Fatty acid transport findings in plants pave the road to better biofuel production and transfer to lipid metabolism research in mammals

Dr Katrin Philippar from the Centre of Human and Molecular Biology (ZHMB) at Saarland University in Germany studies molecular plant physiology, biochemistry, and membrane transport. Her research mainly focuses on ion and metabolite transport in plastid organelles. Dr Philippar's research group made a groundbreaking discovery of the first protein ever to be associated with protein-mediated fatty acid transport from plastid organelles – FAX1. The group determined the localisation, provided detailed characterisation, and developed a model to explain the potential effect of FAX1 on membrane bending.

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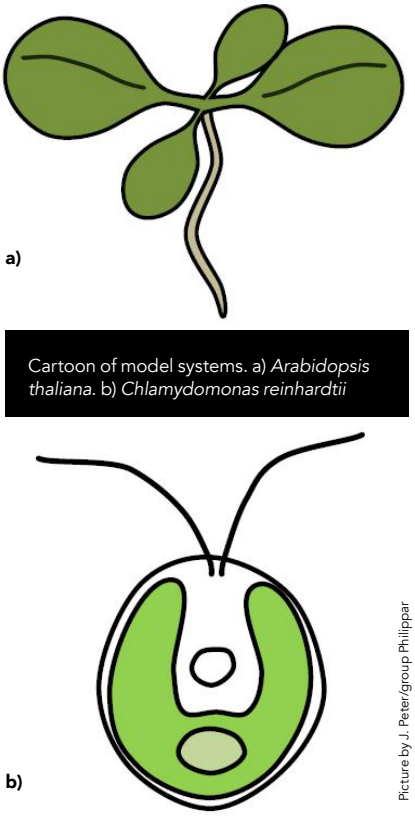
Fatty acids (FAs) are the key component of most lipids and lipid-based polymers found in plant cells, such as phospholipids, galactolipids, triacylglycerol oils, cutin, waxes, suberin, and sporopollenin/tryphine material. These different types of acyl lipids and lipid-based molecules and, by extension, the FAs that are their integral components, have very diverse functions in all life forms, ranging from prokaryotic bacteria to eukaryotic plants and mammals. FAs in phospho-, galacto-, and sphingolipids are assembled into bilayer membranes and are necessary for proper membrane function of all living organisms. Among others, these FAs and lipids are essential for healthy plant membrane biology. In oilseed plants, triacylglycerol oil lipids can be found in seeds where they serve as the dominant form of plant energy storage as well as a food resource for human nutrition or as a base product for biodiesel generation. Waxes and cutin on plant epidermal surfaces and suberin, found in the root endodermis, in bark, and the outer layer of seeds, form barriers that protect the plant from water loss and pathogen attack. Pollen fertility largely depends on the delivery of sporopollenin/tryphine material, composed of modified FAs and lipids, to outer pollen cell walls. In plants, FA/lipid remodelling and turnover are necessary to sequester carbon energy during senescence, protect against freezing during cold temperature, or replace phospholipids with galactolipids during phosphate starvation. Taken together, proper lipid metabolism is essential for growth, development, and performance of all beings, including plants.

WHAT MAKES FATTY ACID TRANSPORT CHALLENGING?

Plant lipid metabolism requires hundreds of enzymatic reactions that occur in several compartments of the cell. Therefore, the exchange of FAs, lipids, and metabolic intermediates between various organelles and membrane systems is necessary. *De novo* synthesis of FAs takes place in plastid organelles. These are compartments that are surrounded by two membranes and include chloroplasts, the cellular location in which photosynthesis, and thus CO_2 consumption as well as oxygen and sugar production, takes place. After synthesis, FAs are assembled into acyl lipids either inside plastid organelles or in the endoplasmic reticulum (ER). Because synthesis and assembly occur

in different parts of the cell, and because ER-derived lipids are part of plastid membranes, extensive lipid and FA transport between these two organelles is required (see Li et al., 2016; Li-Beisson et al., 2017). Lipophilic molecules such as FAs cannot move freely in the aqueous environment of the cell. Although it is generally accepted that free, unbound FAs are shuttled across plastid envelope membranes, the exact mode of export was largely unknown. Most of the knowledge on this subject to date has been acquired in the plant model organism *Arabidopsis thaliana*.



