Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Gram-negative synthase-dependent exopolysaccharide biosynthetic machines

Kristin E Low[1] and P Lynne Howell[1,2]

Bacteria predominantly exist as matrix embedded communities of cells called biofilms. The biofilm matrix is made up of a variety of self-produced extracellular components including DNA, proteins, and exopolysaccharides. Bacterial exopolysaccharides have been implicated in surface adhesion, resistance to antibiotics, and protection from host immune systems. Herein we review the structure and function of the proteins involved in the production of the Gram-negative synthase-dependent exopolysaccharides: alginate, poly-β(1,6)-*N*-acetyl-D-glucosamine (PNAG), cellulose, and the Pel polysaccharide. We highlight the similarities and differences that exist at the molecular level in these synthase systems.

Addresses
[1] Program in Molecular Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada
[2] Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

Corresponding author: Howell, P Lynne (howell@sickkids.ca)

Current Opinion in Structural Biology 2018, **53**:xx–yy

This review comes from a themed issue on **Catalysis and regulation**

Edited by **Alice Vrielink** and **Hazel Holder**

https://doi.org/10.1016/j.sbi.2018.05.001

Introduction

Bacteria exist in a wide variety of environments as matrix embedded communities of cells called biofilms. The key components of the biofilm matrix are extracellular DNA, secreted proteins and exopolysaccharides. Exopolysaccharides (EPSs) provide structural integrity to the matrix and have been implicated in cell–cell and cell–surface adherence, colonization, antibiotic resistance, and providing protection from the host immune system.

The chemical composition of the EPS produced by Gram-negative bacteria varies across species and environmental niche, with many bacteria having the genetic capability to produce more than one type of polymer. The molecular mechanisms used to produce EPSs have been classified into three distinct pathways: the Wzx/Wzy-dependent flippase pathway; the ATP-binding cassette (ABC) transporter-dependent pathway; and the synthase-dependent pathway [1,2]. Synthase-dependent systems use a membrane embedded, multi-protein complex typically consisting of an inner membrane embedded glycosyltransferase (GT) and co-polymerase, often called the synthase complex, which polymerizes the EPS and facilitates translocation across the inner membrane. Binding of the bacterial secondary messenger bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) to the synthase complex post-translationally regulates polymer synthesis [1] (Figure 1a). Polymer secretion is dependent on tetratricopeptide repeat (TPR) domains coupled to a β-barrel porin, while additional enzymes present in the periplasm modify the polymer as necessary.

