Microbial fatty acid transport proteins and their biotechnological potential

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Abstract

Fatty acid metabolism has been widely studied in various organisms. However, fatty acid transport has received less attention even though it plays vital roles for the cells, such as export of toxic free fatty acids or uptake of exogenous fatty acids. Hence, there are important knowledge gaps in how fatty acids cross biological membranes and many mechanisms and proteins involved in these processes still need to be determined. The lack of information is more predominant in microorganisms, even though the identification of fatty acids transporters in these cells could lead to new drug targets or improvements in microbial cell factories. This review provides a thorough analysis of the current information on fatty acid transporters in microorganisms, including bacteria, yeasts and microalgae species. Most information available relates to the model organisms Escherichia coli and Saccharomyces cerevisiae, but transport systems of other species are also discussed. Intracellular trafficking of fatty acids and their transport through organelle membranes in eukaryotic organisms is described as well. Finally, applied studies and engineering efforts using fatty acids transporters are presented to show the applied potential of these transporters and to stress the need for further identification of new transporters and their engineering.

Keywords:

Fatty acid

Membrane transport engineering

Bacteria

Yeast

Microalgae

Introduction

Transport processes are essential to living organisms and the control of transport across lipid membranes was one of the milestones to the generation of life on Earth (Lancet, Zidovetzki, & Markovitch, 2018; Mansy, 2010). Transport proteins play a big role in cell physiology, such as nutrient uptake and export of toxic compounds, and are predicted to make up 13.7% and 5.8% of the *Escherichia coli* and*Saccharomyces cerevisiae* proteome, respectively (Claus, Jezierska, & Bogaert, 2019).

However, research on transport processes has always be limited due to technical difficulties in comparison with enzymes. Most transport proteins are membrane integrated or membrane associated proteins. The membrane environment necessary for the correct folding of membrane proteins complicates their purification and the following *in vitro* studies or crystallizations for structural studies. This results in an underrepresentation of structures of membrane proteins in databases, as they represent less than 3% of protein structures in the

Protein Data Bank while they comprise around 25% of all known proteins (Newport, Sansom, & Stansfeld, 2019). Therefore, when studying membrane proteins, it is necessary to rely on other techniques, such as homology modelling or mutagenesis (Futagi, Kobayashi, Narumi, Furugen, & Iseki, 2019). Besides this, heterologous expression or overexpression of membrane proteins can easily lead to membrane stress in cells, requiring careful strategies to ensure proper expression and activity (Kang & Tullman-Ercek, 2018). Another disadvantage related to transport processes is the lack of chemical change in their activity, which renders transport activity measurement more laborious and less straight-forward than enzymatic activity (Brouwer et al., 2013).

Despite all these limitations, transport proteins have been studied since the last century, when a lactate transporter was first identified in *E. coli* (Jones & Kennedy, 1969). Since then, many transporters have been identified for a wide diversity of molecular structures. However, the transport of hydrophobic substances has been traditionally associated with passive diffusion. During the last years, extensive evidence of protein assisted transport for hydrophobic molecules has led to a paradigm shift in the scientific community (Claus et al., 2019). Although the transport of hydrophobic compounds requires different mechanisms than soluble compounds, the employment of transport proteins allows cells to regulate this process and it opens opportunities for engineering and improving these transport processes.

Among the different hydrophobic cellular compounds, the most prevalent ones in metabolism are fatty acids, serving both as energy storage and membrane constituents. Transport of fatty acids in animals is tightly regulated, both intra and extracellularly. Fatty acids are transported across different organs and cellular compartments via soluble fatty acid binding proteins as well as membrane bound transporters (Glatz, 2015; Glatz, Luiken, & Bonen, 2010). Perturbation in the transport of fatty acids and lipids give place to pathologies, such as adrenoleukodystrophy, which is associated to mutations in the peroxisomal fatty acid transporter (Tarling, Vallim, & Edwards, 2013). This shows the importance of these mechanisms for correct physiological functions. Also in plants, lipid transport plays indispensable roles in processes such as seed development and cutin synthesis. In plants, fatty acids are synthesized in plastids, modified in the endoplasmic reticulum, stored in cytosolic lipid bodies and degraded in peroxisomes. This high compartmentalization of fatty acid metabolism in plants needs the coordinated participation of membrane transporters and carrier proteins for the intracellular trafficking of fatty acids. The transport of lipids and fatty acids in plants has been reviewed elsewhere (N. Li, Xu, Li-Beisson, & Philippar, 2016).

Fatty acid metabolism has been extensively studied in microbial model organisms such as *E. coli* and *S. cerevisiae* , but also in oleaginous bacteria, yeasts and microalgae (Hu, Zhu, Nielsen, & Siewers, 2019; Magnuson, Jackowski, Rock, & Cronan, 1993). Oleaginous organisms, such as *Rhodococcus jostii* or *Yarrowia lipolytica* , are those able to store lipids as 20% or more of their dry cell weight, reaching 80% in some cases (Alvarez et al., 2019). While microbial fatty acid metabolism has been highly studied and engineered, fatty acid transport in microorganisms still present knowledge gaps. The study of fatty acids in microorganisms is relevant because the scientific evidence acquired from the model organisms *E. coli* and*S. cerevisiae* lays the biochemical basis for research in higher organisms. In addition, their easy manipulation allows a more thorough and flexible study of the transport systems and their components. Moreover, microbial production of fatty acids is gaining attention due to their wide range of applications, mainly as biofuels and nutrition supplements, but also in animal feed, cosmetics and lubricants (Vasconcelos, Teixeira, Dragone, & Teixeira, 2019).

Increasing export rates for fatty acids can be highly beneficial for microbial cell factories for two reasons. On the one hand, overproduction of a metabolite causes feedback inhibition as well as toxic effects in the cell that severely affect productivity. The removal of product from the cellular space allows for a continuous production and a higher yield per cell mass (Kell, Swainston, Pir, & Oliver, 2015). On the other hand, the downstream processing of fermentation processes has a significant contribution to the final cost-effectiveness of the production process, due to the need to break open cells to extract the desired product, as well as the subsequent separation process from all cell constituents (Jezierska & Van Bogaert, 2017). The export of fatty acids into the extracellular space allows for a simpler downstream processing, where cells are removed, and

the product can be directly obtained from a media that contains much less by-products (Borodina, 2019).

Despite the limitations associated with the study of membrane proteins and transport, several fatty acid import and export systems have been identified in microorganisms. This review presents the current scientific knowledge on fatty acid transport across biological membranes, as well as examples of engineering fatty acid transport to improve microbial cell factories.

Fatty acid transport in bacteria

Fatty acid uptake systems

Import of fatty acids in *E. coli:*the FadL-FACS system

The first discovered microbial transport systems for fatty acids was the FadL-FACS system from *E. coli* for the import of exogenous long-chain fatty acids. It was described in 1969 that the ability of*E. coli* to degrade exogenous fatty acids was associated to the presence of a fatty acid-CoA synthetase (FACS). Characterization of this enzyme suggested the presence of another transport protein which was discovered later in 1978 (Nunn & Simons, 1978). The gene encoding this protein, named *fadL* , belongs to the fatty acid degrading (*fad)* regulon containing eight catabolic genes for fatty acids (Feng & Cronan, 2009), including *fadD* which codes for the FACS. The regulon is controlled by the *fadR* gene, whose product activates the fad genes upon binding to acyl-CoA. Both *fadL* and*fadD* are expressed in a basal level to allow detection of exogenous fatty acids by *fadR* (Nils Joakim Færgeman & Knudsen, 1997).

FadL is a long-chain fatty acid-specific transporter with a β-barrel structure present in the outer membrane of *E. coli* . The presence of lipopolysaccharides in the outer membrane renders the cell impermeable to hydrophobic molecules such as fatty acids. FadL is necessary for the uptake of exogenous fatty acids so that they can be used both as a source of energy and as a constituent of phospholipids and triacylglycerols, relieving the bacteria from spending energy and resources into synthetizing new fatty acids (Van Den Berg, 2005). The structure and mechanism of FadL has been studied by Van der Berg et al (Van Den Berg, Black, Clemons, & Rapoport, 2004). FadL is a long 14-strand β-barrel that contains special features: in the extracellular region two loops containing α-helices form a hydrophobic groove; in the intracellular region a hatch domain with three α-helices blocks the channel; the N-terminus extends through the barrel towards the extracellular regions; finally, the strand S3 shows a peculiar bend or kink that disrupts the β-sheet formation and forms a lateral opening (Figure 1A). Concerning the mechanism of transport, the authors initially considered at a first stage that the fatty acids would bind first to the hydrophobic groove, then they would diffuse to an internal high-affinity binding pocket where they would lead to conformation changes both in the N terminus and the hatch domain opening a path for their liberation in the periplasmic space (Van Den Berg, 2005). Nonetheless, this model was refuted after showing the rigidity of the hatch domain (Hearn, Patel, Lepore, Indic, & Van Den Berg, 2009). The importance of the lateral opening in the β-barrel caused by the S3 kink led to support the hypothesis of lateral diffusion, in which the fatty acids would not be liberated to the periplasmic space but rather through the opening space in the β-barrel to the outer membrane (Lepore et al., 2011; Figure 1B). A summary of studied residues from FadL can be found in Table 1, all of them conserved in homologue fatty acids transporters from other species.

Once the fatty acids have been transported by FadL through the outer membrane, they move to the inner membrane. Even though there are reports that proteins may be involved in the process (A. Azizan & Black, 1994), it is likely that this step happens spontaneously. A piece of evidence that supports passive diffusion from the outer membrane to the inner membrane, as well as a flip-flop movement inside each membrane, is the necessity of a proton motive force that can generate acidic conditions in the periplasm leading to the protonation of fatty acid and the subsequent increase in liposolubility (Azliyati Azizan, Sherin, DiRusso, & Black, 1999). Once in the inner membrane, these fatty acids are catalysed by FACS to form acyl-CoA molecules, which are destined to further catabolic or anabolic processes. FACS is a soluble protein that is

recruited to the membrane to assist in the transport of fatty acids (Overath, Pauli, & Schaire, 1969). The mechanism of this recruitment is not known and it possibly happens due to conformational changes after binding of ATP and it has been observed to be assisted by the presence of D-lactate, among other conditions (Mangroo & Gerber, 1993). The activity of FACS is necessary for the metabolism of exogenous fatty acids and it is speculated that it promotes the transport of fatty acids through vectorial acylation analogous to the vectorial phosphorylation of sugars (Black & Dirusso, 2003).

Engineering the fatty acid import system of *E. coli* for biotechnological applications

The import of fatty acid into cells is important for several industrial processes, such as fermentation processes using fatty acids and waste oils as carbon source. The cellular catabolism of fatty acids leads to the formation of acetyl-CoA, which delivers the acetyl group to the citric acid cycle providing energy and intermediates to the cell. Furthermore, acetyl-CoA is a precursor for important metabolites, such as branched-amino acids (Amorim Franco & Blanchard, 2017).

