocytosis through protein-protein interactions. Several interactors of mammalian EHDs were identified, including clathrin machinery components. The four human EHD proteins share high homology at the protein level and possess similar domains, but appear to be involved in different stages of intracellular trafficking. EHD1 regulates recycling through the endocytic recycling compartment (ERC). EHD2 has been found to inhibit internalization in mammalians when overexpressed.

We have recently investigated the importance of EH domain containing proteins in plant endocytosis. We were able to show that both of the Arabidopsis EHD homologs, termed AtEHD1 and AtEHD2, play important roles in plant endocytosis. Knockdown of AtEHD1 delayed internalization, and overexpression of AtEHD2 inhibited endocytosis. Thus, the function of plant EHDs is highly homologous to that of mammalian EHDs.

Introduction

Endocytosis is the process by which cells uptake extracellular substances and/or internalize plasma membrane proteins.[1] Mammalian endocytosis regulates many processes, such as homeostasis, nutrient uptake, cell polarity, retrieval of exocytosed vesicle components, downregulation of signaling receptors, localization/abundance of membrane transporters and cell-to-cell signaling.[2-4] From the early/sorting endosomes, plasma membrane proteins are either targeted to the lysosome for degradation or recycled back to the cell surface, through the endocytic recycling compartment.[2-4]

Studies in yeast and mammalian cells revealed that endocytosis depends on regulated interactions between a variety of proteins and lipids through specific modules. One such module is the Eps15 homology (EH) domain, a conserved, protein-interaction domain, found in several endocytic proteins.[5] EH domain containing proteins function as regulators of endocytosis through their ability to interact with other proteins involved in this process. Several interactors of mammalian EHD proteins were identified such as SNAP29 and IGF-1R as well as clathrin machinery components including AP2 and clathrin heavy chain.[6]

Although the four human EHD proteins share high homology at the protein level and possess similar domains, they appear to participate in different aspects of intracellular trafficking.[7-13] The best characterized mammalian EHD is EHD1, which was shown to mediate transport through the endocytic recycling compartment (ERC).[14] EHD2 is a plasma membrane associated protein, and appears to differ from the other mammalian EHDs both in sequence and in subcellular localization. Its overexpression leads to inhibition of endocytosis in mammalian cells (Benjamin S and Horowitz M, unpublished results).[11]

Shared Functions for Mammalian and Plant EHDs

In a recent work,[15] we described the isolation and characterization of two Arabidopsis EH domain containing proteins (AtEHD1 and AtEHD2). AtEHD1 is primarily localized to endosomes while AtEHD2 is plasma membrane associated. The two proteins were shown to be involved in endocytosis in plant systems, and to function similarly to mammalian EHDs. Downregulation of AtEHD1 delays internalization (which may or may not reflect a delay in recycling of endocytic components), while overexpression of AtEHD2 has an inhibitory effect on endocytosis. Based on our results, and considering that Arabidopsis EHDs share similar functions with mammalian EHDs, we would like to propose the model depicted in Figure 1.

Internalized molecules, including plasma memberane (PM) resident receptors, reach the early endosome and/or recycling endosome on which mammalian EHD1 resides. AtEHD1 was found to be co-localized with ARA6, ARA7 and FYVE,[15] indicating that it also resides on early endosomes,[4,16-18] from which recycling back to the plasma membrane occurs in plant cells.[17] Mammalian EHD1 was found to reside primarily in the ERC,[19,20] as well as on early endosomes[21] and vesicular tubular structures.[22] Though evidence of recycling endosomes exists in plants,[23,24] such endosomes have not been well characterized. Previous work in the field has shown that materials are recycled to the TGN and back to the plasma membrane from early endosomes.[25,26] Thus, AtEHD1 resides on early endosomes and recycling endosomes, which may be the same endosomes in the case of plants. From EHD1 positive endosomes, some of the PM receptors are recycled back to the cell surface.

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Mammalian and plant EHD1 clearly function in consort with other endocytic/ recycling proteins, as knockdown of EHD1 results only in a mild recycling phenotype, in both plants and mammalians.[13,15]