Cartoon of model systems. a) *Arabidopsis thaliana*. b) *Chlamydomonas reinhardtii*

Picture by J. Peter/group Philippar

FAX1 – THE FIRST PROTEIN WITH CONFIRMED ROLE IN FATTY ACID TRANSPORT

In 2015, Dr Katrin Philippar and her group (then at LMU Munich) made a breakthrough in our understanding of protein-mediated FA export from plastid organelles by discovering FAX1, the first membrane protein involved in the process (Li et al., 2015). FAX1 protein belongs to the superfamily of membrane proteins called TMEM14, whose function is so far largely unknown.

FAX1 and TMEM14 share four conserved α-helical domains. In *Arabidopsis*, FAX1 is part of a family of seven proteins (FAX1-7), and the loss of function in one of them could likely be compensated by the activity of the others, as ongoing research of the Philippar group on the FAX3 protein suggests. Based on proteomic and phylogenetic data, FAX1 in the model plant *Arabidopsis* was originally predicted to be plant specific, located in plastid membranes, and involved in transport processes. Expression of the FAX1 gene increased in plants with induced early senescence, a process that involves lipid remodelling and turnover. The researchers found *Arabidopsis* FAX1 transcripts to be present at all developmental stages, with highest expression in leaf tissues as well as in early pollen development.

Using fluorescent labelling and microscopy as well as immunoblotting, Dr Philippar and her group empirically demonstrated that FAX1 protein was localised in the plastid envelope of *Arabidopsis*. Similar analyses of pea chloroplasts conducted by the same group pinpointed the location of FAX1 to the inner plastid envelope membrane with its four α-helical membrane-spanning domains.

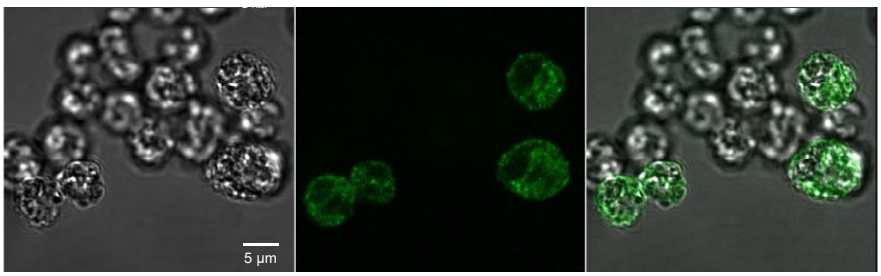
Proper lipid metabolism is essential for growth, development, and performance of all beings, including plants.

FAX1 WAS CHARACTERISED USING MODEL PLANT MUTANTS

To further determine the function of the protein, Dr Philippar's group investigated the characteristics of *Arabidopsis* mutants without a functional FAX1 gene and protein and mutants in which FAX1 was overexpressed (Li et al., 2015). The researchers found FAX1-deficient plants to be smaller than the wild-type plants, with thinner inflorescence stalks and flowers that produce smaller fruit with no seeds. In fact, pollen of the FAX1-deficient mutants lacked the outer pollen cell wall structures, and pollen release by the anthers was impaired. These observations implied that FAX1 protein was essential for biomass production and male fertility. Since components of pollen cell wall and pollen coat are comprised of modified FAs/lipids, mutant phenotype was additionally linked to FA/lipid metabolism. Furthermore, mass spectrometry of FAs, lipids, and waxes showed that deficient mutant plants had more plastid-synthesised lipids and fewer lipids produced in the ER.

