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Editorial

Metabolite transport and its impact on metabolic engineering approaches

Cells are the structural units of life and are separated from the environment by at least one cellular membrane consisting of a lipid bilayer. Thus, metabolite transport across cellular membranes is a key feature of living organisms. Specialized proteins or protein complexes mediate transport processes and are accessible to metabolic engineering approaches.

Genetic modifications in metabolic engineering has mostly involved the deletion or overexpression of genes encoding for enzymes. The role of transporters has received much less attention, but as this special issue shows, it is a key factor to consider when rationally designing microbial cell factories.

Transporters have been employed in metabolic engineering endeavors to target three fundamental aspects:

- Import of substrates
- Export of products
- Modification of intracellular fluxes.

Soaeres-Silva *et al.* (2020) have carried out an extensive literature review on these three approaches focusing on the role of transporters in microbial organic acid production. Metabolic engineering of microbes to improve the import of carbon and energy sources is a key point in microbial cell factory construction. Most of the approaches used have dealt with the necessity of transporters to enable the use of abundant and renewable carbon sources which are not transported efficiently in the cell and hence cannot be metabolized. A well-known example in this regard, is the engineering of *Saccharomyces cerevisiae* for increased lignocellulose-derived xylose transport (Hamacher *et al.* 2002; Farwick *et al.* 2014) or to improve glycerol uptake (Klein *et al.* 2016).

Metabolic engineering of proteins involved in the export of substrates is key to improve the secretion of fermentation products whose intracellular accumulation can be toxic for the cell or can determine a block of the intracellular fluxes. In this issue, Odoni *et al.* (2019) present a strategy for the identification of an organic acid transporter in *Aspergillus niger*. This fungus is well-known for its ability to produce citric acid and the citrate exporter *cexA* was identified using a homology approach using the itaconic acid transporter of *Ustilago maydis* as template (Geiser *et al.* 2016; Steiger *et al.* 2019). Odoni *et al.* (2019) identified the same gene using a transcriptomic approach comparing citrate producing and non-producing conditions. This example shows very well how a novel transporter can be identified with two complementary methods. Recently, also the citrate exporter *cex1* in *Yarrowia lipolytica* was identified, which shares some similarity with *cexA* but is not the closest homolog to the *A. niger* transporter. Thus, homology as the sole criterion can be misleading in identifying the correct transporter gene in another species (Erian *et al.* 2020).

Metabolic engineering approaches involving the manipulation of intracellular transporters are less common. Nonetheless recent data demonstrate that intracellular transporters, mainly mitochondrial carriers, are particularly important in regulating intracellular fluxes. Mitochondria are key organelles for the synthesis of organic acids, amino acids, and fundamental intermediates such as acetyl-CoA. Mitochondrial transporters can deeply affect the intracellular flux distribution of metabolic pathways which are partly or completely localized in this organelle like itaconic acid production (Wierckx *et al.* 2020). Recently, it has been shown that homologs of the *S. cerevisiae* citrate/oxoglutarate mitochondrial carrier Yhm2 are crucial for citrate production in *A. niger* (Kirimura, Kobayashi and Yoshioka 2019) and *Y. lipolytica* (Yuzbasheva *et al.* 2019). The deletion of YlYhm2 along with the overexpression of the newly identified mitochondrial carrier involved in mitochondrial isocitrate export YlSfc1 (Yuzbasheva *et al.* 2020) has allowed to obtain a *Y. lipolytica* strain producing isocitric acid as the main fermentation product.

The interest on mitochondrial transporters for the metabolic engineering of microbial cell factories has been further increased in the recent years following the development of novel approaches such as the 'subcellular metabolic engineering' (Duran, López and Avalos 2020). In this approach a metabolic pathway is confined in an organelle (mostly in the mitochondrion) supplying a protected compartment in which metabolic reaction can take place more efficiently for several factors including confinement of metabolic intermediates, decrease in their possible toxicity, increase in the channeling, abundance of specific cofactors. The subcellular metabolic engineering requires that hydrophilic substrates and products must efficiently cross the organellar membrane and that conversely intermediates are kept in the organelle, highlighting the need of a better knowledge of organellar transport.

New knowledge of transporters is always required to provide new metabolic engineering strategies. In this special issue, Tiukova *et al.* (2019) report the identification of two high-affinity glucose transporters in the spoilage yeast *Brettanomyces bruxellensis*, which represent a constant problem in the winemaking industry for its prevalence during secondary fermentations.

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Figure 1. Engineering of transport proteins (violet, green) increases the transport of hydrophilic metabolites (red) through the lipid bilayer (orange, blue) of biological membranes.

These genes have been identified and characterized expressing them in *Xenopus levis* oocytes. Using this experimental system, the authors have determined that this H^+-dependent transporter has a much higher affinity for glucose than the *S. cerevisiae* Hxt7p explaining the efficiency of *Brettanomyces bruxellensis* as pollutant in industrial low-sugar ethanol fermentations. New knowledge on high-affinity glucose transporters can be extremely useful in the engineering of *S. cerevisiae* for bioethanol production.

Recently, another transporter was identified in the yeast *Torulaspora delbrueckii*, which has the interesting ability to modulate its glucose uptake kinetics depending on the sugar concentration (Pacheco *et al.* 2020). Both papers use an important tool frequently used by many different groups: a yeast strain that lacks all hexose transporters and thus is not able to grow on glucose (Wieczorke *et al.* 1999).

The issue is concluded by a mini review from Mislav Oreb about the emerging field of 'membrane transport metabolons' and how they can be engineered. Transport membrane metabolons are defined as the physical association of membrane transporters with enzymes that metabolize the transported substrates. Examples from this research area demonstrate how structure and function is directly linked when dealing with biochemical reactions (Oreb 2020). Metabolons remind us that up- and down-stream of a transport reaction other biochemical conversions take place: the kinetic properties these enzymes determine if channeling via a transport metabolon makes sense or not. Such concepts are discussed along with information on the artificial design of a transport metabolons.

Overall, we compiled a selection of papers which provide insights both into the discovery and the biotechnological application of transport systems. With the growing knowledge of the molecular identity of transport processes and the ability to efficiently construct new strains through genetic engineering, we can expect a plethora of new studies on transport processes and their impact on metabolic engineering of microbial cells in the coming years.

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