EHD2 resides primarily at the plasma membrane. Though the expression level of AtEHD2 is very low under normal conditions, upon overexpression it acts to diminish internalization of such "classical" endocytic cargos as FM-4-64 and transferrin, in plant and mammalian cells respectively.[11,15] Plant EHD2 most likely inhibits the clathrin-dependent pathway,[15,27] though it could possibly affect other pathways as well. Our recent unpublished results suggest that plant EHD2 inhibits endocytosis of other receptors upon overexpression, including LRR-Receptor like proteins in plant cells. (Bar M and Avni A, unpublished). We have previously shown[28] that signaling of the tomato LeEIX receptor requires the endocytic process. This signaling induces programmed cell death. There is a possibility that plant EHD2 controls LeEIX signaling via modulation of its endocytosis, thereby limiting the level of the response. Plant EHD2 may also serve to modulate other signaling processes in which endocytosis is involved. For example, plant EHD2 may regulate auxin signaling via regulation of PIN receptor endocytosis which is clathrin dependent.[27] This could indicate that plant EHD2 is part of a more general ubiquitous control mechanism of signaling/inhibition associated with receptor mediated endocytosis, and is currently being investigated further. EHD2 was found to be coupled to the actin cytoskeleton in mammalians.[11,29] Our recent evidence suggests that this is the case in plants as well (Bar M and Avni A, unpublished). The picture that emerges from all these data indicates that EHD2 has a crucial function in receptor mediated endocytosis and signaling in eukaryotic cells. It is not clear at this point how EHD2 exerts this function under native conditions, but one clue could be that it possesses plasma membrane and not endosomal localization in both mammalian (Benjamin S and Horwitz M, unpublished results) and plant cells.[11,15] Perhaps fluctuations in local concentration of EHD2 at micro-domains within the plasma membrane can regulate the level of endocytosis at different points throughout the cell. Our inability to obtain EHD2 knock-out plants in our recent work[15] does nevertheless indicate that EHD2 function is extremely important. Soluble Toll-like receptors (TLR) were shown to inhibit the signaling of membrane TLR by binding to the TLR specific ligand,[30] thus serving as decoy receptors. Similarly, the expression level of EHD2 may modulate the endocytic process and provide negative regulation when required.

In conclusion, the model proposed herein and the work on which the model is based demonstrate that EHD proteins play an important role in endocytosis in plants. The role of each protein can be correlated to an extent to the function of mammalian EHDs. Future work concerning EHD1 should focus on deciphering its function in recycling, while EHD2 should be examined in light of its inhibitory functions on many different receptors/ligands in the context of signaling.

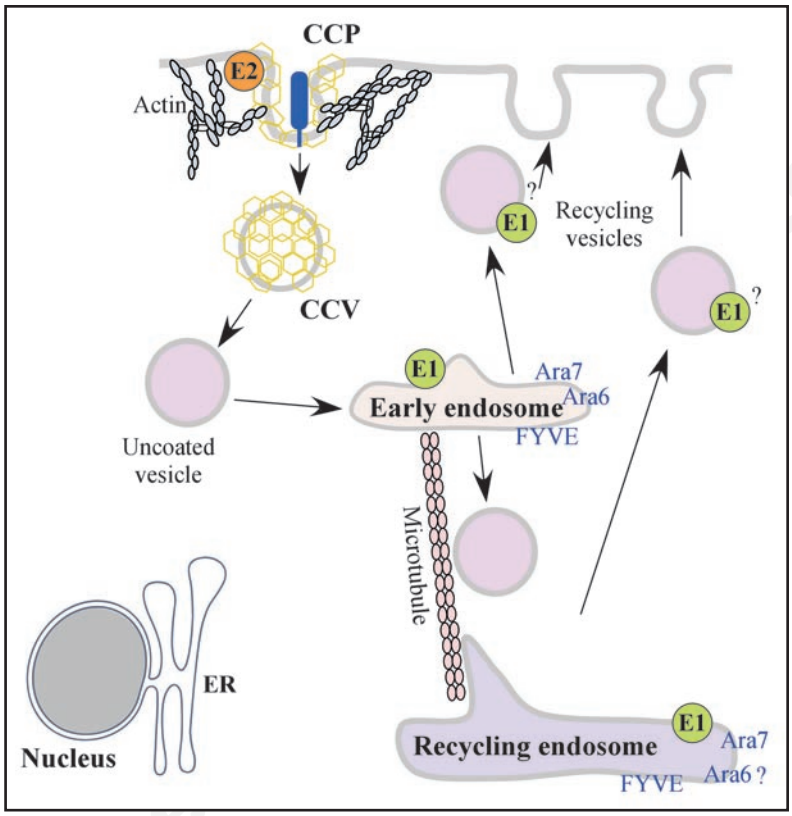

Figure 1. Schematic representation of Plant EHDs localization and function. In Green, localization of AtEHD1; in orange, localization of AtEHD2. Arrows point to trafficking pathways from the membrane to the early/re-cycling endosome and back to the membrane. Localization of known markers is indicated. E1 = AtEHD1, E2 = AtEHD2, CCP = clathrin coated pit; CCV = clathrin coated vesicle.

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