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Recent insights in microbial exopolysaccharide biosynthesis and engineering strategies

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The distinct biosynthesis pathways for microbial exopolysaccharide production provide different engineering strategies to tailor the chemical structures of the final polymers. This review focuses on the latest insights in the various pathways and identifies bottlenecks as well as promising targets for tailoring microbial polysaccharide production. The main engineering strategies includes the combinatorial assembly of glycosyltransferases and engineering of the Wzx and Wzy proteins for flipping of repeating units as well as polymerization. In the case of synthase based polysaccharides, the use of epimerases or engineering approaches of the synthase itself as well as overexpression of c-di-GMP levels is identified as one of the most promising strategies. For sucrase-based biosynthesis, the *in vitro* production by engineered sucrase enzymes or adjusted production conditions is shown as a very promising method.

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Introduction

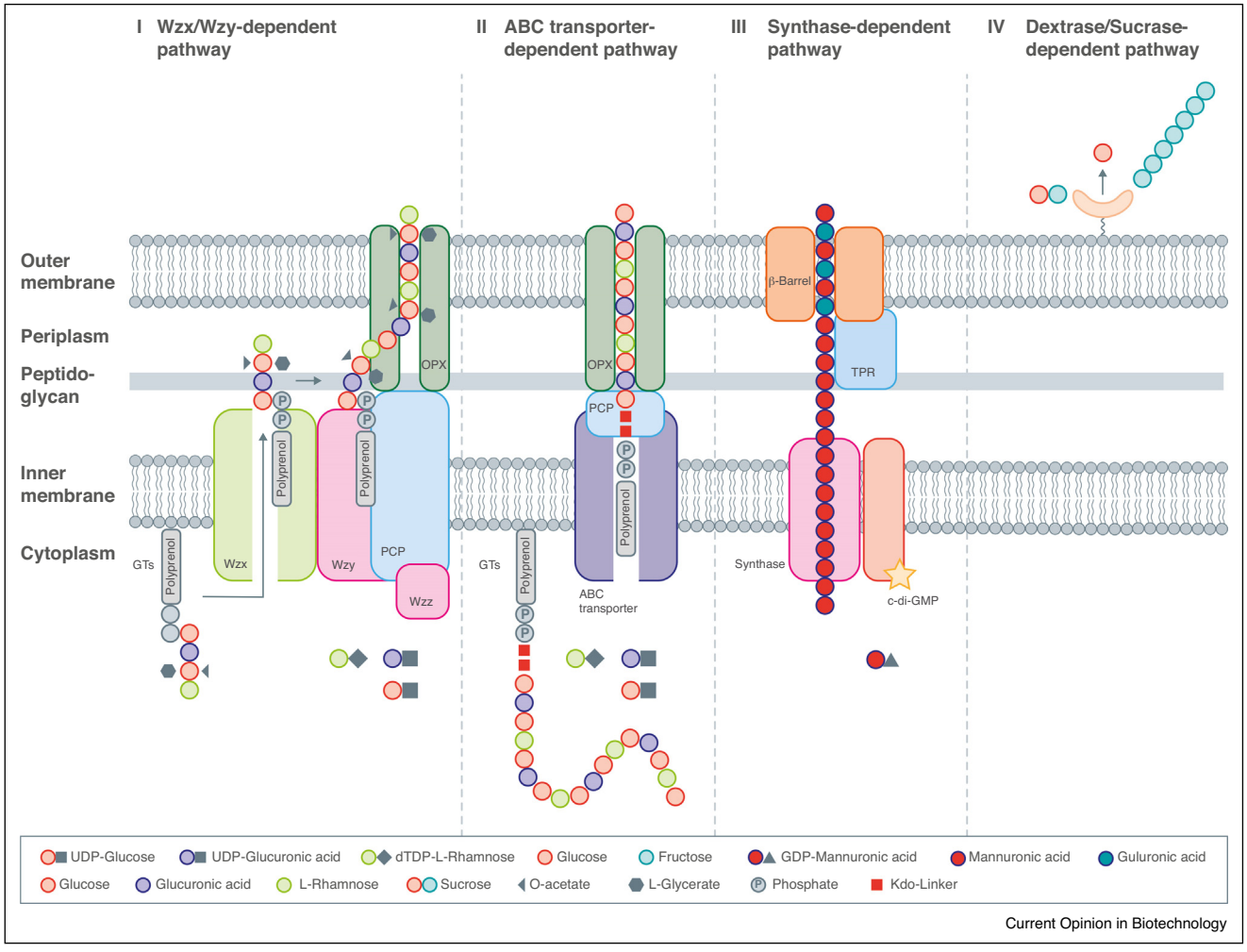
Carbohydrate polymers represent a highly diverse class of biogenic polymers present in all domains of life. In nature, polysaccharides in the different organisms assume several tasks. In plants and algae, cellulose plays an essential role as structural polysaccharide to mediate stability in the cells. Glycogen or starch serve as intracellular storage compounds and some, particularly microbial exopolysaccharides (EPS), serve as natural adhesives. Cells also protect themselves from environmental stress conditions such as extreme pH, antibiotics or desiccation by secreting exopolysaccharides into the environment to encapsulate the individual cells. Additionally, microbial EPS play essential roles in host-pathogen interactions as well as biofilms [1,2]. The different host invading mechanisms have been just recently reviewed by Buttimer *et al.* [3•] and it was shown that capsular polysaccharides mimic host-tissue molecules [4]. In addition to the natural functions of polysaccharides, they have also been used for a long time in different industrial applications such as cosmetics [5], food, feed and pharmacy, as well as technical applications [6,7]. In these applications, they perform impressively as biogenic polymers, due to their unique chemical structures [8–10], which are defined by the highly specific biosynthesis machinery. In general, the biosynthesis of microbial exopolysaccharides can be divided into four distinct categories, depending on the monomer composition as well as localization of the final polymer. For the biosynthesis of most homopolymers, which consist of one type of carbohydrate monomer, the so-called synthase dependent pathway is used. This pathway takes place in the cell and the biosynthesis machinery is part of the whole cell envelope. Another possible biosynthesis for (primarily) homopolymers is production via extracellular enzymes such as dextransucrases or levansucrases. These enzymes cleave sucrose as extracellular substrate and use the energy of the glycosylic bond to polymerize glucose or fructose polymers termed dextrans or levans respectively.