Engineering efforts to produce 3-hydroxypropionate (bioplastic precursor) from fatty acids showed the importance of controlling the expression of the FadL-FACS system (B. Liu et al., 2019). While using a strong promoter for FACS resulted in an increase in productivity, it led to an important decrease when used for FadL. However, the use of medium or weak constitutive promoters for FadL allowed to increase the productivity. This exemplifies the common problem associated to overexpression of membrane proteins, which can increase membrane stress and decrease cell growth and overall productivity (Kang & Tullman-Ercek, 2018). The constitutive expression of FACS and FadL, next to other metabolic modifications, led to a 3-hydroxypropionate yield of 1.56 g/g when grown in a 5L bioreactor using palmitic acid as substrate. In another study where *E. coli* was engineered for the production of lycopene (N. Liu et al., 2020), overexpression of FACS led to a small increase in the lycopene titre (from 29 to 33 mg/g DCW). Nevertheless, growth of the engineered strain in a mix of glucose, waste oil and yeast extract allowed for a total yield of 94 mg/g DCW.

Another important set of applications for the import of fatty acids are whole cell biotransformations. In these, bacteria grown previously to reach a certain biomass density act as catalysers to modify fatty acids or derived compounds through a limited number of enzymatic steps in order to produce a compound of higher value. In such process, cells act as capsules containing enzymes and are not grown during the biotransformation process. Deletion of FACS and overexpression of FadL showed an increase on fatty acid hydroxylation when expressing the cytochrome P450 CYP153A from *Marinobacter aquaeolei,* a heterologous cytosolic enzyme in *E. coli,* suggesting that other proteins can replace the role of FACS in recruiting free fatty acids from the inner membrane and modifying them (Bae, Park, Jung, Lee, & Kim, 2014). Another study involving both fatty acids and hydroxy-fatty acids determined the impact of FadL expression on the biotransformation of these compounds to hydroxy-fatty acids and keto-fatty acids, respectively (Jeon et al., 2018). Enhanced expression of FadL led to a five-fold increase in the single step transformation of oleic acid and 10-hydroxyoctadecanoic acid to 10-hydroxyoctadecanoic acid and 10-keto-octadecanoic, respectively, as well as a two-fold increase in the multistep transformation of ricinoleic acid to the ester molecule ((Z)-11-(heptanoyloxy)undec-9-enoic acid). This study also showed the negative effects that excessive overexpression of FadL can have on overall productivity. Finally, in another study FadL was overexpressed in a strain expressing human Cav1 proteins (Shin et al., 2019). Cav1 proteins stimulate the formation of endosomes that excise from the inner membrane. The formation of endosomes increased the uptake rates of ricinoleic acid two-fold and caused a decrease of fatty acid toxicity in the inner membrane. However, overexpression of FadL in this strain did not lead to a further increase in fatty acid uptake.

Import of fatty acids in other bacteria

Besides the fadL-FACS system from *E. coli* , other systems for the import and assimilation of exogenous fatty acids have been studied in other bacteria. All bacterial fatty acid import systems were found to be dependent on energy supply whether in the form of ATP or in the form of a proton gradient (Calmes & Deal, 1976). One

of the first systems studied was that of other gram-negative bacteria such as*Pseudomonas oleovorans* and *Caulobacter crecentus* , which showed similar characteristics to the fadL-FACS system (Toscano & Hartline, 1973; Zalatan & Black, 2011). Due to the different plasma membrane structure, it has been observed that import of fatty acids in gram positive bacteria occurs differently than in gram-negative bacteria (Figure 2A and 2B). Although fatty acid metabolism and transport has not extensively been studied in gram positive model bacteria, such as *B. subtilis,* some studies have been performed on other gram-positive bacteria. In the lipophilic gram-positive bacteria *Nocardia asteroides* (Calmes & Deal, 1976), the fatty acid uptake system was, in contrast to the previously discussed systems, found to be constitutively expressed and able to import fatty acids as free fatty acids, which would be activated by a soluble FACS at a later and independent stage. With a K_M of 870 μM, the transport process from *N. asteroides* was found to have much less affinity than that of *E. coli* (K_M between 15 and 34 μM). The import of fatty acids in another gram-positive bacterium,*Streptomyces coelicolor* (Banchio & Gramajo, 1997), also showed a constitutive expression, as well as a dependency on pH levels in the environment, decreasing the uptake of long-chain fatty acids with increasing pH. The import system of this bacterium was found to be specific for ionized fatty acids (at pH 7 or higher), while protonated ones were claimed to use a passive process rather than an active one (at a pH lower than 7).

In the fadL-FACS system, FACS is associated to the membrane, linking the formation of acyl-CoA to transport. Nevertheless, the production of acyl-CoA from exogenous fatty acids is not conserved in all bacteria, and other ways of activating and incorporating exogenous fatty acids have been studied, such as acyl-ACP synthase in *Vibrio harveyi,*or fatty acid kinases in gram positive bacteria (Cronan, 2014). The different ways to in which exogenous fatty acids are activated and metabolized has been reviewed elsewhere (Yao & Rock, 2017).

The study of fatty acid import in bacteria can serve different interests, such as improving the fatty acid import capacities of organisms of industrial importance or identifying new drug targets for pathogenic bacteria able to import fatty acids from the host. Among the different bacteria that are of interest for industry, the gram-positive genus *Rhodococcus* has attracted special attention for their ability to withstand and degrade a wide variety of pollutant, including hydrocarbons and lignin-derived compounds (Kim, Choi, Yoo, Zylstra, & Kim, 2018). Some species of this genus, such as *Rhodococcus opacus* and *Rhodococcus jostii,* can be considered oleaginous, being able to accumulate high amounts of triacylglycerol when growing in carbon-excess conditions (Alvarez et al., 2019). A fatty acid importer from *R. jostii* was found in a cluster with genes involved in lipid metabolism after a homology search using known ACB-transporters for hydrophobic compounds. The function of this gene (ro05645 or ltp1) was confirmed after its overexpression, which led to an increased growth in media containing fatty acids as substrate, as well as the uptake of fluorescently labelled fatty acids (Villalba & Alvarez, 2014). Furthermore, the overexpression of *ltp1* was used to increase both the growth (2.2-fold) and the lipid accumulation (3.5-fold) of *R. jostii* when growing in olive mill waste (Herrero, Villalba, Lanfranconi, & Alvarez, 2018).

Mycobacterium tuberculosis is the causing agent of tuberculosis, one of the deadliest diseases in the world. This bacterium is adapted to survive for long periods of time inside the human host, partially thanks to its ability to metabolize host-derived fatty acids. This ability is especially important, as these cells tend to reside within lipid-rich sites, such as inside foamy macrophages (Lovewell, Sassetti, & VanderVen, 2016). *M. tuberculosis* presents a cell envelope containing mycolic acid and glycolipids which acts as a barrier for hydrophobic molecules. A multiprotein complex was observed to be involved in the uptake of fatty acids during macrophage infection as well as in monocultures (Figure 2C). The first member of this complex to be identified was LucA, a protein also involved in the uptake of cholesterol (Nazarova et al., 2017). It was observed that a mutant lacking LucA was unable to incorporate fatty acids, and a transcriptomic analysis of such mutant revealed the involvement of genes from the Mce1 locus in the uptake of fatty acids. From this locus, gene *rv0167*coding for the putative permease YrbE1A was found to be directly involved in fatty acid import. A further screening of mutants lacking the ability to assimilate fatty acids when infecting macrophages revealed the participation of other proteins (Nazarova et al., 2019), such as MceD1, MceG and OmamB. While the function of OmamB is not known, a similar protein OmamA was found to interact with

LucA and stabilize the fatty acid import complex. MceG is a putative ATPase that may be involved in providing the energy for the transport of the fatty acids. Finally, MceD1 and other proteins from the same locus such as MceA1 and MceC1, may have structural roles in the formation of the fatty acid import complex. In addition to the complex Mce1, another gene (*rv1272*) from *M. tuberculosis* showing homology to the*ltp1* gene from *R. jostii* as well as to the lipid A export gene *msbA* from *E. coli* was confirmed to import fatty acids when expressed in *E. coli* (Martin & Daniel, 2018). A Blast search of *rv1272* against the SwissProt database showed that this gene is homologous (39.39% identity) to the uncharacterized transporter YfiC from *Bacillus subtilis,* suggesting that the role of this transporter might be associated to the transport of fatty acids or other hydrophobic compounds.

Fatty acid export systems

The fatty acid export system in *E. coli* and its engineering

In its natural environment, *E. coli* must face high concentrations of fatty acids and other hydrophobic compounds and therefore it must contain mechanisms to prevent toxic effects, such as export proteins. At the same time, secretion of endogenous fatty acids is not detected for wild type strains under normal conditions. Nevertheless, fatty acid secretion in *E. coli* was observed for the first time when expressing a thioesterease from the plant *Umbelleria californica*(Voelker & Davies, 1994). This enzyme was expressed to generate free fatty acids inside the cell. In normal conditions, free fatty acids do not accumulate intracellularly in bacteria, as they are directly transferred from acyl-ACP to glycerol-3-phosphate (Magnuson et al., 1993). On the other hand, plants possess thioesterases to liberate fatty acids from acyl-ACP and these free fatty acids are used for anabolic processes (Gerhardt, 1992). The oilseed from *U. californica* accumulates medium-chain fatty acids, mainly lauric acid ($C_{12:0}$) (Davies, Anderson, Fan, & Hawkins, 1991), and the expression of its thioesterase in *E. coli* leads to the same fatty acid profile (Voelker & Davies, 1994). However, the accumulation of fatty acids was only observed when the β-oxidation pathway was disabled. Secretion of lauric acid was shown by the observation of extracellular laurate crystals when growing on solid media. Later engineering of *E. coli* showed that saturated and monounsaturated fatty acids with 12 till 18 carbon atoms can be secreted in an efficient way reaching 40 mg/L of extracellular fatty acids, but no specific efflux protein for fatty acids was identified (H. Liu et al., 2012).

The accumulation of medium-chain fatty acids is toxic to bacteria due to their ability to destabilize the membrane and interfere with essential activities, such as creation of proton gradients (Lennen et al., 2011). This toxic activity was used to identify proteins involved in fatty acid export that would protect the cells from the accumulation of medium-chain fatty acids when expressing the thioesterase from *U. californica* (Lennen, Politz, Kruziki, & Pfleger, 2013). The genes whose knock out led to important effects on cell viability under these conditions were *rob, acrAB, tolC* and, at a lower extent,*emrAB* . The activity of the first three genes is tightly linked, as *rob* is a regulatory gene that induced *acrAB* expression among other genes, and TolC is an unspecific porin from the outer membrane that forms a complex with *acrAB.* The AcrAB-TolC complex is a transporter from the Resistance-Nodule-Division (RND) superfamily that spans from the intracellular space until the outer side of the bacterial membrane, forming a channel whose mechanism is regulated by allosteric changes and a proton gradient (Wang et al., 2017; Zgurskaya & Nikaido, 1999). The complex is formed by three TolC components, six AcrA components and three AcrB components (Figure 3). The mechanism behind the binding of ligands to AcrB is not precisely known, but due to the large number of different substrates that the AcrAB-TolC complex can transport it is thought that there might be several binding sites in the same binding pocket (Nakashima, Sakurai, Yamasaki, Nishino, & Yamaguchi, 2011). In any case, the binding of a substrate changes the conformation of the AcrB monomer from the relaxed or access state to a binding state, which induces conformational changes in the rest of the complex. Through several interactions with AcrB, AcrA continues the conformational changes to TolC, so that it can change from the closed state to the open state, opening the channel. Finally, the AcrB subunit bound to the ligand changes to another conformation (open state), liberating the substrate into the open channel for its liberation to the extracellular medium (Wang et al., 2017).

