Kentaro Hanada

Profile

Kentaro Hanada received his Bachelor degree of Pharmaceutical Science from The University of Tokyo in 1983, and Ph.D. degree (supervisor: Prof. Yasuhiro Anraku) from the same university in 1988. During undergraduate period, he succeeded in purification and reconstitution of an amino acid transporter from bacteria, which is probably the first instance for functional purification of membrane proteins with multiple TMD by using a biotechnologically linked 'tag.' In 1988, he joined Department of Chemistry, National Institute of Health of Japan (afterward renamed to Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases) as a research staff and started biochemical and genetic studies on sphingolipid metabolism with Drs. Yuzuru Akamatsu and Masahiro Nishijima. He isolated a CHO cell mutant defective in serine palmitoyltransferase, the enzyme for the first step of the sphingolipid biosynthesis, and showed that sphingolipids are essential for the growth of mammalian cells. From 1993 to 1995, he stayed in the late Dr. Richard Pagano's laboratory in USA with Long-term Fellowship of Human Frontier Science Program and worked on isolation of CHO cell mutant defective in uptake of a fluorescent phosphatidylserine analog. After return to Japan, he attempted to isolate various types of CHO cell mutants for sphingomyelin metabolism and found a new mutant with dysfunction of ceramide trafficking. Then, he identified CERT, the molecular machinery for ER-to-Golgi transport of ceramide, and opened a way to understand molecular mechanisms underlying intracellular sorting of lipids. Currently, he is the Director of Department of Biochemistry and Cell Biology, NIID.

Intracellular trafficking of ceramide by the ceramide trafficking protein CERT

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Synthesis and sorting of lipids are essential events for membrane biogenesis. Intracellular trafficking of lipids may also be important for lipid-mediated signaling events. Ceramide is synthesized at the endoplasmic reticulum (ER), and transported to the Golgi compartment for conversion to sphingomyelin (SM). We have identified a 68-kDa cytosolic protein named CERT to be a key factor for ER-to-Golgi trafficking of ceramide[1]. CERT consists of three parts (Figure.1). The amino terminal ~120 amino acid region forms a pleckstrin homology (PH) domain, which has a Golgi-targeting function by recognizing phosphatidylinositol-4-monophosphate (PI4P). The carboxyl terminal ~230 amino acid region forms a START domain capable of extracting ceramide from membranes and transferring the bound ceramide to membranes. CERT can transfer various molecular species of ceramide but not other lipid types including sphingosine and SM, indicating its substrate specificity to ceramide and also flexibility to natural isoforms of ceramide[1)2)]. X-ray structural analysis of co-crystals of the CERT START domain in complex with ceramide has revealed how CERT exhibits the substrate specificity and flexibility[3)]. The middle region between the PH and START domains appears to form no globular domains, but has the short peptide FFAT motif to interact with VAP, an ER membrane protein. Mutations in the FFAT motif of CERT impair not only the VAP interaction but also ER-to-Golgi trafficking of ceramide, although the FFAT mutations do not affect the activity of CERT to catalyze ceramide transfer between artificial phospholipid vesicles[4)]. The middle region receives phosphorylations that regulate the function of CERT[5)]. On the basis of these results, we have proposed that CERT extracts ceramide from the ER and carries it to the Golgi apparatus in a non-vesicular manner and that efficient CERT-mediated trafficking of ceramide occurs at membrane contact sites between the ER and the Golgi apparatus (Figure.2)[4)6)].

In addition, we had previously developed the chemical inhibitor HPA12 of intracellular trafficking of ceramide, and found that the HPA compound is an antagonist of CERT[7)8)]. The CERT START domain has a pair of tryptophan residues likely responsible for interaction with phospholipid membranes[3)9)]. Comparison of co-crystals revealed that two side chains of the tryptophan pair are protruded outwards in the complex with ceramide, but not with the inhibitor, suggesting an unexpected mode of inhibition mechanism by HPA series[10)].