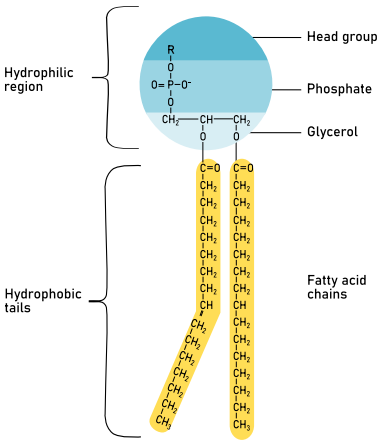
When the scientists investigated the characteristics of *Arabidopsis* mutants with overexpressed FAX1 protein, they observed the opposite trend relative to the deficient mutants. Overexpression mutants were slightly bigger, produced more fresh weight, and had thicker inflorescence stalks than the wild type. They also produced a normal yield of viable seed. Less plastid-synthesised lipids but more ER-derived lipids were detected in the overexpression mutants compared to the wild-type plants. In particular, plants with overexpressed FAX1 had more ER-produced triacylglycerol oils in flowers and leaves. These findings demonstrated the role of FAX1 in FA export from plastids. An additional piece of evidence toward the deduced role of FAX1 was the ability of FAX1 from *Arabidopsis* to restore FA uptake into yeast cells with impaired FA transport after heterologous expression.

Based on what Dr Philippar's group discovered so far, we can be certain that salad oil that we regularly consume or plant-derived oil that we use as hair treatment once has been transported by the activity of FAX1 proteins.

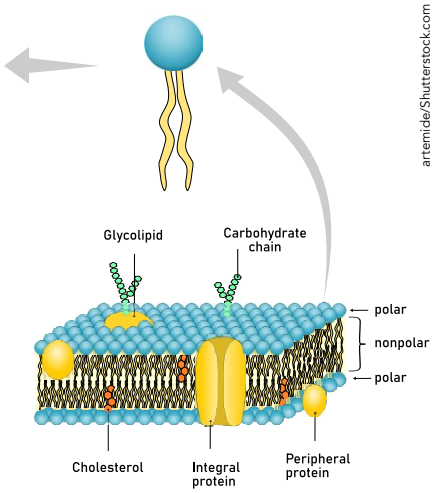


Subcellular localisation of FAX protein in *Chlamydomonas* cells by GFP-targeting.

Image by J. Peter/group Philippar



Phospholipid molecule with fatty acid chains assembled into bilayer membranes.



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content in *Arabidopsis* by other research groups (Li et al., 2020; Tian et al., 2019). Apart from plastid FAX proteins, Dr Philippar's group is now evaluating the contribution of FAX proteins located in membranes of the ER/secretory pathway to lipid metabolism and signalling. In parallel to studies in *Arabidopsis*, plastid- and ER-localised FAX relatives in the green microalga *Chlamydomonas reinhardtii*, a model organism for plant oil metabolism and biofuel production, are of particular interest.

Moreover, TMEM14 relatives of the FAX family of proteins can be found in mitochondria of vertebrates. Therefore, there is potential for the knowledge on the function of these proteins involved in lipid metabolism, membrane bending/shaping in plants to be transferred to mammalian cells.

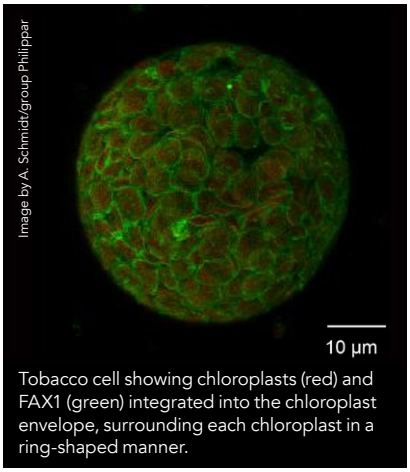
MEMBRANE PROTEINS SUCH AS FAX1 AFFECT MEMBRANE BENDING

The FAX1 protein inserts with four α-helical domains into the lipid bilayer membrane of the inner plastid envelope. Two of these α-helices are entirely hydrophobic, while the other two display an amphiphilic (both hydrophilic and hydrophobic) character. By binding to and asymmetrically wedging into the lipid bilayer, amphiphilic α-helices of proteins such as FAX1 can affect the curvature of biological membranes in all organisms. Particularly, these α helices play a role in determining the intricate build of membranes in intracellular organelles such as the ER, Golgi apparatus, mitochondria, and chloroplasts. Therefore, amphiphilic α-helices are indispensable for maintaining cellular metabolism and organismal fitness. Dr Philippar's group developed a new model on the possible role of FAX1 as a membrane-spanning protein in the bending of membranes and/or mediation of contacts between inner and outer plastid envelope membranes. The model was based on the discovery of extensively bended and honeycomb-shaped plastid inner envelope membranes in *Arabidopsis* plants overexpressing FAX1 protein (Könnel et al., 2018).