Due to the broad specificity of the AcrAB-TolC system, the AcrB component has been engineered through directed evolution to improve the export rate of different hydrophobic molecules, such as medium-chain alcohols, alkanes and alkenes (Chen, Ling, & Chang, 2013; Fisher et al., 2014; Mingardon et al., 2015). However, no protein engineering of AcrB to improve fatty acid export has been documented. Genetic engineering to improve medium-chain fatty acid export in *E. coli*was performed by overexpressing several potential transporters (J. Wu et al., 2019). Overexpression of either of three transport proteins, namely AcrE, MdtC and MdtE, was found to increase medium-chain fatty acid extracellular concentrations from 600 mg/L to values between 800 and 1100 mg/L. One must realize that these proteins are part of larger protein complexes whose other components were not overexpressed in the study.

The AcrEF complex displays homology to the well-studied AcrAB-TolC complex. Yet, the AcrEF complex has been observed to be expressed at lower levels than the AcrAB complex and it has shown an important function in cell division and chromosome segregation (Lau & Zgurskaya, 2005). Nevertheless, the AcrAB and AcrEF complexes are from a structural point of view highly related: the amino acid sequence of acrF shares 87% similarity with acrB, and acrE is homologous to acrA (80% AA similarity). Therefore, it is likely that acrE fulfils the same role as acrA in the acrEF-TolC complex as the channel that connects acrF and TolC. Yet, when overexpressing acrA and acrB from the acrAB-TolC complex separately, no increased fatty acid export was observed. Hence, the mechanism behind the overexpression leading to increased export activity of a channel protein that connects the inner membrane pump (which contains the substrate binding) and the outer membrane porin remains unsolved. Furthermore, in the same study three-fold higher extracellular medium-chain fatty acid concentrations were achieved when overexpressing the three transport proteins (acrE, mdtC and mdtE) simultaneously, while observing only a 10% reduction in the OD (J. Wu et al., 2019).

Export of fatty acids in other bacteria

As observed in the previous section, fatty acid export in *E. coli*seems to be mainly associated to protection against membrane-related toxic effects caused by large concentrations of medium and long-chain fatty acids (Desbois & Smith, 2010). Medium-chain fatty acids have antimicrobial effects affecting a wide range of bacteria (Huang, Alimova, Myers, & Ebersole, 2011). Therefore, it is expected that many bacteria present export systems similar to AcrAB-TolC to export toxic fatty acids, although the specific systems have not been studied to date.

Besides medium chain fatty acids, polyunsaturated fatty acids display antimicrobial properties and in reaction to this, several pathogenic bacteria have developed systems to prevent their toxicity. Some of these systems are the degradation of exogenous fatty acids or their incorporation into phospholipids (Jiang et al., 2019). However, the environments where some pathogenic bacteria must thrive in, such as the skin or the mouth, contain unsaturated fatty acids and therefore they need more advanced protection mechanisms such as fatty acid export systems (Choi et al., 2013; Parsons, Yao, Frank, Jackson, & Rock, 2012). In the gram-positive bacterium *Staphylococcus aureus* , an opportunistic pathogen found on the skin, a fatty acid export system was found when screening for strains resistant to linoleic acid (Alnaseri et al., 2019). The strain *S. aureus* FAR7 showed a mutation in the transcription factor *farR,* which led to the upregulation of the*farE* gene*,* encoding an efflux pump from the RND superfamily. Also the physiological response to PUFAs of the gram-negative pathogenic bacteria *Acinetobacter baumannii,* was studied and revealed the upregulation of the *adeJ* gene, a component of the multidrug efflux pump adeIJK (Jiang et al., 2019). It was observed that the deletion of this gene increased the susceptibility to PUFAs, leading to a six-fold increased growth delay in the presence of docosahexaenoic acid. However, the mutation did not lead to an accumulation of PUFAs in the cell. Nevertheless, growth experiments showed the ability of adeIJK to affect membrane lipid homeostasis and to export lipids to the extracellular medium. These results suggested that the adeIJK pump fulfils a similar role as the emhABC pump from*Pseudomonas fluorescens* , which controls lipid homeostasis through the efflux of endogenous long-chain fatty acids, both saturated and monounsaturated, in response to temperature changes (Adebusuyi & Foght, 2011). Another studied fatty acid export system from a pathogenic bacterium is the farAB system from *Neisseria gonorrhoeae* , which shows high

similarity to the emrAB system from *E. coli,* and whose deletion leads to susceptibility to the long-chain fatty acids oleic acid, linoleic acid and palmitic acid. The minimal inhibitory concentration decreased from 1600 μg/ml to 50 μg/ml in the case of unsaturated fatty acids and from 100 μg/ml to 12.5 μg/ml in the case of palmitic acid (Lee & Shafer, 1999).

Photosynthetic microorganisms are of special interest due to their ability to fix carbon from the atmosphere to produce industrially relevant compounds in a more efficient way than plants.*Synechocystis* sp. PCC 6803 is the model organism for cyanobacteria and it has been observed to secrete long-chain fatty acids, up to 13% of the cellular biomass, without any genetic modifications (X. Liu, Sheng, & Curtiss, 2011). The deletion of*Synechocystis* sp. PCC 6803 genes *sll0180* and*slr2131,* homologous to the respective *E. coli* genes*acrA* and *acrB,* showed an effect in the fatty acid secretion of an engineered strain of *Synechocystis* sp. PCC 6803 specialized in the extracellular production of fatty acids (Bellefleur, Wanda, & Curtiss, 2019). While the complementation with *acrA* did not lead to a recovery of the fatty acid secretion rates, the complementation of *slr2131* with *acrB* allowed for an increase in both extracellular and intracellular fatty acid concentrations.

Another fatty acid export mechanism has been identified in another cyanobacteria, *Synechococcus elongatus* a. This system was found through the genomic and transcriptomic analysis of a mutant able to produce free fatty acids but resistant to their toxicity. Inactivation of this export system, composed by the genes *rndA1*and *rndB1* , led to susceptibility to exogenous saturated medium-chain fatty acids and unsaturated long-chain fatty acids. Orthologs of *rndB1* are found in most genomes from cyanobacteria, but not in those of *Synechocystis* sp. PCC 6803. The RndA1B1 system from *S. elongatus* allows for efficient secretion of oleic acid, but palmitate is not transported and therefore it accumulates in the cells (Kato et al., 2015).

Fatty acid transport in yeast

Uptake of fatty acids in yeast

Fatty acid import in yeast follows the same principle of vectorial acylation described for *E. coli.* In *Saccharomyces cerevisiae,* the import system is composed of Fat1 and Faa1 or Faa4. Mutations in these genes show hampered growth under fatty acid auxotrophic conditions (Black & Dirusso, 2003). Although *S. cerevisiae* cannot use fatty acids as growth substrate, it needs to incorporate exogenous fatty acids in case of inhibition of fatty acid synthesis by cerulenin or during anaerobic growth as desaturases require molecular oxygen to produce unsaturated fatty acids.

Fat1 is an ortholog of the mammal fatty acid transport proteins identified in murine species (Nils J. Færgeman, DiRusso, Elberger, Knudsen, & Black, 1997), and it has been described to be involved both in the import of fatty acids and in the activation of very-long-chain fatty acids (Zou, Dirusso, Ctrnacta, & Black, 2002). Fat1 is an integral membrane protein with two transmembrane domains (Obermeyer, Fraisl, DiRusso, & Black, 2007; Figure 4). The conserved ATP/AMP binding region characteristic of acyl-CoA synthetases is separated by a portion of the protein that is inserted into the membrane. The intracellular C-terminus contains a region conserved among other fatty acid transport proteins with very-long-chain acyl-CoA synthetase activity (VLACS). Finally, the soluble Faa1 has been observed to interact with the C-terminus of this protein to activate the imported fatty acids. The transport mechanism of Fat1 is unknown but several residues from Fat1 have been mutated to study their effects (Zou et al., 2002). These experiments have shown that although most residues affect both transport and acyl-CoA synthetase activity, the mutation of certain residues separates these two activities, suggesting that they follow different mechanisms. On one hand, F528A and L669R mutations abolish transport function, but retain some acyl-CoA synthetase activity. On the other hand, S258A and D508A mutations abolish acyl-CoA synthetase activity while retaining some transport activity. Fat1 has been proposed to be situated not only in the plasma membrane but also in lipid bodies, endoplasmic reticulum and peroxisomes (Van Roermund et al., 2012).

S. cerevisiae contains two main acyl-CoA synthetases for long-chain fatty acids, Faa1 and Faa4. These

proteins interact with Fat1 to form a complex that combines transport and activation of fatty acids. Faa1 is responsible for most of the acyl-CoA synthetase activity observed in *S. cerevisiae* and it has been observed to interact with the carboxy terminal of Fat1 as described in several studies, including yeast two-hybrid experiments (Zou et al., 2003). Although the activity of Faa4 is lower than Faa4, it is the only acyl-CoA synthetase gene that can rescue fatty acid import activity in a ΔFaa1 mutant, suggesting that its mode of action is identical (Johnson, Knoll, Levin, & Gordon, 1994). Next to vectorial acylation, endocytosis plays a significant role in the uptake of exogenous fatty acids (Jacquier & Schneiter, 2010). The deletion of Ypk1, a protein-kinase involved in endocytosis was found to affect fatty acid import by reducing it by half compared to the wild-type. These results led to investigate the involvement of other proteins associated to endocytosis End3, Vrp1 and Srv2, whose deletion also hampered fatty acid import at the same extent. The involvement of all these genes stresses the importance of endocytosis for fatty acid import, probably due to the internalization of fatty acid-rich membrane domains.

