ORGANELLE "HUGGING"

Regulation of peroxisome-ER interactions in mammalian cells

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1. INTRODUCTION & SUMMARY

Cells consist of different compartments, which are called **organelles**. They provide specialized environments for specific tasks. Most people have heard of the nucleus – DNA storage, and mitochondria – powerhouses of the cell. But are less familiar with other important organelles:

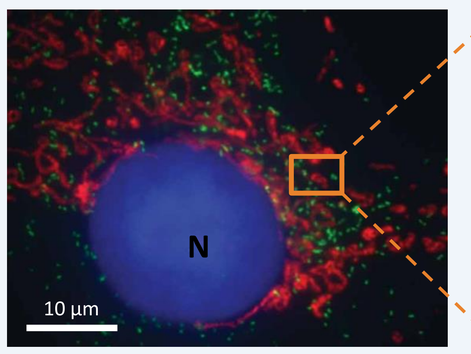
- **Peroxisomes (PO)**: small, membrane-bound organelles – degradation of fatty acids/lipids (VLCFAs), the synthesis of other lipids (e.g. plasmalogens), detoxification (ROS)
- **Endoplasmic reticulum (ER)**: a network of membranous tubules – protein folding and modification, the synthesis of lipids (e.g. membrane lipids, plasmalogens)

Each organelle has its own role, but does not function as an isolated, static entity. Peroxisomes and the ER work together for certain processes, such as the synthesis of plasmalogens – nerve/brain lipids. **They physically interact ("hugging")** to make communication and metabolite (e.g. lipids) exchange efficient. We discovered that this interaction is mediated by two tether proteins; ACBD5 at the peroxisomal membrane interacts with VAPB at the ER membrane, and that **their interaction is regulated by negatively charged groups i.e. phosphorylation.** The less ACBD5 binds to VAPB, the less peroxisomes and the ER "hug", which means that they collaborate less to make these important nerve lipids. Hence, ACBD5 deficient patients present with severe neurological problems.

METHODS The interaction between ACBD5 and VAPB can be explored using **binding assays [immunoprecipitation, IP]**. In the experiments, Myc-ACBD5 was expressed in COS-7 cells, a monkey kidney fibroblast-like cell line, and subsequently, pulled down using its tag (Myc). Its interaction with endogenous VAPB was examined using western blotting (bands represent protein amounts).

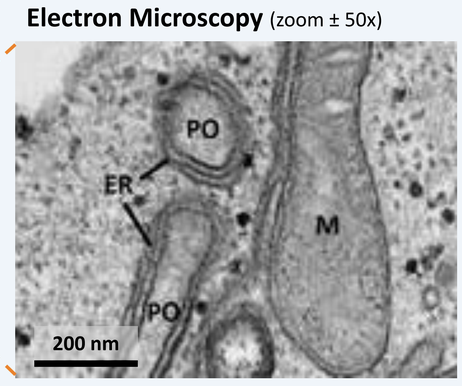
2. BACKGROUND (1) - PEROXISOME-ER "HUGGING"

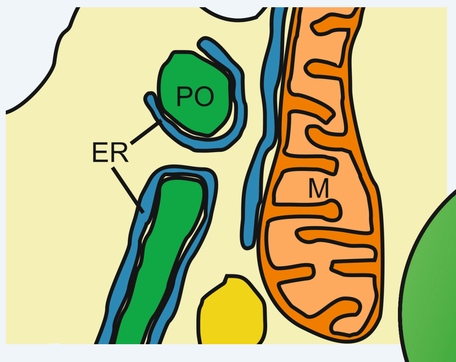
Immunofluorescence



Different compartments of the cell, organelles, are fluorescently labelled.

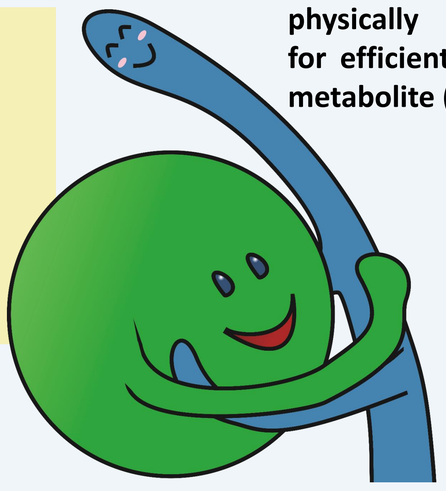
Electron Microscopy (zoom ± 50x)





Organelles are dynamic, interact with each other and cooperate to coordinate physiological functions.

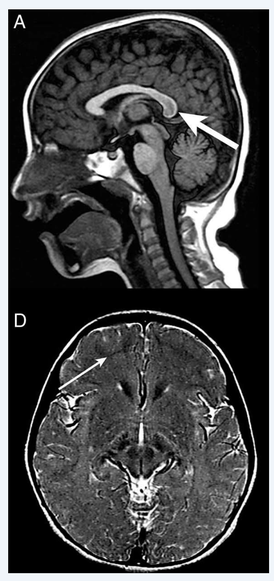
N, nucleus; PO, peroxisome; M, mitochondria; ER, endoplasmic reticulum



Peroxisomes and the ER work together in the cell. They physically interact ("hugging") for efficient communication and metabolite (e.g. lipids) exchange.

