Analyzing the Intersections Between the Endoplasmic Reticulum and Lipid Droplets

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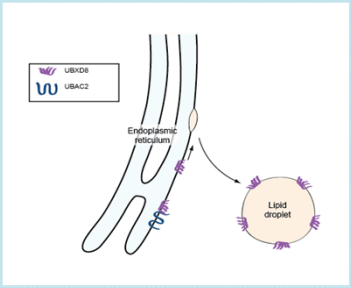
Abstract

The membrane embedded protein UBXD8 has been connected to ERAD and Lipid Droplet synthesis/breakdown. Our research has shown that the thioredoxin-like domain (THL) of UBXD8 is necessary to the binding of UBAC2, an ER protein that restricts trafficking of UBXD8 to lipid droplets. We hypothesize that the THL domain of UBXD8 alone is sufficient for binding UBAC2. The THL domain of UBXD8 was cloned to test this hypothesis through affinity purification and western blotting. As a second verification, fluorescence microscopy will be used. By elucidating protein to protein interactions that regulate ERAD and Lipid Droplet metabolism, it could develop into important implications for our understanding of lipid droplet function in cell biology and disease.



Introduction

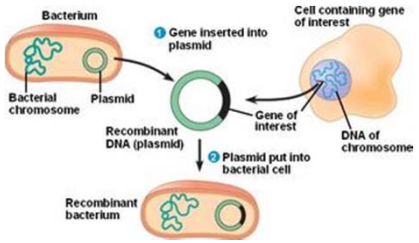
- Many cardiovascular diseases have been traced back to inadequate storage of nutrients inside cells.
- Nearly every cell type stores nutrients as triacylglycerol in lipid droplets, an endoplasmic reticulum-derived organelle.
- Our research has discovered an ubiquitination complex that is a part of ERAD and connected to Lipid Droplets.
- The ubiquitination complex plays many important roles in the organelle that include signaling protein for degradation.

Methods and Results

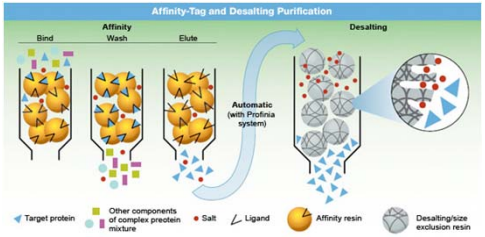
Full length UBXD8 protein

Isolate the THL domain of UBXD8

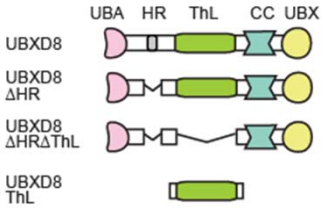
Cloning of UBXD8 THL-domain



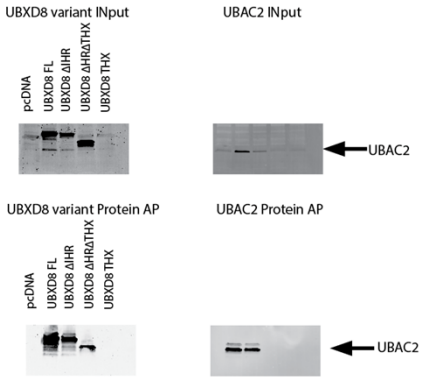
Affinity Co-Purification uses S-protein agarose bead to bind to S-tagged protein complexes

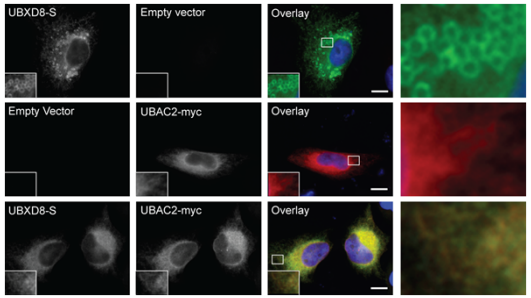


UBXD8 deletion constructs



Western Blot Affinity Purification Results

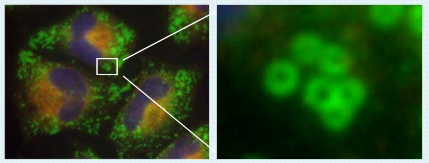




Fluorescence microscopy images of UBXD8 and UBAC2 overexpressed and co-expressed.

Conclusion and Discussion

In order to assess the domains that are directly related to UBXD8 tethering to UBAC2 we generated different deletion constructs of UBXD8 and assessed their ability to bind to UBAC2. All UBXD8 fragments missing the THL domain were unable to bind to UBAC2. Thus the THL domain is necessary for UBXD8 to bind to UBAC2.



Fluorescent microscopy image of Lipid Droplets stained in the cell by Bodipy.

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References

1. Olzmann JA, Richter CM, Kopito RR (2013) Spatial regulation of UBXD8 and p97/VCP controls ATGL-mediated lipid droplet turnover. Proc Natl Acad Sci U S A 110: 1345- 1350.

2. Olzmann JA, Kopito RR, Christianson JC (2013) The mammalian endoplasmic reticulum- associated degradation system. Cold Spring Harb Perspect Biol 5.

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