Besides *S. cerevisiae,* the uptake of exogenous fatty has been studied in other yeasts. *Cryptococcus neoformans* is an important fungal pathogen that infects alveolar macrophages and it is responsible for increasing deaths in immunosuppressed individuals. This yeast has been observed to import exogenous fatty acids for the formation of lipid droplets; and the presence of oleic acid stimulates its replication, both in extracellular form and during macrophage infection (Nolan, Fu, Coppens, & Casadevall, 2017). However, no molecular mechanism or components has been described for this process. The acquisition of exogenous fatty acids in *Candida albicans,* another important fungal pathogen, has been studied at the molecular level. CaFaa4, the ortholog gene for Faa4 and Faa1 from *S. cerevisiae,* was characterized and observed to be essential for fatty acid import (Tejima, Ishiai, Murayama, Iwatani, & Kajiwara, 2018). Note that in contrast to *S. cerevisiae* , in *C. albicans* only one Faa gene seems to be involved. The same holds true for Y. lipolytica. Moreover, the mechanism behind fatty acid transport is not conserved across yeasts. Although *Y. lipolytica* possesses an ortholog of Fat1 from *S. cerevisiae,* this protein is not associated to fatty acid import and it has been suggested to be involved in fatty acid export from lipid bodies (R. Dulermo, Gamboa-Meléndez, Dulermo, Thevenieau, & Nicaud, 2014). Furthermore, the acyl-CoA synthetase from *Y. lipolytica* (YlFaa1), while being the only gene involved in fatty acid activation, it is not essential for growth on fatty acids (R. Dulermo, Gamboa-meléndez, & Ledesma-amaro, 2015).

Intracellular trafficking of fatty acids in yeast

Fatty acids must be activated to acyl-CoA before they can enter a specific metabolic pathway. As described in the previous section, exogenous fatty acids are imported and converted to acyl-CoA nearly simultaneously. Acyl-CoA can also be derived from *de novo*synthesised fatty acids and from fatty acids contained in the neutral lipids stored in lipid bodies. The joint action of lipases and acyl-CoA synthetases, such as the Fat1 from *S. cerevisiae* and its ortholog in *Y. lipolytica,* leads to the mobilisation of fatty acids from lipid bodies (T. Dulermo, Thevenieau, & Nicaud, 2014). Acyl-CoA molecules must reach the destination inside the cell where they will be degraded, stored or used to build other molecules (DiRusso & Black, 1999). The intracellular trafficking routes for fatty acids and acyl-CoA molecules are shown in Figure 5. In *S. cerevisiae* , this intracellular transport is facilitated by an acyl-CoA binding protein coded by the gene *acb1* . Although it has been observed that this protein facilitates transport of acyl-CoA to lipid bodies for the formation of triacylglycerol, it is not necessary for the survival of the cell (Schjerling et al., 1996). Therefore, either there are other transport mechanisms, or they are not needed for the diffusion of acyl-CoA molecules across the cytosol.

In yeast, fatty acids are catabolized by β-oxidation in peroxisomes. The transport of fatty acids in the peroxisome of *S. cerevisiae* has been studied, revealing the involvement of several proteins. Free fatty acids, mainly medium-chain fatty acids, can be imported into the peroxisome by passive diffusion or an unidentified system (Hettema et al., 1996). Long-chain fatty acids, in the form of acyl-CoA, are transported into the peroxisome through the heterodimeric transporter formed by Pxa1 and Pxa2 (Shani, Sapag, Watkins, & Valle, 1996). It has been proposed that the Pxa1/Pxa2 transporter would cleave the fatty acyl-CoA prior to

transport and it would introduce only the free fatty acid portion into the peroxisome (Van Roermund et al., 2012). This mechanism prevents accumulation of CoA in the peroxisome and it has already been described in plants (Fulda, Schnurr, Abbadi, Heinz, & Browse, 2004). Fatty acids transported by Pxa1/Pxa2 need to be activated by re-conversion to acyl-CoA to enter the β-oxidation cycle. Two acyl-CoA synthetases have been associated to the peroxisomal activation of fatty acids: Fat1 and Faa2. Substitution of the Pxa1/Pxa2 transporter with the human orthologue showed that Fat1 must interact with the yeast Pxa1/Pxa2 transporter to be active (Van Roermund et al., 2012). While Fat1 is also found in other regions of the cell, such as the plasma membrane, Faa2 is found exclusively in peroxisomes. Faa2 accepts a wide range of fatty acids as substrate, but it has a preference for medium-chain fatty acids (Knoll, Johnson, & Gordon, 1994). The general model of peroxisomal fatty acid transport can be observed in Figure 5. The peroxisomal transport of fatty acids in *Y. lipolytica* has also been studied (R. Dulermo et al., 2015). The overall system seems similar to that of *S. cerevisiae* , with distinct routes for long and medium-chain fatty acids. Both Pxa1/Pxa2 and Fat1 are involved in the peroxisomal transport. However, *Y. lipolytica* lacks the Faa2 protein for the activation of medium-chain fatty acids, and it has been proposed that a coumarate ligase-like protein might fulfil this role instead (R. Dulermo et al., 2015).

Export of fatty acids in yeast

Just as for prokaryotic organisms, secretion of free fatty acids is no common natural process in yeasts or fungi. Yet, upon the engineered intracellular accumulation of free fatty acids, yeasts are able to secrete them to the extracellular medium (Arhar & Natter, 2019; Scharnewski, Pongdontri, Mora, Hoppert, & Fulda, 2008). Some proteins involved in the export of fatty acids in *S. cerevisiae* have been described. An omics analysis of fatty acid secreting mutants of *S. cerevisiae* identified potential fatty acid export protein Mrp8, though no experimental results are available for this transporter (Fang et al., 2016). An experimentally proven fatty acid export protein is Tpo1, from the MFS superfamily, that was initially identified as a polyamine transporter involved in the resistance of yeast towards spermidine (Albertsen, Bellahn, Krämer, & Waffenschmidt, 2003; Tomitori, Kashiwagi, Sakata, Kakinuma, & Igarashi, 1999). Further studies identified different substrates for this transporter, including medium-chain fatty acids (Legras et al., 2010). Other proteins involved in fatty acid export are the pathogen-related yeast (Pry) proteins.*S. cerevisiae* contains three Pry proteins, two of which are involved in the export of sterol molecules (Darwiche, El Atab, Cottier, & Schneiter, 2018). One of these proteins, Pry1, is also able to bind fatty acids and its deletion hampers the secretion of fatty acids in a fatty acid accumulating mutant strain (Darwiche, Mène-Saffrané, Gfeller, Asojo, & Schneiter, 2017).

Fatty acid export in yeast has been engineered to improve productivity of microbial cell factories. The heterologous expression of human transporter FATP1 in *Y. lipolytica* increased extracellular fatty acid titre from 60mg/L to 190 mg/L. Furthermore, this transporter also showed activity towards fatty alcohols, rising the percentage of extracellular fatty alcohols from 9% to 29% of total fatty alcohols produced (Hu, Zhu, Nielsen, & Siewers, 2018). In *S. cerevisiae,*Tpo1 has been engineered through directed evolution to increase medium-chain fatty acid export rate (Zhu et al., 2020). The mutants were obtained by selecting for increased resistance against medium-chain fatty acids through two rounds of enrichment in selective media containing decanoate of a library of Tpo1 mutants obtained by error-prone PCR. The mutations F322L, T45S, and I432N increased resistance against both decanoate and octanoate and F322L was identified as the mutation with the highest impact. Integration of two copies of this engineered Tpo1 in *S. cerevisiae* increased the extracellular fatty acids with a chain length of 6 to 10 carbon atoms about 2-fold and those with 12 and 14 carbon atoms about 4-fold.

Fatty acid transport in microalgae

Export of fatty acids in microalgae

Microalgae release fatty acids to the extracellular medium in their natural habitat, proposedly as allelopathic compounds (Allen, Ten-hage, & Leflaive, 2018; Sushchik, Kalacheva, Zhila, Gladyshev, & Volova, 2003). Although allelopathic compounds can be have a wide chemical diversity, fatty acid and derivatives are common in water ecosystems and their production is increased under conditions that do not allow for optimal growth (such as nitrogen or phosphate limitation) but allow for efficient photosynthesis (Allen et al., 2018). Microalgae fatty acid production has been linked to allelopathic effects on competitor organisms due to the capacity of certain fatty acids to alter membrane permeability (J. T. Wu, Chiang, Huang, & Jane, 2006). For example, the green algae *Uronema confervicolum* can secrete 1.45 µg/L of free fatty acids, 77% of which correspond to linoileic and linolenic acid (Allen et al., 2018). In the same study, these fatty acids were observed to inhibit growth of the diatom *Fistulifera saprophila* , although at higher concentrations that those produced by *U. confervicolum* . The green alga *Chlorella vulgaris* has also been observed to produce fatty acids at a concentration of 0.85 mg/L/10^6 cells under phosphate limiting conditions (DellaGreca et al., 2010). The mixture of fatty acids changes drastically when comparing two scientific reports and is reflected in the production of palmitic acid, which can be absent from the fatty acid mixture or be the most abundant depending on the growth conditions, possibly due to the mode of CO_2 supply or different medium compositions (DellaGreca et al., 2010; Sushchik et al., 2003). The fatty acid mixture produced by *C. vulgaris* has been observed to be toxic to the alga *Raphidocelis subcapitata* , resulting in the extinction of this alga when grown in a coculture with *C. vulgaris* (DellaGreca et al., 2010). While microalgae are observed to secrete fatty acids, no uptake has been described, probably due to the autotropic nature of microalgae.

Fatty acid secretion in microalgae can be increased by means of genetic engineering. Knockdown of long-chain acyl-CoA synthetase genes cracs1 and cracs2 in *Chlamydomonas reinhardtii* cc849 (a cell wall deficient strain) increased extracellular fatty acid production from 2.93 mg/10^9 cells to 8.19 mg/10^9cells and 9.66 mg/10^9 cells, respectively (Jia et al., 2016). Overexpression of transcription factor NobZIP1 in*Nannochloropsis oceanica* increased the extracellular fatty acid content by 40%(D. Li et al., 2019). The study of the effects of NobZIP1 revealed that one of the negatively regulated targets, UDP-glucose dehydrogenase, an enzyme involved in cell wall polymer metabolism, is linked to lipid metabolism. Silencing this enzyme through interference RNA increased the extracellular fatty acid content by 20% (D. Li et al., 2019). Although the secretion of fatty acids in algae has been widely studied, no export system has been identified to date.

Intracellular trafficking of fatty acids in microalgae

Microalgae, analogous to plants, synthesize fatty acids in plastids. However, synthesis of triacylglycerol and other lipids takes place in the endoplasmic reticulum and therefore the newly synthesized fatty acids must be export from the plastid to the cytosol. A plastid fatty acid exporter acting on free fatty acids, AtFAX1, has been identified and studied in *Arabidopsis thaliana* (N. Li et al., 2015).Orthologues of this protein can be found in microalgae, and their function has been studied in some of them. The green-alga model*Chlamydomonas reinhardtii* contains two orthologues, CrFAX1 and CrFAX2, whose overexpression respectively increased neutral lipid content 15% and 17% under nitrogen limiting conditions (N. Li et al., 2019). Overexpression of these genes produced more and larger lipid droplets, increasing the content of intracellular triacylglycerol by 38%. The fatty acid composition did not show significant variation, showing that these transporters are involved in the transport of both saturated and unsaturated fatty acids. A fatty acid plastid exporter, CmFAX1, has also been identified in an extremophilic red microalgae,*Cyanidioschyzon merolae* , that inhabits sulfuric acid hot springs (Takemura, Imamura, & Tanaka, 2019). This exporter was confirmed to be located in the plastid membrane through targeted immunofluorescence and its fatty acid transport activity was verified through complementation experiments in yeast cells lacking the FAT1 transporter. Deletion of CmFAX1 in *C. merolae* increased free fatty acid content in the total cell extract 2.5-fold and CmFAX1 overexpression increased lipid droplet formation 2.4-fold. In contrast to the CrFAX

transporters, the overexpression of CmFAX1 introduced significant changes in the fatty acid composition of the triacylglycerol molecules, incorporating C14:0, C14:1 and C20:0 fatty acids and increasing the content of C18:2 (Takemura et al., 2019).