Disruption of this interaction affects for instance the synthesis of brain lipids (plasmalogens), causing neurological problems in patients.

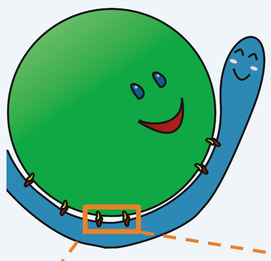
(Right image: brain MRI scan of ACBD5 deficient patient.)



Ferdinandusse *et al.* 2017

3. BACKGROUND (2)

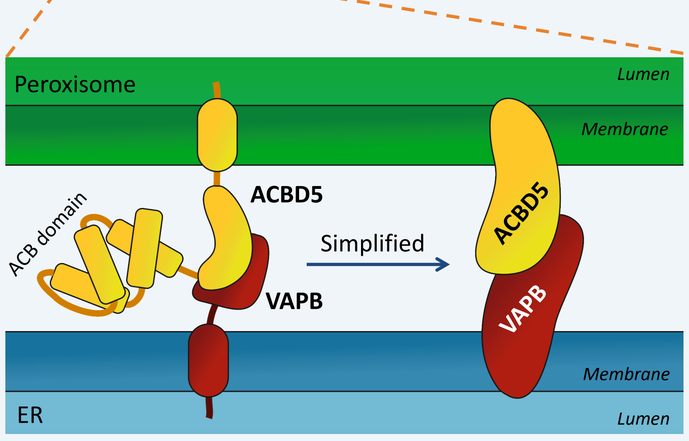
HOW DO PEROXISOMES AND THE ER INTERACT?



The narrow contacts [membrane contact sites] between peroxisomes and the ER are mediated by the interaction of two proteins:

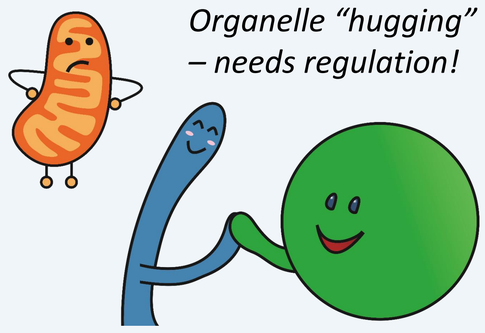
- **ACBD5**, at the peroxisomal membrane
- **VAPB**, at the ER membrane.

(Costello *et al.* 2017)



ACBD5, acyl-CoA binding domain containing protein 5;
VAPB, vesicle-associated membrane protein-associated protein B

4. RESEARCH QUESTION



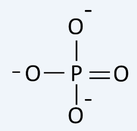
Organelle "hugging" – needs regulation!

In recent years it is has become evident that **all organelles physically interact with other organelles** in cells. As these interactions are very likely dependent on the needs of the cell and so, the major function of an organelle at that moment, **organelle contacts need to be dynamic and thus, regulated.**

HOW IS THE PEROXISOME-ER INTERACTION REGULATED?

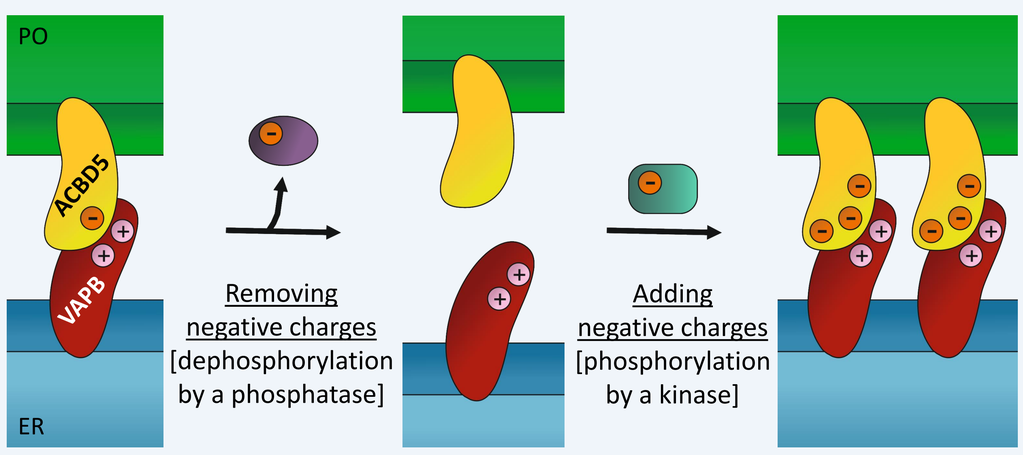
5. HYPOTHESIS/MODEL

The attraction between positive and negative charges plays a role in the interaction between VAPB and ACBD5.



PHOSPHATE GROUP
Negatively charged

VAPB has a positively charged surface. Removing and adding negatively charged phosphate groups to ACBD5 may regulate the VAPB-ACBD5 interaction.