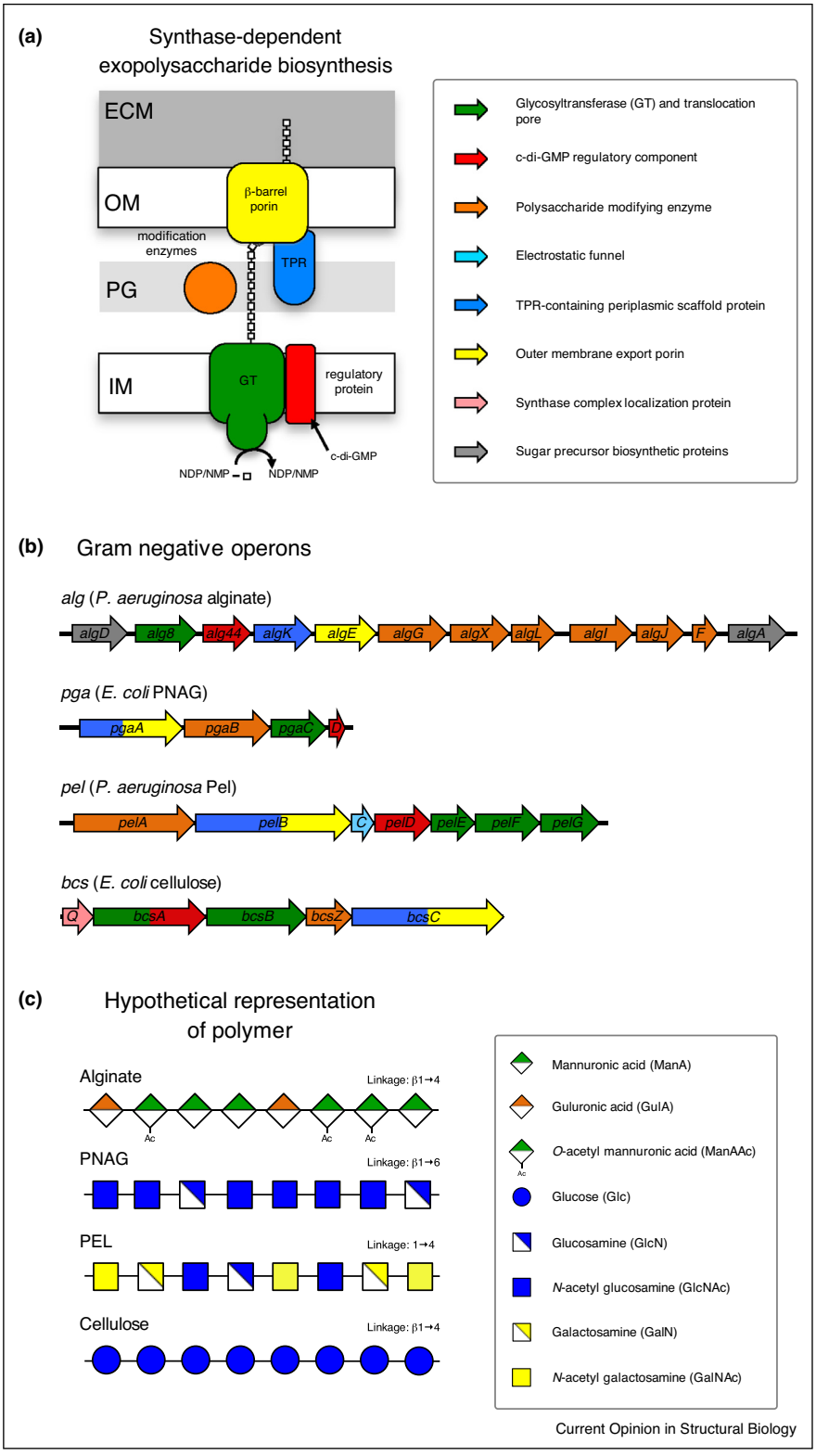
EPSs that have been classified as using the synthase-dependent mechanism include: alginate, poly-β(1,6)-*N*-acetyl-D-glucosamine (PNAG), cellulose, acetylated cellulose, and more recently the Pel polysaccharide (Figure 1b). Except for the Pel polysaccharide, which contains *N*-acetyl glucosamine and *N*-acetyl galactosamine, these polymers are synthesized as linear homopolymers. All of the polymers can be chemically modified in a random fashion (Figure 1c). Typically, the proteins required for polymerization, modification, and export of each of these polymers are encoded on a single operon (Figure 1b). While the alginate operon contains some of the genes required for synthesis of the nucleotide-sugar precursor molecules [3], more typically these proteins are encoded elsewhere on the chromosome. Representative operons from commonly studied species for each polymer (Figure 1b) reveal that the order in which the genes are arranged is not conserved. In addition, the different functionalities required for biosynthesis are not always fulfilled by the same number of proteins or even the same type of protein fold.

In the last seven years, the structures of many of the proteins involved in synthase-dependent EPS biosynthesis have been determined (Table 1). These structures have helped to elucidate not only the individual protein's mechanism of action but have also served to highlight both the similarities and differences that exist at the molecular level between the various synthase systems. This review will describe our current understanding of the molecular mechanisms involved in the synthesis of alginate, PNAG, Pel, and cellulose.

Regulation of EPS biosynthesis by c-di-GMP

Synthase-dependent EPS biosynthesis is post-translationally regulated by the bacterial secondary messenger

Figure 1



Bacterial synthase-dependent biosynthetic systems and operons. Legends for the role of the gene product and saccharide symbols are in boxed inserts. **(a)** Schematic of simplified synthase-dependent EPS biosynthesis system depicting key protein components including the glycosyltransferase (GT), regulatory protein, modification enzymes, TPR domain, and β-barrel porin. An inner membrane synthase complex is composed of the GT and translocation pore proteins coupled with regulation components. Activated sugar precursors function as substrates for