After the free fatty acids have abandoned the plastid, they are incorporated into lipid droplets in the form of glycerolipids. For this, they need to be activated to acyl-CoA by a long-chain acyl-CoA synthetase. *C. reinhardtii* encodes three putative long-chain acyl-CoA synthetase and two of them were found to be associated to lipid droplets in a proteomic study (Nguyen et al., 2011). The deletion of one of them, LCS2, led to a 2-fold increase in triacylglycerol rich in polyunsaturated fatty acids (e.g. 52:10 or 54:9) showing that this enzyme is mainly associated to the activation of saturated fatty acids produced *de novo* in the plastids (X. Li et al., 2015).

Once the growth conditions change and the lipid reserves are needed for survival and growth, fatty acids get mobilised from lipid droplets by lipases and long-chain acyl-CoA synthetases. The resulting acyl-CoA molecules must be incorporated into peroxisomes for their degradation (Kong et al., 2017; Figure 6). This is speculated to happen in similar way to plants and yeast, via a transporter with thioesterase activity and the later participation of a peroxisomal long-chain acyl-CoA synthetase. While there have been no studies to date on microalgae to support this hypothesis, *C. reinhardtii* possesses a putative fatty acid peroxisomal transporter, ABCD1 (Kong, Romero, Warakanont, & Li-Beisson, 2018). An overview of the intracellular trafficking of fatty acids and acyl-CoA molecules in *C. reinhardtii* can be observed in Figure 6.

Conclusion and future perspectives

Even though the role of passive diffusion of fatty acids through membrane is only lately being put into question, the study of fatty acid transport systems during the last years has shed light on several of these systems and their mechanism (Table 2). Differences in membrane structure and divergence of physiological needs cause a wide diversity of fatty acid transport systems. This diversity is reflected on the existence of both single protein systems and multiprotein complexes as well as transport systems with or without acyl-CoA synthetase activity. Furthermore, the higher intracellular complexity of eukaryotic organisms needs the presence of transporters for the organelle membranes and trafficking systems between different hotspots for fatty acid metabolism and storage. Although many proteins involved in the microbial transport of fatty acids have already been identified, there are still important knowledge gaps, such as the transport mechanism of most of these proteins, the identification of fatty acid export proteins for microalgae or the study of fatty acid transport systems in filamentous fungi, such as *Aspergillus niger* or other model microorganisms such as *B. subtilis*. Finally, the engineering examples showed in this review are expected to keep increasing in number as more fatty acid transport proteins are identified and their mechanisms are elucidated. This will be an essential step in the optimization of microbial cell factories that consume or produce fatty acids and related compounds.

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Conflict of Interest

The authors declare no conflict of interest

References

Adebusuyi, A. A., & Foght, J. M. (2011). An alternative physiological role for the EmhABC efflux pump in Pseudomonas fluorescens cLP6a.*BMC Microbiology* , *11* , 252. https://doi.org/https://doi.org/10.1186/1471-2180-11-252

Albertsen, M., Bellahn, I., Krämer, R., & Waffenschmidt, S. (2003). Localization and function of the yeast multidrug transporter Tpo1p.*Journal of Biological Chemistry* , *278* (15), 12820–12825. https://doi.org/10.1074/jbc.M210715200

Allen, J. L., Ten-hage, L., & Leflaive, J. (2018). Regulation of Fatty Acid Production and Release in Benthic Algae : Could Parallel Allelopathy Be Explained with Plant Defence Theories ? *Microbial Ecology* , *75* , 609–621.

Alnaseri, H., Kuiack, R. C., Ferguson, K. A., Schneider, J. E. T., Heinrichs, D. E., & McGavin, M. J. (2019). DNA binding and sensor specificity of FarR, a novel tetr family regulator required for induction of the fatty acid efflux pump FarE in staphylococcus aureus.*Journal of Bacteriology* , *201* (3), 1–16. https://doi.org/10.1128/JB.00602-18

Alvarez, H. M., Herrero, O. M., Silva, R. A., Hernandez, M. A., Lanfranconi, M. P., & Villalba, M. S. (2019). Insights into the metabolism of oleaginous Rhodococcus spp. *Applied and Environmental Microbiology* , *85* (18), 1–12. https://doi.org/10.1128/AEM.00498-19

Amorim Franco, T. M., & Blanchard, J. S. (2017). Bacterial Branched-Chain Amino Acid Biosynthesis: Structures, Mechanisms, and Drugability. *Biochemistry* , *56* (44), 5849–5865. https://doi.org/10.1021/acs.biochem.7b00849

Arhar, S., & Natter, K. (2019). Common aspects in the engineering of yeasts for fatty acid- and isoprene-based products. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* ,*1864* (12), 158513. https://doi.org/10.1016/j.bbalip.2019.08.009

Azizan, A., & Black, P. N. (1994). Use of transposon TnphoA to identify genes for cell envelope proteins of Escherichia coli required for long-chain fatty acid transport: The periplasmic protein Tsp potentiates long-chain fatty acid transport. *Journal of Bacteriology* ,*176* (21), 6653–6662. https://doi.org/10.1128/jb.176.21.6653-6662.1994

Azizan, Azliyati, Sherin, D., DiRusso, C. C., & Black, P. N. (1999). Energetics underlying the process of long-chain fatty acid transport.*Archives of Biochemistry and Biophysics* , *365* (2), 299–306. https://doi.org/10.1006/abbi.1999.1171

Bae, J. H., Park, B. G., Jung, E., Lee, P. G., & Kim, B. G. (2014). fadD deletion and fadL overexpression in Escherichia coli increase hydroxy long-chain fatty acid productivity. *Applied Microbiology and Biotechnology* , *98* (21), 8917–8925. https://doi.org/10.1007/s00253-014-5974-2

Banchio, C., & Gramajo, H. C. (1997). Medium- and long-chain fatty acid uptake and utilization by Streptomyces coelicolor A3(2): First characterization of a Gram-positive bacterial system.*Microbiology* , *143* (7), 2439–2447. https://doi.org/10.1099/00221287-143-7-2439

Bellefleur, M. P. A., Wanda, S. Y., & Curtiss, R. (2019). Characterizing active transportation mechanisms for free fatty acids and antibiotics in Synechocystis sp. PCC 6803. *BMC Biotechnology* ,*19* (1), 1–17. https://doi.org/10.1186/s12896-019-0500-3

Black, P. N., & Dirusso, C. C. (2003). Transmembrane Movement of Exogenous Long-Chain Fatty Acids: Proteins, Enzymes, and Vectorial Esterification. *Microbiology and Molecular Biology Reviews* ,*67* (3), 1–11. https://doi.org/10.1128/MMBR.67.3.454

Borodina, I. (2019). Understanding metabolite transport gives an upper hand in strain development. *Microbial Biotechnology* ,*12* (1), 69–70. https://doi.org/10.1111/1751-7915.13347

Brouwer, K. L. R., Keppler, D., Hoffmaster, K. A., Bow, D. A. J., Cheng, Y., Lai, Y., ... Evers, R. (2013). In vitro methods to support transporter evaluation in drug discovery and development. *Clinical Pharmacology and Therapeutics* , *94* (1), 95–112. https://doi.org/10.1038/clpt.2013.81

Calmes, R., & Deal, S. J. (1976). Fatty acid transport by the lipophilic bacterium Nocardia asteroides. *Journal of Bacteriology* , *126* (2), 751–757. https://doi.org/10.1128/jb.126.2.751-757.1976

Chen, B., Ling, H., & Chang, M. W. (2013). Transporter engineering for improved tolerance against alkane biofuels in Saccharomyces cerevisiae.*Biotechnology for Biofuels* , *6* (1), 1–10. https://doi.org/10.1186/1754-6834-6-21

Choi, J., Park, N., Hwang, S., Sohn, J. H., Kwak, I., Cho, K. K., & Choi, I. S. (2013). The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens. *Journal of Environmental Biology* , *34* (July), 673–676.

Claus, S., Jezierska, S., & Bogaert, I. N. A. Van. (2019). Protein-facilitated transport of hydrophobic molecules across the yeast plasma membrane. *FEBS Letters* , *593* , 1508–1527. https://doi.org/10.1002/1873-3468.13469

Cronan, J. E. (2014). A new pathway of exogenous fatty acid incorporation proceeds by a classical phosphoryl transfer reaction.*Molecular Microbiology* , *92* (2), 217–221. https://doi.org/10.1111/mmi.12558

Darwiche, R., El Atab, O., Cottier, S., & Schneiter, R. (2018). The function of yeast CAP family proteins in lipid export, mating, and pathogen defense. *FEBS Letters* , *592* (8), 1304–1311. https://doi.org/10.1002/1873-3468.12909

Darwiche, R., Mene-Saffrane, L., Gfeller, D., Asojo, O. A., & Schneiter, R. (2017). The pathogen-related yeast protein Pry1, a member of the CAP protein superfamily, is a fatty acid-binding protein.*Journal of Biological Chemistry* , *292* (20), 8304–8314. https://doi.org/10.1074/jbc.M117.781880

Davies, H. M., Anderson, L., Fan, C., & Hawkins, D. J. (1991). Developmental induction, purification, and further characterization of 12:0-ACP thioesterase from immature cotyledons of Umbellularia californica. *Archives of Biochemistry and Biophysics* ,*290* (1), 37–45. https://doi.org/10.1016/0003-9861(91)90588-A

DellaGreca, M., Zarrelli, A., Fergola, P., Cerasuolo, M., Pollio, A., & Pinto, G. (2010). Fatty acids released by Chlorella vulgaris and Their Role in Interference with Pseudokirchneriella subcapitata: Experiments and Modelling. *Journal of Chemical Ecology* , *36* (3), 339–349. https://doi.org/10.1007/s10886-010-9753-y

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids : activities , mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol* , *85* , 1629–1642. https://doi.org/10.1007/s00253-009-2355-3

DiRusso, C. C., & Black, P. N. (1999). Long-chain fatty acid transport in bacteria and yeast. Paradigms for defining the mechanism underlying this protein-mediated process. *Molecular and Cellular Biochemistry* , *192* (1–2), 41–52. https://doi.org/10.1007/978-1-4615-4929-1_5

Dulermo, R., Gamboa-Melendez, H., Dulermo, T., Thevenieau, F., & Nicaud, J. M. (2014). The fatty acid transport protein Fat1p is involved in the export of fatty acids from lipid bodies in Yarrowia lipolytica.*FEMS Yeast Research* , *14* (6), 883–896. https://doi.org/10.1111/1567-1364.12177