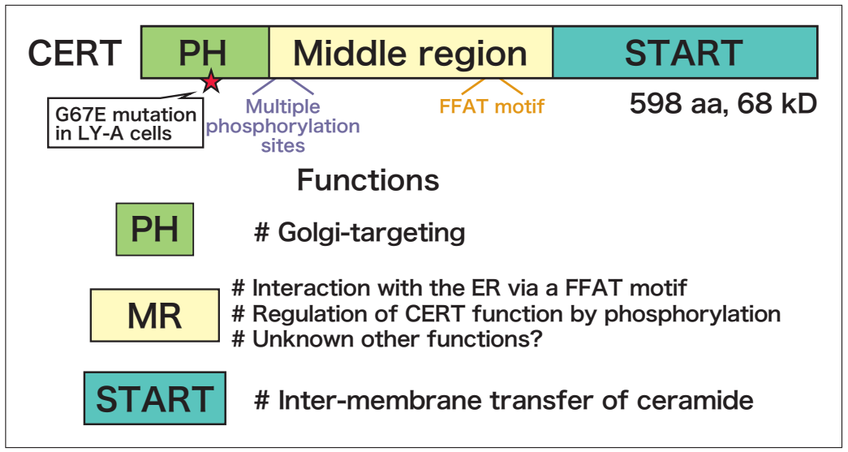

Figure 1 Structure of CERT

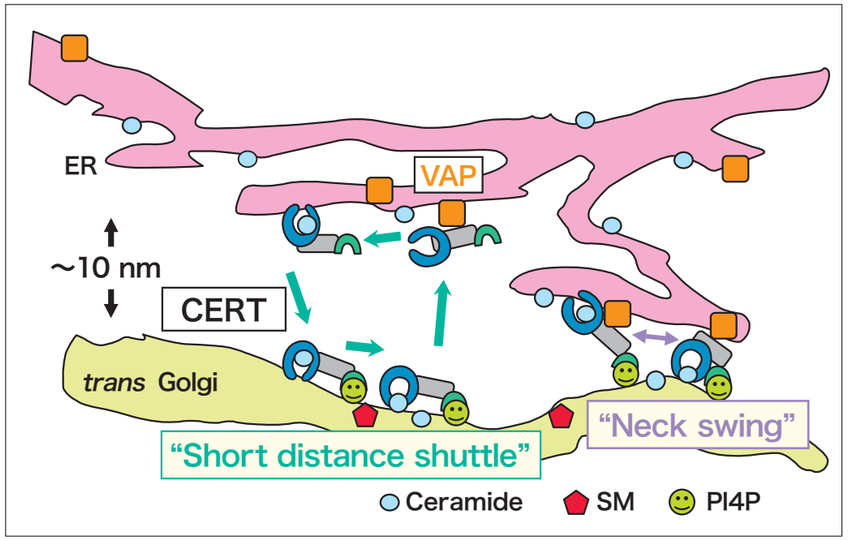

Figure 2. Possible models for CERT-mediated trafficking of ceramide at the ER-Golgi membrane contact sites

References

1) Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, Nishijima M. Molecular machinery for non-vesicular trafficking of ceramide. Nature (246) : 803-809, 2003.
2) Kumagai K, Yasuda S, Okemoto K, Nishijima M, Kobayashi S, Hanada K. CERT mediates intermembrane transfer of various molecular species of ceramides. J. Biol. Chem. (280): 6488-6495, 2005.
3) Kudo N, Kumagai K, Tomishige N, Yamaji T, Wakatsuki S, Nishijima M, Hanada M, Kato R. Structural basis for specific lipid recognition by CERT responsible for nonvesicular trafficking of ceramide. Proc. Natl. Acad. Sci. USA (105) 488-493, 2008.
4) Kawano M, Kumagai K, Nishijima M, Hanada K. Efficient trafficking of ceramide from the endoplasmic reticulum to the Golgi apparatus requires a VAMP-associated protein-interacting FFAT motif of CERT. J. Biol. Chem. (281) 30279-30288, 2006.
5) Kumagai K, Kawano M, Shinkai-Ouchi F, Nishijima M, Hanada K. Interorganelle trafficking of ceramide is regulated by phosphorylation-dependent co-operativity between the PH and START domains of CERT. J. Biol. Chem. (282): 17758-17766, 2007.
6) Hanada K. Kumagai K, , Tomishige N, Yamaji T. CERT-mediated trafficking of ceramide. *Biochim. Biophys. Acta* (1791): 684-691, 2009
7) Yasuda S, Kitagawa H, Ueno M, Ishitani H, Fukasawa M, Nishijima M, Kobayashi S, Hanada K. A novel inhibitor of ceramide trafficking from endoplasmic reticulum to the site of sphingomyelin synthesis. *J. Biol. Chem.* (276): 43994-44002, 2001
8) Kumagai K, Yasuda S, Okemoto K, Nishijima M, Kobayashi S, Hanada K. CERT mediates intermembrane transfer of various molecular species of ceramides. *J. Biol. Chem.* (280): 6488-6495, 2005
9) Sugiki T, Takahashi H, Nagasu M, Hanada K, Shimada I. Real-time assay method of lipid extraction activity. *Anal. Biochem.* (399): 162-167, 2010
10) Kudo N, Kumagai K, Matsubara R, Kobayashi S, Hanada K, Wakatsuki S, Kato R. Crystal structures of the CERT START domain with inhibitors provide insights into the mechanism of ceramide transfer. *J. Mol. Biol.* (396) : 245-251, 2010