Lipid flippases: towards structure, function and a better understanding

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The asymmetric distribution of lipids within eukaryotic cellular membranes are of vital importance for signaling, vesicle formation and apoptosis. The distribution of lipid between the membrane leaflets is tightly controlled and the translocation is carried out by three groups of membrane proteins: flippases, floppases and scramblases. Floppases translocate lipids towards the exocytosolic leaflet whereas scramblases are bidirectional transporters, that serve to scramble the lipids distribution.

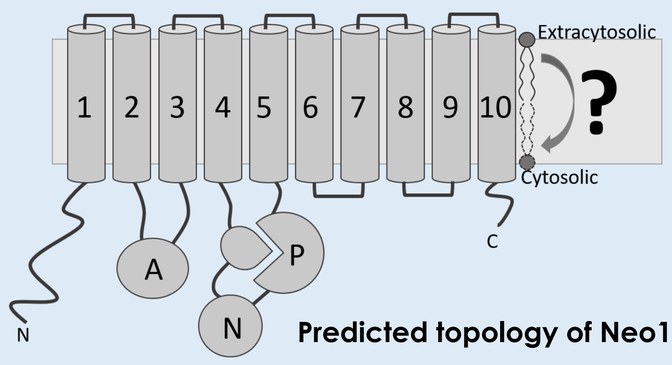
Lastly, the flippases translocate towards the cytosol and the majority are P4-ATPases, a P-type ATPase subfamily(1).

Mutations in P4-ATPases have been linked to neuronal degeneration, cerebral ataxia, mental retardation and Alzheimers'(2), highlighting the importance of gaining a better understanding of the membrane protein family.

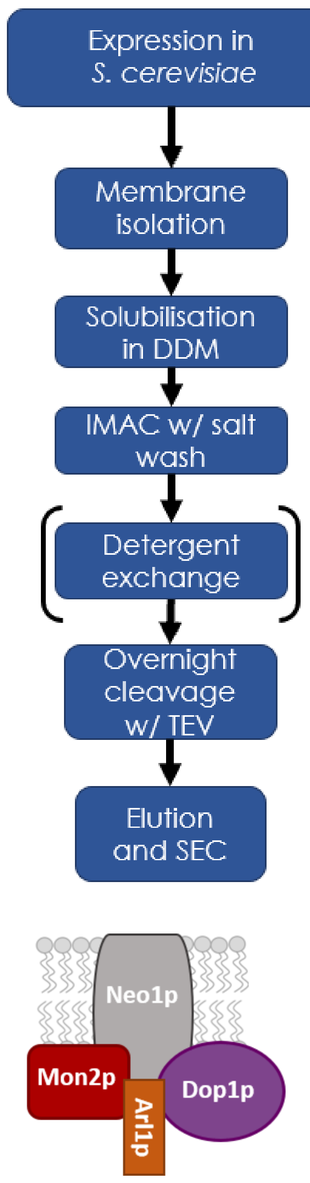
Neo1p

Unlike most P4-ATPases that exists as a heterodimeric complex with a CDC50 protein, Neo1p does not. To date, activity has not been measured *in vitro* and the lipid substrate(s) have yet to be identified.

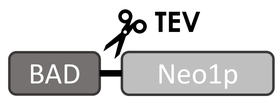
However, Neo1p knockout is terminal for cells(3).