Dulermo, R., Gamboa-melendez, H., & Ledesma-amaro, R. (2015). Unraveling fatty acid transport and activation mechanisms in Yarrowia lipolytica. *BBA - Molecular and Cell Biology of Lipids* ,*1851* (9), 1202–1217. https://doi.org/10.1016/j.bbalip.2015.04.004

Dulermo, T., Thevenieau, F., & Nicaud, J. (2014). The fatty acid transport protein Fat1p is involved in the export of fatty acids from lipid bodies in Yarrowia lipolytica. *FEMS Yeast Research* ,*14* , 883–896. https://doi.org/10.1111/1567-1364.12177

Faergeman, Nils J., DiRusso, C. C., Elberger, A., Knudsen, J., & Black, P. N. (1997). Disruption of the Saccharomyces cerevisiae homologue to the murine fatty acid transport protein impairs uptake and growth on long-chain fatty acids. *Journal of Biological Chemistry* ,*272* (13), 8531–8538. https://doi.org/10.1074/jbc.272.13.8531

Faergeman, Nils Joakim, & Knudsen, J. (1997). Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling.*Biochemical Journal* , *323* (1), 1–12. https://doi.org/10.1042/bj3230001

Fang, F., Dai, B., Zhao, G., Zhao, H., Sun, C., Liu, H., & Xian, M. (2016). In depth understanding the molecular response to the enhanced secretion of fatty acids in Saccharomyces cerevisiae due to one-step gene deletion of acyl-CoA synthetases. *Process Biochemistry* ,*51* (9), 1162–1174. https://doi.org/10.1016/j.procbio.2016.05.017

Feng, Y., & Cronan, J. E. (2009). A New Member of the Escherichia coli fad Regulon : Transcriptional Regulation of fadM (ybaW) ?.*Journal of Bacteriology* , *191* (20), 6320–6328. https://doi.org/10.1128/JB.00835-09

Fisher, M. A., Boyarskiy, S., Yamada, M. R., Kong, N., Bauer, S., & Tullman-Ercek, D. (2014). Enhancing tolerance to short-chain alcohols by engineering the Escherichia coli AcrB efflux pump to secrete the non-native substrate n-butanol. *ACS Synthetic Biology* ,*3* (1), 30–40. https://doi.org/10.1021/sb400065q

Fulda, M., Schnurr, J., Abbadi, A., Heinz, E., & Browse, J. (2004). Peroxisomal Acyl-CoA Synthetase Activity Is Essential for Seedling Development in Arabidopsis thaliana. *Plant Cell* , *16* (2), 393–405. https://doi.org/10.1105/tpc.019646

Futagi, Y., Kobayashi, M., Narumi, K., Furugen, A., & Iseki, K. (2019). Homology modeling and site-directed mutagenesis identify amino acid residues underlying the substrate selection mechanism of human monocarboxylate transporters 1 (hMCT1) and 4 (hMCT4). *Cellular and Molecular Life Sciences* , *76* (24), 4905–4921. https://doi.org/10.1007/s00018-019-03151-z

Gerhardt, B. (1992). Fatty acid degradation in plants. *Progress in Lipid Research* , *31* (4), 417–446.

Glatz, J. F. C. (2015). Lipids and lipid binding proteins: A perfect match. *Prostaglandins Leukotrienes and Essential Fatty Acids* ,*93* , 45–49. https://doi.org/10.1016/j.plefa.2014.07.011

Glatz, J. F. C., Luiken, J. J. F. P., & Bonen, A. (2010). Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. *Physiological Reviews* , *90* (1), 367–417. https://doi.org/10.1152/physrev.00003.2009

Hearn, E. M., Patel, D. R., Lepore, B. W., Indic, M., & Van Den Berg, B. (2009). Transmembrane passage of hydrophobic compounds through a protein channel wall. *Nature* , *458* (7236), 367–370. https://doi.org/10.1038/nature07678

Herrero, O. M., Villalba, M. S., Lanfranconi, M. P., & Alvarez, H. M. (2018). Rhodococcus bacteria as a promising source of oils from olive mill wastes. *World Journal of Microbiology and Biotechnology* ,*34* (8), 1–10. https://doi.org/10.1007/s11274-018-2499-3

Hettema, E. H., van Roermund, C. W., Distel, B., van den Berg, M., Vilela, C., Rodrigues-Pousada, C., ... Tabak, H. F. (1996). The ABC transporter proteins Pat1 and Pat2 are required for import of long-chain fatty acids into peroxisomes of Saccharomyces cerevisiae. *The EMBO Journal* , *15* (15), 3813–3822. https://doi.org/10.1002/j.1460-2075.1996.tb00755.x

Hu, Y., Zhu, Z., Nielsen, J., & Siewers, V. (2018). Heterologous transporter expression for improved fatty alcohol secretion in yeast.*Metabolic Engineering* , *45* (August 2017), 51–58. https://doi.org/10.1016/j.ymben.2017.11.008

Hu, Y., Zhu, Z., Nielsen, J., & Siewers, V. (2019). Engineering Saccharomyces cerevisiae cells for production of fatty acid-derived biofuels and chemicals. *Open Biology* , *9* (5). https://doi.org/10.1098/rsob.190049

Huang, C. B., Alimova, Y., Myers, T. M., & Ebersole, J. L. (2011). Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of Oral Biology* , *56* (7), 650–654. https://doi.org/10.1016/j.archoralbio.2011.01.011

Jacquier, N., & Schneiter, R. (2010). Ypk1, the yeast orthologue of the human serum- and glucocorticoid-induced kinase, is required for efficient uptake of fatty acids. *Journal of Cell Science* ,*123* (13), 2218–2227. https://doi.org/10.1242/jcs.063073

Jeon, E. Y., Song, J. W., Cha, H. J., Lee, S. M., Lee, J., & Park, J. B. (2018). Intracellular transformation rates of fatty acids are influenced by expression of the fatty acid transporter FadL in Escherichia coli cell membrane. *Journal of Biotechnology* ,*281* (April), 161–167. https://doi.org/10.1016/j.jbiotec.2018.07.019

Jezierska, S., & Van Bogaert, I. N. A. (2017). Crossing boundaries: the importance of cellular membranes in industrial biotechnology.*Journal of Industrial Microbiology and Biotechnology* ,*44* (4–5), 721–733. https://doi.org/10.1007/s10295-016-1858-z

Jia, B., Song, Y., Wu, M., Lin, B., Xiao, K., Hu, Z., & Huang, Y. (2016). Characterization of long - chain acyl - CoA synthetases which stimulate secretion of fatty acids in green algae Chlamydomonas reinhardtii. *Biotechnology for Biofuels* , 1–11. https://doi.org/10.1186/s13068-016-0598-7

Jiang, J. H., Hassan, K. A., Begg, S. L., Rupasinghe, T. W. T., Naidu, V., Pederick, V. G., ... Eijkelkamp, B. A. (2019). Identification of Novel Acinetobacter baumannii Host Fatty Acid Stress Adaptation Strategies. *MBio* , *10* (1), 1–6. https://doi.org/10.1128/mBio.02056-18

Johnson, D. R., Knoll, L. J., Levin, D. E., & Gordon, J. I. (1994). Saccharomyces cerevisiae contains four fatty acid activation (FAA) genes: An assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism. *Journal of Cell Biology* , *127* (3), 751–762. https://doi.org/10.1083/jcb.127.3.751

Jones, T. H., & Kennedy, E. P. (1969). Characterization of the membrane protein component of the lactose transport system of Escherichia coli.*Journal of Biological Chemistry* , *244* (21), 5981–5987.

Kang, M. K., & Tullman-Ercek, D. (2018). Engineering expression and function of membrane proteins. *Methods* , *147* (January), 66–72. https://doi.org/10.1016/j.ymeth.2018.04.014

Kato, A., Takatani, N., Use, K., Uesaka, K., Ikeda, K., Chang, Y., ... Omata, T. (2015). Identification of a Cyanobacterial RND-Type Efflux System Involved in Export of Free Fatty Acids. *Plant and Cell Physiology* , *56* (12), 2467–2477. https://doi.org/10.1093/pcp/pcv150

Kell, D. B., Swainston, N., Pir, P., & Oliver, S. G. (2015). Membrane transporter engineering in industrial biotechnology and whole cell biocatalysis. *Trends in Biotechnology* , *33* (4), 237–246. https://doi.org/10.1016/j.tibtech.2015.02.001

Kim, D., Choi, K. Y., Yoo, M., Zylstra, G. J., & Kim, E. (2018). Biotechnological potential of rhodococcus biodegradative pathways.*Journal of Microbiology and Biotechnology* , *28* (7), 1037–1051. https://doi.org/10.4014/jmb.1712.12017

Knoll, L. J., Johnson, D. R., & Gordon, J. I. (1994). Biochemical studies of three Saccharomyces cerevisiae acyl-CoA synthetases, Faa1p, Faa2p, and Faa3p. *Journal of Biological Chemistry* ,*269* (23), 16348–16356.

Kong, F., Liang, Y., Légeret, B., Beyly-Adriano, A., Blangy, S., Haslam, R. P., ... Li-Beisson, Y. (2017). Chlamydomonas carries out fatty acid β-oxidation in ancestral peroxisomes using a bona fide acyl-CoA oxidase. *Plant Journal* , *90* (2), 358–371. https://doi.org/10.1111/tpj.13498

Kong, F., Romero, I. T., Warakanont, J., & Li-Beisson, Y. (2018). Lipid catabolism in microalgae. *New Phytologist* , *218* (4), 1340–1348. https://doi.org/10.1111/nph.15047

Lancet, D., Zidovetzki, R., & Markovitch, O. (2018). Systems protobiology: Origin of life in lipid catalytic networks. *Journal of the Royal Society Interface* , *15* (144). https://doi.org/10.1098/rsif.2018.0159

Lau, S. Y., & Zgurskaya, H. I. (2005). Cell Division Defects in Escherichia coli Deficient in the Multidrug Efflux Transporter AcrEF-TolC. *Journal of Bacteriology* , *187* (22), 7815–7825. https://doi.org/10.1128/JB.187.22.7815

Lee, E. H., & Shafer, W. M. (1999). The farAB-encoded efflux pump mediates resistance of gonococci to long-chained antibacterial fatty acids. *Molecular Microbiology* , *33* (4), 839–845. https://doi.org/10.1046/j.1365-2958.1999.01530.x

Legras, J. L., Erny, C., Le Jeune, C., Lollier, M., Adolphe, Y., Demuyter, C., ... Karst, F. (2010). Activation of two different resistance mechanisms in saccharomyces cerevisiae upon exposure to octanoic and decanoic acids. *Applied and Environmental Microbiology* , *76* (22), 7526–7535. https://doi.org/10.1128/AEM.01280-10

Lennen, R. M., Kruziki, M. A., Kumar, K., Zinkel, R. A., Burnum, K. E., Lipton, M. S., ... Pfleger, B. F. (2011). Membrane stresses induced by overproduction of free fatty acids in Escherichia coli. *Applied and Environmental Microbiology* , *77* (22), 8114–8128. https://doi.org/10.1128/AEM.05421-11

Lennen, R. M., Politz, M. G., Kruziki, M. A., & Pfleger, B. F. (2013). Identification of transport proteins involved in free fatty acid efflux in Escherichia coli. *Journal of Bacteriology* , *195* (1), 135–144. https://doi.org/10.1128/JB.01477-12

Lepore, B. W., Indic, M., Pham, H., Hearn, E. M., Patel, D. R., & Van Den Berg, B. (2011). Ligand-gated diffusion across the bacterial outer membrane. *Proceedings of the National Academy of Sciences of the United States of America* , *108* (25), 10121–10126. https://doi.org/10.1073/pnas.1018532108

Li, D., Balamurugan, S., Yang, Y., Zheng, J., Huang, D., Zou, L., ... Li, H. (2019). Transcriptional regulation of microalgae for concurrent lipid overproduction and secretion. *Science Advances* ,*5* (January), eaau3795.

