論文の内容の要旨

The novel membrane deformation ability of the ankyrin-repeat-containing protein ANKHD1 and its involvement on the early endosome

(アンキリンリピート含有タンパク質 ANKHD1 の新規脂質膜小胞化活性と
その初期エンドソームへの関与の同定)

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Membrane tubulation and vesicle formation are required for various intracellular events such as endocytosis, vesicle transport, and organelle division. Lipid membrane is deformed to tubules and vesicles by lipid-binding proteins. Membrane curvature is thought to be generated by the insertion of the portion of the proteins including amphipathic helix as well as by the scaffolding proteins that bind to the membrane surface for bending. However, the number of membrane-deforming proteins is limited.

The ankyrin repeat domain (ARD) are conserved in approximately 600 proteins in human. The ARDs are composed of the sequence of several numbers of the ankyrin repeats (ANKs), which consist of two antiparallel α-helices. The number of ANKs varies among ARDs, and the sequence of ANKs often adopt the curved structures reminiscent of the Bin-Amphiphysin-Rvs (BAR) domain, which is the dimeric membrane scaffold for membrane tubulation. The BAR domains sometimes have the amphipathic helices, that enables the membrane scission leading to small vesicle formation

(vesiculation). There are several ARD-containing proteins that have lipid-binding ability. However, the membrane binding and deformation abilities of most of the ARD-containing proteins had been unclear.

In this study, I analyzed 18 ARDs of highly transcribed proteins for their membrane binding and vesiculation abilities by quantitative liposome co-sedimentation assay. I found that the ARD of ankyrin repeat and KH domain-containing protein 1 (ANKHD1) dimerized and efficiently deformed the membrane into tubules and vesicles. ANKHD1 contains 25 ANKs that are divided into the first 15 ANKs and the latter 10 ANKs (Figure 1). I found that the first 15 ANKs formed dimer, and the latter 10 ANKs had the membrane tubulation and vesiculation abilities. The latter 10 ANKs were predicted to have a curved structure with positively charged protein surface like the BAR domain. Furthermore, similar to the BAR domain proteins with membrane vesiculation ability, there is the amphipathic helix (1400-1415 aa) adjacent to the latter 10 ANKs. I found that the mutations of the positively charged amino-acid residues in the latter 10 ANKs and the deletion of the amphipathic helix (1400-1415 aa) decreased the membrane vesiculation ability in vitro. Interestingly, the combination of the first 15 ANKs and the latter 10 ANKs resulted in the dimeric 25 ANKs, and the dimeric 25 ANKs had significantly higher membrane vesiculation ability than the latter 10 ANKs alone. These results showed that ANKHD1 vesiculated the liposome in a similar manner as the dimeric BAR domain protein with membrane vesiculation ability (Figure 2).

I then examined the cellular function of ANKHD1. The number of early endosomes was increased in ANKHD1-knockdown cells. The early endosomes are regulated by the small GTPase Rab5, that induces membrane fusion to enlarge the early endosomes. Rab5 was co-localized with ANKHD1 at the possible membrane scission sites of the early endosomes, and the enlargement of early endosomes by constitutive active mutant of Rab5 was inhibited by ANKHD1. However, the knockdown of ANKHD1 did not induce significant changes in the maturation of early endosomes to late endosomes or lysosomes. These results suggest that the membrane vesiculation by ANKHD1 negatively regulates the enlargement of early endosomes through the membrane vesiculation ability of the ANKHD1.

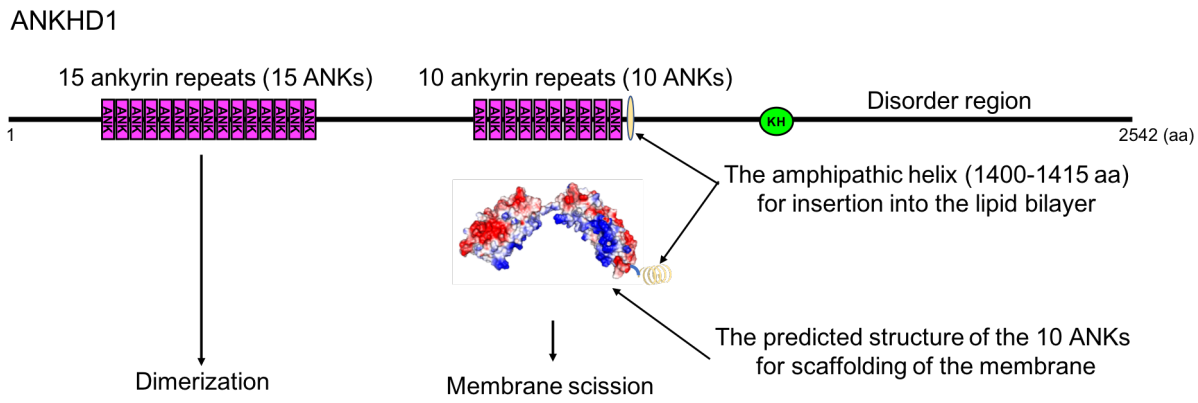

Figure 1. Domain structures and their functions of ANKHD1

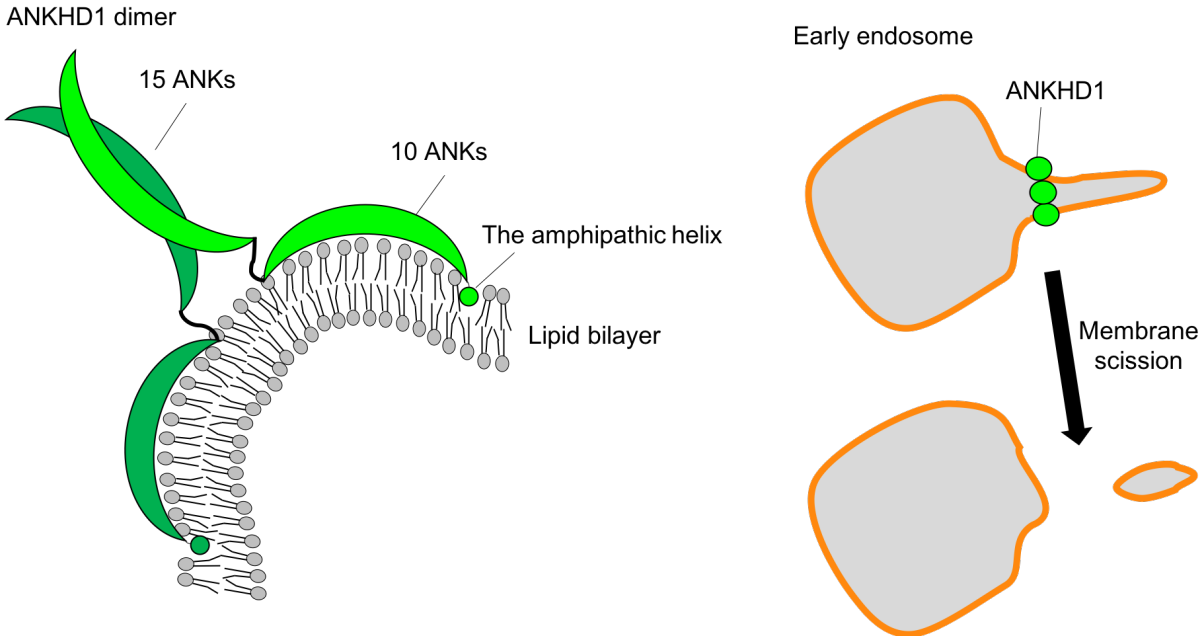

Figure 2. The membrane scission by the dimerized ANKHD1 at the early endosomes