6. RESULTS (1)

PHOSPHATASE-SENSITIVE ACBD5-VAPB BINIDING

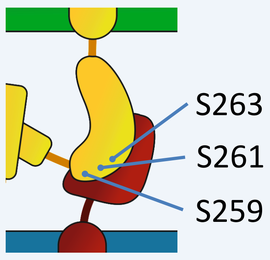
Figure 1. Phosphatase treatment inhibits the ACBD5-VAPB binding. The input shows Myc-ACBD5 and VAPB in COS-7 cells at the beginning of the experiment. IP shows the ACBD5-VAPB interaction, with and without λ-phosphatase (λPP) treatment of cell lysates. Normally VAPB binds to ACBD5 (-). After the λPP treatment [removing the negatively charged phosphate groups] VAPB does not bind (+).

The ACBD5-VAPB binding appears to be phosphatase sensitive.

However, λ-phosphatase acts on all proteins present in the cell lysate, not specifically on ACBD5. Therefore, we decided to explore phosphorylation of the VAPB-binding site of ACBD5.

7. RESULTS (2)

PHOSPHORYLATION SITES CLOSE TO THE VAPB-BINDING SITE AFFECT THE ACBD5-VAPB BINDING



Phosphate groups bind to specific sites of a protein, for instance to Serine residues (S). **ACBD5 has several Serines close to the VAPB-binding site. To generate phospho-dead mutants we replaced them for Alanine (A)**, which cannot be phosphorylated.

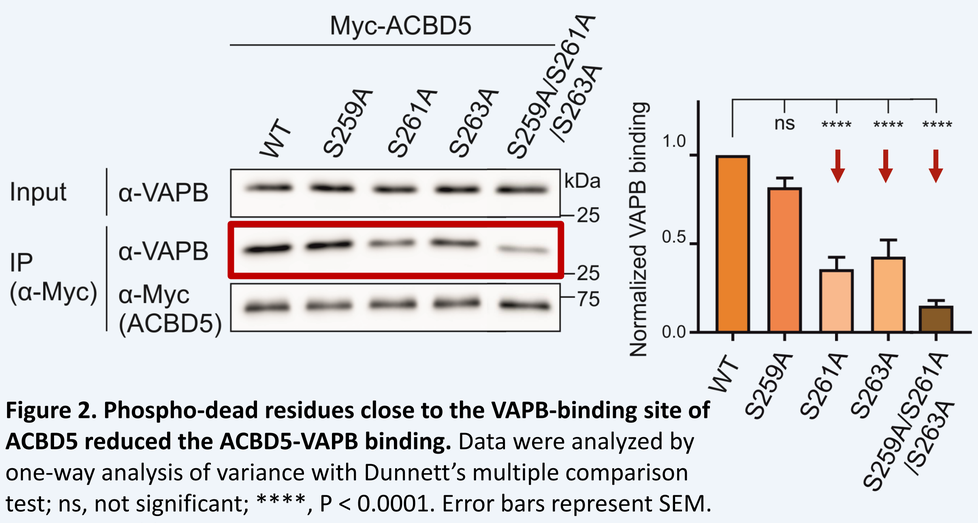
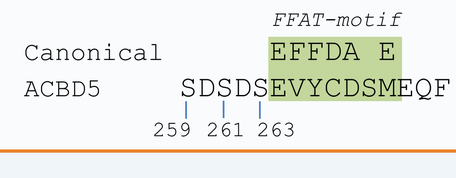


Figure 2. Phospho-dead residues close to the VAPB-binding site of ACBD5 reduced the ACBD5-VAPB binding. Data were analyzed by one-way analysis of variance with Dunnett's multiple comparison test; ns, not significant; ****, $P < 0.0001$. Error bars represent SEM. n = 3. WT, wild type.

IN MORE DETAIL: THE ACBD5/FFAT-VAPB BINDING

ACBD5 binds VAPB with its **FFAT-like motif** (two phenylalanines (**FF**) in an **a**cidic **t**ract). Different variations of this motif can be found in many proteins. Thus, the FFAT-VAPB interaction is a common way to target proteins to the ER membrane. Protein-specific phosphorylation might be a mechanism to regulate the different interactions under certain cellular conditions.



8. CONCLUSION & DISCUSSION

Phosphorylation sites close to the VAPB-binding site of ACBD5 affect the ACBD5-VAPB binding (Fig. 2), which likely contributes to the phospho-sensitivity of this interaction (Fig. 1). This supports our proposed model.

To explore this data further:

- Test ACBD5 phospho-mimetics
- Study the effect on peroxisome-ER interactions
- Identify phosphatase(s)/kinase(s)
- Identify conditions affecting peroxisome-ER contacts

References

Costello JL *et al.* (2017) J Cell Biol 216:331–342.
Ferdinandusse S *et al.* (2017) J Med Genet 54:330-337.

IMF image: Schrader, M (2019) *SciTech Eu* 32:194-195.
EM image: Costello et al. 2017

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