Li, N., Gügel, I. L., Giavalisco, P., Zeisler, V., Schreiber, L., Soll, J., & Philippar, K. (2015). FAX1, a Novel Membrane Protein Mediating Plastid Fatty Acid Export. *PLoS Biology* , *13* (2), 1–37. https://doi.org/10.1371/journal.pbio.1002053

Li, N., Xu, C., Li-Beisson, Y., & Philippar, K. (2016). Fatty Acid and Lipid Transport in Plant Cells. *Trends in Plant Science* ,*21* (2), 145–158. https://doi.org/10.1016/j.tplants.2015.10.011

Li, N., Zhang, Y., Meng, H., Li, S., Wang, S., Xiao, Z., ... Luo, F. (2019). Characterization of Fatty Acid Exporters involved in fatty acid transport for oil accumulation in the green alga Chlamydomonas reinhardtii. *Biotechnology for Biofuels* , *12* (1), 1–12. https://doi.org/10.1186/s13068-018-1332-4

Li, X., Zhang, R., Patena, W., Gang, S. S., Blum, S. R., Ivanova, N., ... Jonikasa, M. C. (2015). An indexed, mapped mutant library enables reverse genetics studies of biological processes in chlamydomonas reinhardtii. *Plant Cell* , *28* (2), 367–387. https://doi.org/10.1105/tpc.15.00465

Liu, B., Xiang, S., Zhao, G., Wang, B., Ma, Y., Liu, W., & Tao, Y. (2019). Efficient production of 3-hydroxypropionate from fatty acids feedstock in Escherichia coli. *Metabolic Engineering* ,*51* (October 2018), 121–130. https://doi.org/10.1016/j.ymben.2018.10.003

Liu, H., Yu, C., Feng, D., Cheng, T., Meng, X., Liu, W., ... Xian, M. (2012). Production of extracellular fatty acid using engineered Escherichia coli. *Microbial Cell Factories* , *11* , 1–13. https://doi.org/10.1186/1475-2859-11-41

Liu, N., Liu, B., Wang, G., Soong, Y. H. V., Tao, Y., Liu, W., & Xie, D. (2020). Lycopene production from glucose, fatty acid and waste cooking oil by metabolically engineered Escherichia coli.*Biochemical Engineering Journal* , *155* (September 2019), 107488. https://doi.org/10.1016/j.bej.2020.107488

Liu, X., Sheng, J., & Curtiss, R. (2011). Fatty acid production in genetically modified cyanobacteria. *PNAS* , *108* (17), 6899–6904. https://doi.org/10.1073/pnas.1103014108

Lovewell, R. R., Sassetti, C. M., & VanderVen, B. C. (2016). Chewing the fat: Lipid metabolism and homeostasis during M. tuberculosis infection. *Current Opinion in Microbiology* , *29* , 30–36. https://doi.org/10.1016/j.mib.2015.10.002

Magnuson, K., Jackowski, S., Rock, C. O., & Cronan, J. E. (1993). Regulation of fatty acid biosynthesis in Escherichia coli.*Microbiological Reviews* , *57* (3), 522–542. https://doi.org/10.1128/mmbr.57.3.522-542.1993

Mangroo, D., & Gerber, G. E. (1993). Fatty acid uptake in Escherichia coli: regulation by recruitment of fatty acyl-CoA synthetase to the plasma membrane. *Biochemistry and Cell Biology* , *71* (1–2), 51–56. https://doi.org/10.1139/o93-008

Mansy, S. S. (2010). Membrane Transport in Primitive Cells. *Cold Spring Harb Perspect Biol* , 2:a002188. https://doi.org/10.1002/9780470015902.a0021630

Martin, A., & Daniel, J. (2018). The ABC transporter Rv1272c of Mycobacterium tuberculosis enhances the import of long-chain fatty acids in Escherichia coli. *Biochemical and Biophysical Research Communications* , *496* (2), 667–672. https://doi.org/10.1016/j.bbrc.2018.01.115

Mingardon, F., Clement, C., Hirano, K., Nhan, M., Luning, E. G., Chanal, A., & Mukhopadhyay, A. (2015). Improving Olefin Tolerance and Production in E . coli Using Native and Evolved AcrB.*Biotechnology and Bioengineering* , *112* (5), 879–888. https://doi.org/10.1002/bit.25511

Nakashima, R., Sakurai, K., Yamasaki, S., Nishino, K., & Yamaguchi, A. (2011). Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket. *Nature* , *480* (7378), 565–569. https://doi.org/10.1038/nature10641

Nazarova, E. V., Montague, C. R., Huang, L., La, T., Russell, D., & Vanderven, B. C. (2019). The genetic requirements of fatty acid import by mycobacterium tuberculosis within macrophages. *ELife* ,*8* , 1–12. https://doi.org/10.7554/eLife.43621

Nazarova, E. V., Montague, C. R., La, T., Wilburn, K. M., Sukumar, N., Lee, W., ... VanderVen, B. C. (2017). Rv3723/LucA coordinates fatty acid and cholesterol uptake in Mycobacterium tuberculosis. *ELife* ,*6* , 1–22. https://doi.org/10.7554/eLife.26969

Newport, T. D., Sansom, M. S. P., & Stansfeld, P. J. (2019). The MemProtMD database : a resource for membrane-embedded protein structures and their lipid interactions. *Nucleic Acids Research* ,*47* (November 2018), 390–397. https://doi.org/10.1093/nar/gky1047

Nguyen, H. M., Baudet, M., Cuine, S., Adriano, J. M., Barthe, D., Billon, E., ... Li-Beisson, Y. (2011). Proteomic profiling of oil bodies isolated from the unicellular green microalga Chlamydomonas reinhardtii: With focus on proteins involved in lipid metabolism.*Proteomics* , *11* (21), 4266–4273. https://doi.org/10.1002/pmic.201100114

Nolan, S. J., Fu, M. S., Coppens, I., & Casadevall, A. (2017). Lipids affect the Cryptococcus neoformans-macrophage interaction and promote nonlytic exocytosis. *Infection and Immunity* , *85* (12), 1–18. https://doi.org/10.1128/IAI.00564-17

Nunn, W. D., & Simons, R. W. (1978). Transport of long-chain fatty acids by Escherichia coli: mapping and characterization of mutants in the fadL gene. *Proceedings of the National Academy of Sciences of the United States of America* , *75* (7), 3377–3381. https://doi.org/10.1073/pnas.75.7.3377

Obermeyer, T., Fraisl, P., DiRusso, C. C., & Black, P. N. (2007). Topology of the yeast fatty acid transport protein Fat1p: Mechanistic implications for functional domains on the cytosolic surface of the plasma membrane. *Journal of Lipid Research* , *48* (11), 2354–2364. https://doi.org/10.1194/jlr.M700300-JLR200

Overath, P., Pauli, G., & Schaire, H. U. (1969). Fatty Acid Degradation in Escherichia coli: An Inducible Acyl-CoA Synthetase, the Mapping of old-Mutations, and the Isolation of Regulatory Mutants. *European Journal of Biochemistry* , *7* , 559–574. https://doi.org/10.1016/0163-7827(92)90004-3

Parsons, J. B., Yao, J., Frank, M. W., Jackson, P., & Rock, C. O. (2012). Membrane Disruption by Antimicrobial Fatty Acids Releases Low- Molecular-Weight Proteins from Staphylococcus aureus. *Journal of Bacteriology* , *194* (19), 5294–5304. https://doi.org/10.1128/JB.00743-12

Scharnewski, M., Pongdontri, P., Mora, G., Hoppert, M., & Fulda, M. (2008). Mutants of Saccharomyces cerevisiae deficient in acyl-CoA synthetases secrete fatty acids due to interrupted fatty acid recycling.*FEBS Journal* , *275* (11), 2765–2778. https://doi.org/10.1111/j.1742-4658.2008.06417.x

Schjerling, C. K., Hummel, R., Hansen, J. K., Borsting, C., Mikkelsen, J. M., Kristiansen, K., ... Acb, T. (1996). Disruption of the Gene Encoding the Acyl-CoA-binding Protein (ACB1) Perturbs Acyl-CoA Metabolism in Saccharomyces cerevisiae *. *The Journal of Biological Chemistry* , *271* (37), 22514–22521.

Shani, N., Sapag, A., Watkins, P. A., & Valle, D. (1996). An S. cerevisiae Peroxisomal Transporter, Orthologous to the Human Adrenoleukodystrophy Protein, appears to be a Heterodimer of Two Half ABC Transporters: Pxalp and Pxa2p : Pxalp and Pxa2p. *Annals New York Academy of Sciences* , *804* (1), 770–772.

Shin, J., Yu, J., Park, M., Kim, C., Kim, H., Park, Y., ... Kweon, D. H. (2019). Endocytosing Escherichia coli as a Whole-Cell Biocatalyst of Fatty Acids. *ACS Synthetic Biology* , *8* (5), 1055–1066. https://doi.org/10.1021/acssynbio.8b00519

Sushchik, N. N., Kalacheva, G. S., Zhila, N. O., Gladyshev, M. I., & Volova, T. G. (2003). A Temperature Dependence of the Intra- and Extracellular Fatty-Acid Composition of Green Algae and Cyanobacterium.*Russian Journal of Plant Physiology* , *50* (3), 374–380.

