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Getting pumped: membrane efflux transporters for enhanced biomolecule production

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Small molecule production in microbial hosts is limited by the accumulation of the product inside the cell. Efflux transporters show promise as a solution to removal of the often-toxic products. Recent advances in transporter identification through expression profiling, heterologous expression, and knockout studies have identified transporters capable of secreting compounds of biotechnological interest. In addition, engineering of well-studied transporters has shown that substrate specificity in these transporters is malleable. Future work in identification, engineering, and expression of small molecule exporters can be instrumental in expanding the biocatalysis portfolio.

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Introduction

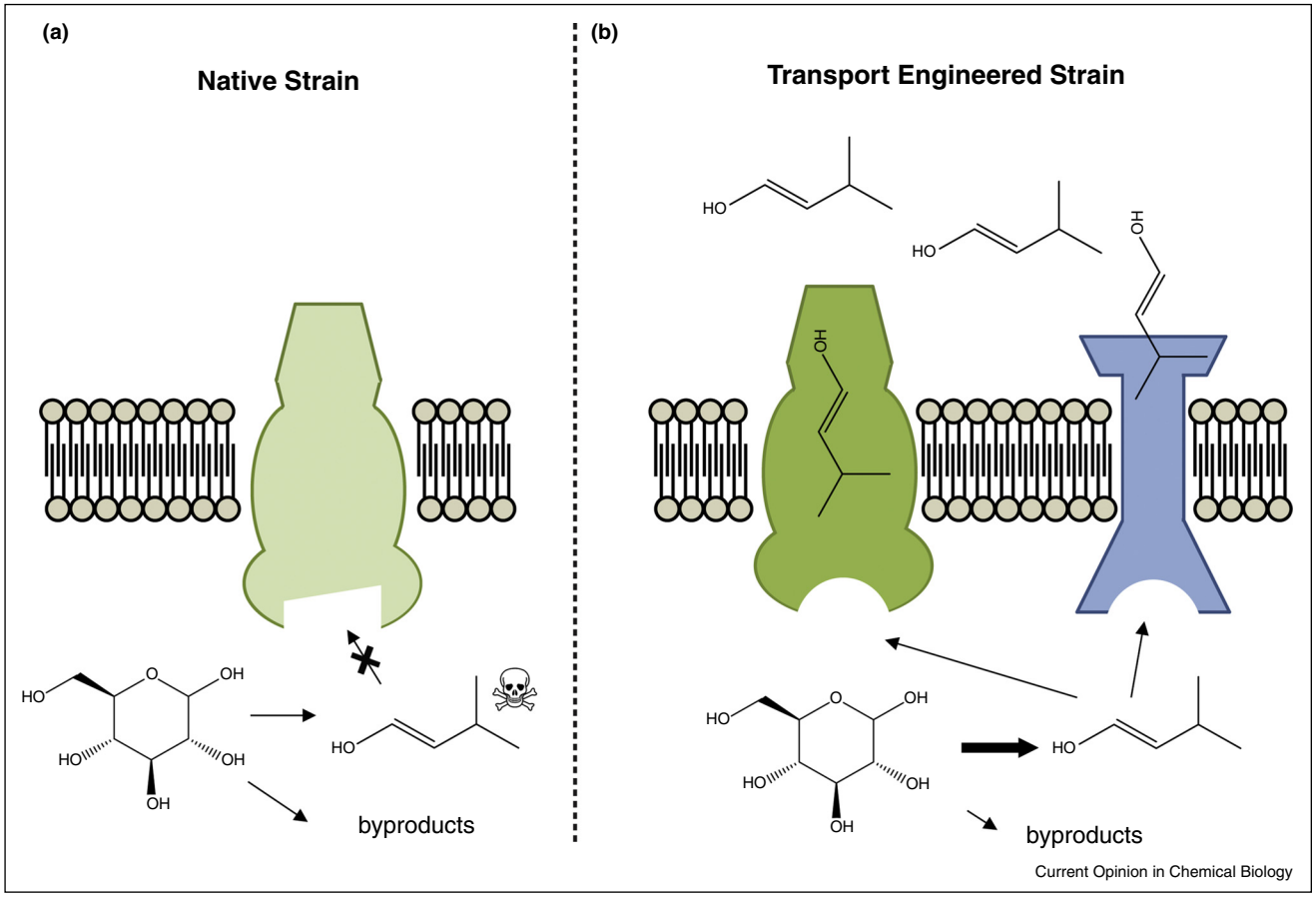
Over the past decade, the biocatalytic production of chemicals has gained increased traction in the scientific community. Organisms provide a large repertoire of enzymes capable of performing unique and specific chemistry in mild reaction conditions. Moreover, a series of reactions can be linked to create a synthetic pathway by expressing multiple enzymes simultaneously in genetically tractable microbial hosts. Synthetic biology has been used to modify all aspects of biocatalysis, from engineering enzymes with new chemical function [1,2], to controlling reactant flux on the host [3] and pathway [4] scale. Nonetheless, this biochemical production method suffers from the drawback that all reactions must proceed in the cell interior, and all intermediates and final products therefore remain in the reaction pool. This can create problems due to both unintended side-reactions and product inhibition. Thus biocatalysis *in vivo* has been most successful for producing chemicals of high value, for which high yield is not a consideration, and/or for which the product easily moves out of the cell. The recent focus on microbial production of biofuels [5] and commodity chemicals [6] has considerably altered this paradigm, and subsequently created a need for developing mechanisms of sequestering and removing small molecules from the cell. The utilization of active transporters is a particularly promising strategy for the efflux of biochemicals (Figure 1). Secretion permits the removal of toxic products, allows for lower recovery costs for all products, and can drive reactions forward as product is separated from the rest of the cell.