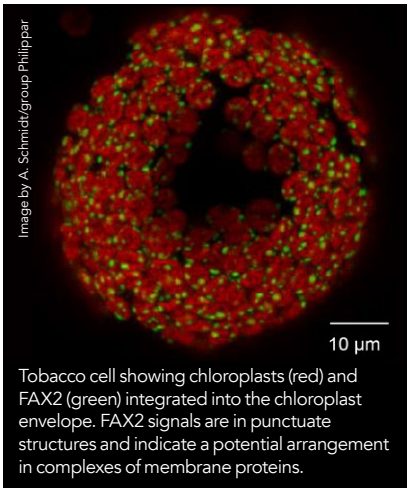
HOW CAN WE USE THE KNOWLEDGE ON LIPID TRANSPORT AND METABOLISM?

Apart from basic research focused on subcellular localisation and structure–function relationships of the FAX/TMEM14 proteins in the membranes of organelles, Dr Philippar's projects also have a practical aspect. Her research group is striving to develop novel strategies for biofuel production. For example, building on the basic research established for FAX1, FAX2 and FAX4 in plastids already have been shown as targets for modulating seed oil

Dr Philippar's research group is striving to develop strategies for biofuel production by basic research on fatty acid transport proteins.



Tobacco cell showing chloroplasts (red) and FAX1 (green) integrated into the chloroplast envelope, surrounding each chloroplast in a ring-shaped manner.



Tobacco cell showing chloroplasts (red) and FAX2 (green) integrated into the chloroplast envelope. FAX2 signals are in punctuate structures and indicate a potential arrangement in complexes of membrane proteins.

WHAT'S NEXT IN FAX PROTEIN RESEARCH?

As mentioned above, the function of FAX proteins in ER/secretory pathway membranes needs to be evaluated to complete the picture of the role of FAX in cellular lipid metabolism. Another focus is the direct comparison between the function of FAX proteins in the model plant *Arabidopsis* and the model alga *Chlamydomonas* and clarification of evolutionary aspects of the FAX/TMEM14 protein family origin. Some additional unanswered questions include the exact molecular mechanism for FAX-mediated FA transport, substrate specificity, and how FAs in plastids traverse the intermembrane space/outer membrane and are finally delivered into the ER. Therefore, development of *in vitro* and *in vivo* assays for detailed structural and functional analysis of FAX/TMEM14 proteins in FA transport and/or lipid membrane bending is underway. Moreover, Dr Philippar's group is striving to discover FAX interaction partners in the plant cell and to integrate research on mammalian TMEM14 proteins.

Behind the Research

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Research Objectives

Professor Philippar's group at Saarland University studies subcellular localisation and structure/function relations of FAX/TMEM14 proteins in organellar membranes.

Detail

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Bio
Katrin Philippar gained a Diploma in Biochemistry (University of Hannover), a doctorate (*Dr. rer. nat.*) in Biology (University of Würzburg, 1999) and a Habilitation in Botany and Cell Biology from the Ludwig-Maximilians-University Munich in 2010. Since her time as group leader at LMU Munich (2004–2014), her research focus has been on ion and metabolite transport in plastid organelles in plant cells. From 2015–2016 she held a DFG Heisenberg fellowship and, since 10/2016, she has been full Professor for Plant Biology at Saarland University, Saarbrücken.

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Collaborators
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Personal Response

Can you expand on your favourite application of your research?

"The favourite application of my research would be a sustainable production of lipids for human nutrition and/or biofuel production in green microalga. In future setups, this could be accomplished by the simultaneous overexpression of FAX proteins in plastid and ER/secretory pathway membranes together with respective enzymes for FA and lipid synthesis."

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