Predicted topology of Neo1p



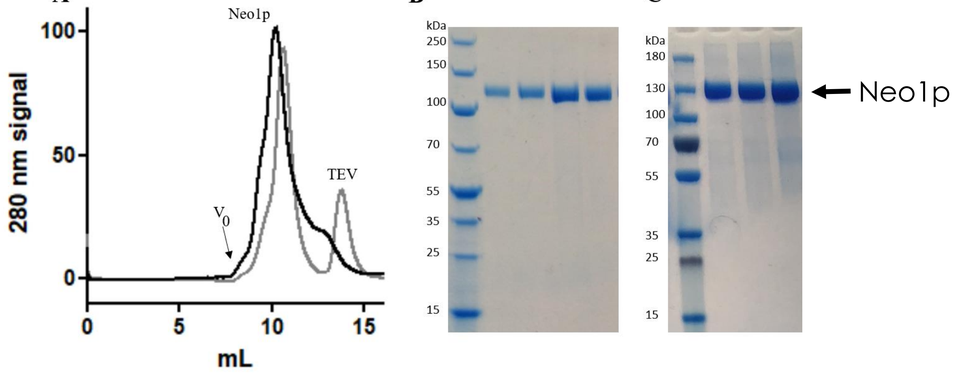
Purification strategy



Above: Neo1p construct with N-terminal BAD-tag

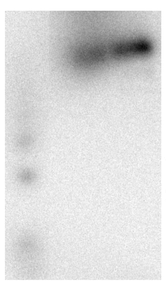
Left: established purification protocol.

Below: SEC profile on Superdex 200*inc* and SDS-PAGE analysis of peak fractions in DDM (gray and B)and LMNC (black and C)



Phosphorylation study

No ATPase activity has been observed towards a variety of tested yeast phospholipids, but a phosphoenzyme formation assay on purified protein (figure →) demonstrated Neo1p's ability to be phosphorylated.



Phospho-assay

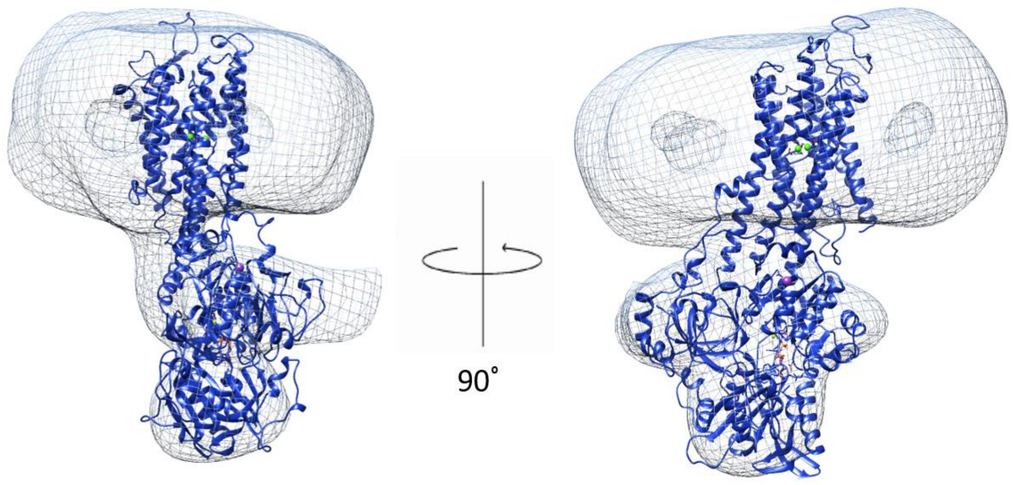
Radioactive ATP was added to two samples purified protein to test phosphoenzyme formation

Importance of complex formation

According to *in vivo* studies, Neo1p forms a complex with Dop1p, Mon2p and Arl1p (4). The function of the complex is not well studied. A similar complex has been indentified in *C. elegans* and connected to Wntless recycling (5).