Small molecule movement in and out of the cell is based on two distinct mechanisms. The first, passive diffusion, is a function of the interaction between the molecule and the lipid bilayer(s) of the cell. For small nonpolar or slightly polar molecules, the diffusion rate is proportional to the product of the partition coefficient of the molecule into an oil phase (the interior of the bilayer) and the diffusion coefficient in that phase [7]. Smaller molecules move faster through the membrane, and more hydrophobic molecules partition more easily. Thus, the majority of bioactive molecules are too large or hydrophilic to easily pass through the membrane. At the same time, many hydrophobic solvents pass through the membrane relatively quickly, which often contributes to their cytotoxicity [8]. To better control the passage of small molecules, cells have a second, active mechanism for moving them across the membrane. Transporters exist for both import and export for most types of molecules including alkanes [9], amines [10], acids [11], esters [12], and many others. Therefore, active transporters provide an engineering platform for designing the transfer of small molecules into and out of the cell. In this review, we will focus on methods for controlling biochemical secretion; other reviews [13–15] offer a comprehensive examination of small molecule import.

Types of efflux transporters

It is estimated that up to 7% of all inner membrane proteins in the model bacteria *Escherichia coli* are involved in efflux, while more than four times that number are associated with import of small molecules [16]. This disparity highlights the different approaches the cell takes to controlling the chemical content of the cytosol. While importers are known to be quite specific and often act only on a small class of chemicals or just a single molecule [17,18], exporters have a range of substrate profiles. Some efflux pumps are known to be quite

Figure 1



Schematic of transporter use for efflux of small molecules. **(a)** Biocatalysis can convert sugar feedstocks to a variety of organic molecules which may remain in the cell if no transporter acts on the molecules. These products can be toxic to the cell and cause product inhibition. **(b)** Strains can be engineered through mutagenesis of existing transporters (dark green) or by expression of heterologous transporters (blue) to secrete the product. Such transporters have been shown to increase production and decrease toxicity of the desired product molecule.

promiscuous [19] in overall activity, while others maintain high specificity towards their targets [20]. Perhaps the best-characterized class of small molecule exporters in microbes are those responsible for the secretion of antibiotics and other toxic agents, known as multidrug resistance (MDR) pumps [21]. Studies of these transporters have strong relevance for combating increased antibiotic resistance in pathogenic bacteria, but also provide insight into ways that they can be used to modulate the internal chemical environment of the cell. MDR pumps can be classified into several transporter families, grouped by their topology and energy source. For example, Gram-negative bacteria must transport small molecules across two lipid membranes, and use only a single pump to accomplish this action. Pumps in these bacteria utilize one of two primary mechanisms: first, secreting the small molecule into the periplasm, from which transport is accomplished through diffusion across the more porous membrane, or second, employing accessory proteins to channel the substrate through the outer membrane [22]. Most transporters also rely on two distinct mechanisms for obtaining the energy required for transport — hydrolyzing ATP, or using an ion concentration gradient across the membrane. For pumps that use ion gradients, the proton motive force is well-represented as a driving force, but pumps that utilize Na^+ or K^+ gradients are common as well. For most pumps, one ATP or one ion is required to move one molecule out of the cell. Thus, in systems where production yield of the small molecule is of high importance, ion pumps may be the preferred method for efflux of desired product due to lower total energy cost associated with transport, although other considerations such as total kinetic activity and substrate specificity may favor ATP-based transporters.

Finding the right transporters

To find the right transporters for secretion of a desired biochemical, two tactics can be taken. The first tactic is a systems biology/transcriptomic approach of discovering transporters which act on the molecule of interest. A