Insight into the topology of the monotopic hairpin protein UBXD8 in endoplasmic reticulum bilayer and lipid droplet monolayer membranes

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Abstract

Lipid droplets (LDs) are ubiquitous organelles that act as an “energy sink” to sequester excess metabolic energy in the form of neutral lipids, particularly triacylglycerides and sterol esters. They originate from the endoplasmic reticulum where the local accumulation of neutral lipids within the phospholipid bilayer membrane triggers the formation of new LDs that eventually consist of a hydrophobic neutral lipid core that is encapsulated by a phospholipid monolayer. The surface of LDs is decorated with several proteins, including metabolic enzymes that regulate LD functions. Class I LD proteins are initially inserted into the ER bilayer membrane from where they can partition to the LD monolayer surface. They can associate with the membranes using amphipathic helix motifs or by adopting a monotopic hairpin topology. How such proteins are targeted and topologically arranged in ER and LD membranes is still not fully understood. In this study, we combined a cysteine solvent accessibility method (PEGylation assay) with molecular dynamics (MD) simulations to obtain structural insight into the topology of the ER/LD protein UBXD8 in both, ER and LD membranes. We could precisely map the membrane-embedded UBXD8 hairpin domain that is buried inside the ER bilayer and revealed that this domain is more solvent exposed on the LD monolayer. MD simulations corroborate our experimental observations and suggest that UBXD8 adopts a deeply embedded V-shape topology in the phospholipid bilayer with two anti-parallel α-helices facing each other. In contrast, on the LD monolayer, UBXD8 is more solvent exposed by adopting a shallow conformation resulting from the opening of α-helices. These findings allow us to raise new working hypothesis on the molecular mechanisms underlying ER-to-LD protein partitioning.