Takemura, T., Imamura, S., & Tanaka, K. (2019). Identification of a chloroplast fatty acid exporter protein, CmFAX1, and triacylglycerol accumulation by its overexpression in the unicellular red alga Cyanidioschyzon merolae. *Algal Research* , *38* (August 2018), 101396. https://doi.org/10.1016/j.algal.2018.101396

Tarling, E. J., Vallim, T. Q. d. A., & Edwards, P. A. (2013). Role of ABC transporters in lipid transport and human disease. *Trends in Endocrinology and Metabolism* , *24* (7), 342–350. https://doi.org/10.1016/j.tem.2013.01.006

Tejima, K., Ishiai, M., Murayama, S. O., Iwatani, S., & Kajiwara, S. (2018). Candida albicans fatty acyl-CoA synthetase, CaFaa4p, is involved in the uptake of exogenous long-chain fatty acids and cell activity in the biofilm. *Current Genetics* , *64* (2), 429–441. https://doi.org/10.1007/s00294-017-0751-2

Tomitori, H., Kashiwagi, K., Sakata, K., Kakinuma, Y., & Igarashi, K. (1999). Identification of a gene for a polyamine transport protein in yeast. *Journal of Biological Chemistry* , *274* (6), 3265–3267. https://doi.org/10.1074/jbc.274.6.3265

Toscano, W. A., & Hartline, R. A. (1973). Transport of octanoate by Pseudomonas oleovorans. *Journal of Bacteriology* , *116* (2), 541–547. https://doi.org/10.1128/jb.116.2.541-547.1973

Van Den Berg, B. (2005). The FadL family: Unusual transporters for unusual substrates. *Current Opinion in Structural Biology* ,*15* (4), 401–407. https://doi.org/10.1016/j.sbi.2005.06.003

Van Den Berg, B., Black, P. N., Clemons, W. M., & Rapoport, T. A. (2004). Crystal structure of the long-chain fatty acid transporter FadL.*Science* , *304* (5676), 1506–1509. https://doi.org/10.1126/science.1097524

Van Roermund, C. W. T., Ijlst, L., Majczak, W., Waterham, H. R., Folkerts, H., Wanders, R. J. A., & Hellingwerf, K. J. (2012). Peroxisomal fatty acid uptake mechanism in saccharomyces cerevisiae.*Journal of Biological Chemistry* , *287* (24), 20144–20153. https://doi.org/10.1074/jbc.M111.332833

Vasconcelos, B., Teixeira, J. C., Dragone, G., & Teixeira, J. A. (2019). Oleaginous yeasts for sustainable lipid production—from biodiesel to surf boards, a wide range of "green" applications.*Applied Microbiology and Biotechnology* , 3651–3667. https://doi.org/10.1007/s00253-019-09742-x

Villalba, M. S., & Alvarez, H. M. (2014). Identification of a novel ATP-binding cassette transporter involved in long-chain fatty acid import and its role in triacylglycerol accumulation in Rhodococcus jostii RHA1. *Microbiology (United Kingdom)* , *160* (PART 7), 1523–1532. https://doi.org/10.1099/mic.0.078477-0

Voelker, T. A., & Davies, H. M. (1994). Alteration of the Specificity and Regulation of Fatty Acid Synthesis of Escherichia coli by Expression of a Plant Medium- Chain Acyl-Acyl Carrier Protein Thioesterase. *Journal of Bacteriology* , *176* (23), 7320–7327.

Wang, Z., Fan, G., Hryc, C. F., Blaza, J. N., Serysheva, I. I., Schmid, M. F., ... Du, D. (2017). An allosteric transport mechanism for the AcrAB-TolC multidrug efflux pump. *ELife* , *6* , 1–19. https://doi.org/10.7554/eLife.24905

Wu, J. T., Chiang, Y. R., Huang, W. Y., & Jane, W. N. (2006). Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. *Aquatic Toxicology* , *80* (4), 338–345. https://doi.org/10.1016/j.aquatox.2006.09.011

Wu, J., Wang, Z., Zhang, X., Zhou, P., Xia, X., & Dong, M. (2019). Improving medium chain fatty acid production in Escherichia coli by multiple transporter engineering. *Food Chemistry* ,*272* (March 2018), 628–634. https://doi.org/10.1016/j.foodchem.2018.08.102

Yao, J., & Rock, C. O. (2017). Exogenous fatty acid metabolism in bacteria. *Biochimie* , *141* , 30–39. https://doi.org/10.1016/j.biochi.2017.06.015

Zalatan, F., & Black, P. (2011). Characterization of long-chain fatty acid uptake in Caulobacter crescentus. *Archives of Microbiology* ,*193* (7), 479–487. https://doi.org/10.1007/s00203-011-0694-9

Zgurskaya, H. I., & Nikaido, H. (1999). Bypassing the periplasm: Reconstitution of the AcrAB multidrug efflux pump of Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America* , *96* (13), 7190–7195. https://doi.org/10.1073/pnas.96.13.7190

Zhu, Z., Hu, Y., Teixeira, P. G., Pereira, R., Chen, Y., Siewers, V., & Nielsen, J. (2020). Multidimensional engineering of Saccharomyces cerevisiae for efficient synthesis of medium-chain fatty acids. *Nature Catalysis* , *3* (1), 64–74. https://doi.org/10.1038/s41929-019-0409-1

Zou, Z., Dirusso, C. C., Ctrnacta, V., & Black, P. N. (2002). Fatty acid transport in Saccharomyces cerevisiae: Directed mutagenesis of FAT1 distinguishes the biochemical activities associated with Fat1p. *Journal of Biological Chemistry* , *277* (34), 31062–31071. https://doi.org/10.1074/jbc.M205034200

Zou, Z., Tong, F., Faergeman, N. J., Borsting, C., Black, P. N., & Dirusso, C. C. (2003). Vectorial Acylation in Saccharomyces cerevisiae. *The Journal of Biological Chemistry* , *278* (18), 16414–16422. https://doi.org/10.1074/jbc.M210557200

Tables

Table 1: FadL residues which have been shown or suggested to be involved in transport of fatty acids

Residue	*Region*	*Effect on transport*	*Reference*
F3	Binding Pocket	Abolished	(Lepore et al., 2011)
N33/P34/A35	Hatch Domain	Reduced	(Hearn et al., 2009)
S100/N101/Y102/G103	S3 kink	Abolished	(Hearn et al., 2009)
G143	Hatch Domain	-	(Van Den Berg, 2005)
R157	Binding Pocket	-	(Van Den Berg, 2005)
G212	Hatch Domain	No effect	(Hearn et al., 2009)
D348	Binding Pocket	Abolished	(Lepore et al., 2011)

Table 2: Proteins involved in microbial fatty acid transport processes

Protein Name	*Organism*	*UniProt identifier*	*Act*
FadL	*E. coli*	P10384	Fat
FACS	*E. coli*	P69451	Lor
Ltp1	*R. jostii*	Q0S4W2	Fat
Mce1 complex	*M. tuberculosis*	O07412 (YrbE1A)	Fat
Rv1272c	*M. tuberculosis*	P9WQJ3	Fat
AcrAB-TolC Complex	*E. coli*	P0AE06 (AcrA) P31224 (AcrB) P02930 (TolC)	Fat
AcrEF-TolC complex	*E. coli*	P24180 (AcrE) P24181 (AcrF) P02930 (TolC)	Fat
FarE	*S. aureus*	A0A114B8E3+	Fat
AdeIJK complex	*A. baumannii*	Q2FD95+ (AdeI) Q2FD94+ (AdeJ) Q24LT6 (AdeK)	Fat
EmhABC complex	*P. fluorescens*	Q4KH22+ (EmhA) Q4KH23+ (EmhB) A0A4P7I039 (EmhC)	Fat
FarAB complex	*N. gonorrhoeae*	Q9RQ30 (FarA) Q9RQ29 (FarB)	Fat
Slr2131	*Synechocystis* sp. PCC 6803	P73998	Fat
RndA1B1 complex	*S. elongatus*	Q31KM1 (RndA1) Q31KM0 (RndB1)	Fat
Fat1	*S. cerevisiae*	P38225	Fat
Faa1	*S. cerevisiae*	P30624	Lor
Faa4	*S. cerevisiae*	P47912	Lor
Ypk1	*S. cerevisiae*	P12688	Pro
End3	*S. cerevisiae*	P39013	Act
Vrp1	*S. cerevisiae*	P37370	Act
Srv2	*S. cerevisiae*	P17555	Act
CaFaa4	*C. albicans*	A0A1D8PU56	Lor
YlFat1	*Y. lipolytica*	Q6C5Q8	Fat
YlFaa1	*Y. lipolytica*	Q6C8Q3	Lor
Acb1	*S. cerevisiae*	P31787	Acy
Pxa Complex	*S. cerevisiae*	P41909 (Pxa1) P34230 (Pxa2)	Fat
Faa2	*S. cerevisiae*	P39518	Acy
Tpo1	*S. cerevisiae*	Q07824	Fat
Cracs1	*C. reinhardtii*	A8JH58	Lor
Cracs2	*C. reinhardtii*	A0A3S6D659	Lor
NobZIP1	*N. oceanica*	A0A3S7YS51	Tra
CrFAX1	*C. reinhardtii*	Cre10.g421750++	Fat
CrFAX2	*C. reinhardtii*	Cre08.g366000++	Fat
CmFAX1	*C. merolae*	M1V3K0	Fat
LCS2	*C. reinhardtii*	Cre13.g566650++	Lor
ABCD1	*C. reinhardtii*	Cre15.g637761++	Put

+ Multiple Entries available in UniProt

++ AlgaePath identifier (no uniprot identifier available)*http://algaepath.itps.ncku.edu.tw/index.html*

List of Figures with captions

Figure 1 A) Structure of FadL: The ∝-helices of the extracellular region forming the opening of a hydrophobic groove are shown in orange. Inside the protein, several residues that can interact from the binding pocket are shown in red. The hatch domain of the intracellular region is shown in blue. Finally, one of the β-strands (strand 3 or S3), presents a bend or a kink, shown in green, that leads to the formation of

an opening in the intramembrane surface of the protein. **B) Transport mechanism of FadL:** Schematic representation of the transport mechanism of FadL proposed by Lepore et al., 2011. The yellow box represents the bilipid layer and the red hexagons the lipopolysaccharide layer.

Figure 2 Bacterial fatty acid import systems: A) In certain Gram-negative bacteria a membrane-associated acyl-CoA synthetase imports the fatty acids to the cytosol in the form of acyl-CoA, a process known as vectorial acylation. **B)** In Gram-positive bacteria, single proteins have been identified in organisms such as *R. jostii* and*M. tuberculosis* which may be able to import free fatty acids into the cells, which will be later activated for their metabolization.**C)** In *M. tuberculosis,* the multiprotein complex Mce has been identified. The figure shows a hypothetical model adapted from Nazarova et al., 2019.

Figure 3 Structure of the AcrAB-TolC complex: The multiprotein complex AcrAB-TolC spans from the inner to the outer membrane.

Figure 4 Structure of Fat1: Fat1 presents two transmembrane domains and two domains which are partially integrated in the membrane. ATP/AMP binding domains are found near the two latter domains. A VLACS domain is found further in the C-terminal region. Finally, the soluble Faa1 has been observed to interact with the C-terminus of this protein to activate the imported fatty acids.

Figure 5 Intracellular trafficking of fatty acids in yeast cells: Simplified fatty acid transport routes in *S. cerevisiae*based on current knowledge. Both plasma membrane transport processes and intracellular trafficking routes are showed.

Figure 6 Intracellular trafficking of fatty acids in microalgae cells: Fatty acid intracellular trafficking routes in *C. reinhardtii.* No transport system has been described for the outer plastid membrane and for the export of free fatty acids to the extracellular media.