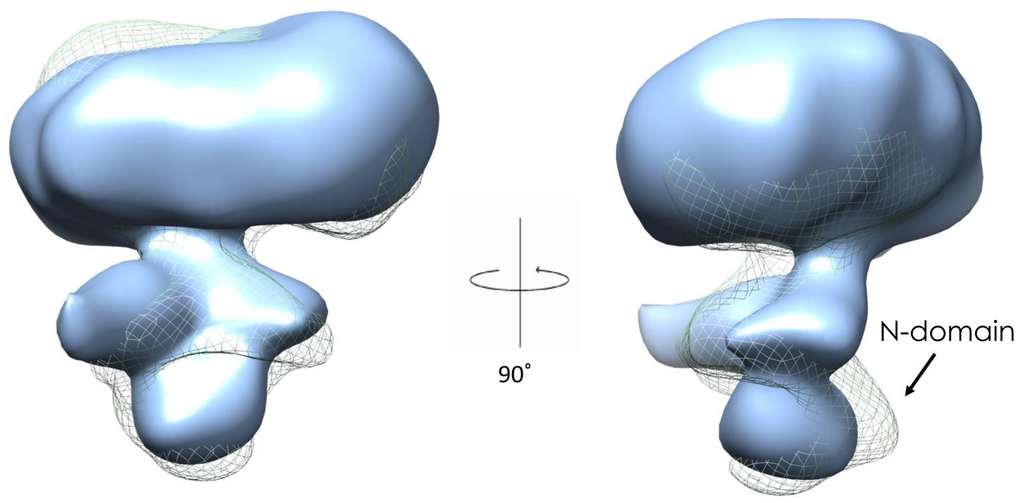
Negative stain EM

Different ATPases states of Neo1p were investigated using negative stain EM and state specific inhibitors: AMPPCP to capture an E1-ATP-like state and BeFx to capture an E2P state.



A previous Neo1p envelope with Neo1p-BeFx (E2P state) was aligned to the Neo1p-AMPPCP envelope by aligning manually placed SERCA structures.

A clear shift of the N-domain is seen, which is expected between E1 and E2 states.



Top: negative stain envelope with AMPPCP and SERCA in a E1-ATP manually docked.

Bottom: AMPPCP (mesh) and BeFx (blue) Neo1p envelopes compared. Aligned by manual docking to aligned SERCA structures. Uranyl formate was used as a stain in both cases.

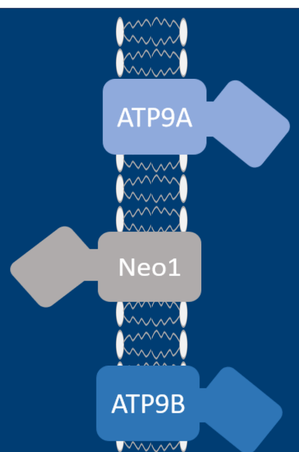
Future

Neo1p

- Is Neo1p a lipid flippase and what is its substrate?
- Inactivety is possible due to auto-inhibitory state of Neo1p.
  - Phosphorylation?
  - Unknown binding partner?
  - Regulatory lipids?
- Does Neo1p require an unknown accessory subunit?

Next

- Reconstitution into detergent-free system
- CryoEM to determine high-resolution structure



ATP9A/ATP9B

The mammalian Neo1p orthologs are predicted to be 118-128 kDa and function without a CDC50 accessory subunit (6). No *in vitro* data is availiable.

- Are they essential like Neo1p, either combined or individually?
- Substrate(s)?: *C. elegans* ortholog TAT-5 and Neo1p are suggested to translocate PE
- Are they auto-inhibited as Neo1p might be?

Next

- Clone and establish an expression and purification protocol
- Biochemical determination of ATPase activity and substrate(s)

REFERENCES

(1) Review: Holthuis, J. C. *Lipid traffic: floppy drives and a superhighway*. Nat. Rev. Mol. Cell Biol. (2005).

(2) Andersen, J. P. et al. *P4-ATPases as phospholipid flippases-structure, function, and enigmas.* Front. *Physiol.*(2016).

(3) Hua, Z., et al. *Requirement for Neo1p in Retrograde Transport from the Golgi Complex to the Endoplasmic Reticulum*. Mol. Bio. Cell. 2003

(4) Barbosa, S. et al. *Oligomeric Dop1p is part of the endosomal Neo1p-Ysl2p-Arl1p membrane remodeling complex. Traffic* (2010).

(5) Mcgough, I. J. et al. *SNX3-retromer requires an evolutionary conserved MON2:DOPEY2:ATP9A complex to mediate Wntless sorting and Wnt secretion. Nat. comm* (2018).

(6) Takatsu, H. et al. *ATP9B, a P4-ATPase, localizes to the trans-Golgi network in a CDC50 protein-independent manner.* J. Biol. Chem. (2011).

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