More complex is the assembly of heteropolysaccharides, by stepwise transfer of various activated sugar monomers and derivatives thereof, such as sugar alcohols or aminated sugars. In principle two different pathways are described which differ in the final localization of the polymers. The so-called ABC transporter pathways produces heteropolysaccharides which are still bound to the cell envelope by a poly-2-keto-3-deoxyoctulosonic acid (Kdo) linker, and are mainly known as capsular polysaccharides (CPS), whereas the so-called Wzx/Wzy pathway results primarily in EPS which are secreted into the environment. A schematic overview of the different pathways is given in Figure 1.

Wzx/Wzy pathway

This pathway includes the assembly of repeating units by the action of highly specific glycosyltransferases (GTs) which transfer activated sugar nucleotide monomers toward the undecaprenyl diphosphate (Und-P) linker anchored at the cytosolic side of the inner membrane (Figure 2). Thus, the sequence of the final, mostly branched, polymers is determined by the GTs available. Adjacent to the backbone assembly, the side chain and substituents such as acetate, glycerol, pyruvate, succinate

Figure 1



Schematic representation of the distinct polysaccharide pathways found in microorganisms. **(I)** The Wzx/Wzy pathway for the biosynthesis of mainly branched heteropolysaccharides, which are finally secreted into the environment. After assembly of the repeating unit by the action of GTs, the Und-P linked oligomer is flipped into the periplasm by the Wzx protein, followed by polymerization via Wzy and transport by action of the OPX proteins. **(II)** In the ABC transporter pathway, the whole polymer chain is assembled within the cytoplasm by the action of different GTs and then flipped toward the periplasm. **(III)** The synthase based pathway polymerizes just one type of monosaccharides by a highly specific GT like domain and the final polymer is transported through the cell envelope. **(IV)** The extracellular sucrase based biosynthesis assembles homopolymers or specific oligosaccharides by substrate cleavage and using the monomer for polymerization.

or even sulphate will be incorporated before the polymer is finally assembled, but it is not very clear at which stage this incorporation occurs [11,12]. The flippase protein, which is encoded by the *wzx* gene, transfers the repeating unit into the periplasmic space by an H^+-dependent antiporter mechanism as proposed for *Pseudomonas aeruginosa* [13]. However, not all repeating units are charged and the structures of the different Wzx proteins show various numbers of transmembrane sequences, as well as lacking similarity at the sequence level. This indicates that diverse types of Wzx exist [14••]. There is evidence for Wzx substrate preference on cognate O-units beyond the first sugar, but our insights are still limited since some Wzx proteins show just low specificity [13,14••,15]. Thus, the Wzx proteins represent one of the main targets for tailored polysaccharide production by use of synthetic repeating units. Once the repeating units are transferred toward the periplasm, they are recognized by a polymerase, which polymerizes the repeating units by backbone assembly, while at the same time transferring the mutual polysaccharide to the outside of the cell. This step is performed by the polymerase Wzy, sometimes in combination with a co-polymerase, which can be involved in chain length determination of the polymer [16,17]. Final secretion is realized by action of proteins such as outer membrane export proteins (OPX) which show relative low substrate specificity as impressively reviewed by Cuthbertson *et al.* [18]. Therefore, the name Wzx/Wzy