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CHAPTER 1

Capsular Polysaccharides in *Escherichia coli*

David Corbett and **Ian S. Roberts**[1]

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I. INTRODUCTION

The expression of extracellular polysaccharide (EPS) material is a common feature of many bacteria. This EPS coats the outside of the bacterial cell and as a consequence plays an intimate role in mediating interactions between the bacterium and its immediate environment. In certain cases the polysaccharide may be tightly associated with the cell surface forming a discrete structure termed a capsule, or it may be shed in the form of EPS or slime. A number of roles have been assigned to polysaccharide capsules and it is clear that in a variety of environments the expression of a capsule confers a selective advantage to the host. The major components of bacterial capsules are highly hydrated, high molecular weight acidic polysaccharides that confer upon bacteria an overall negative charge and hydrophilic properties. There is great structural diversity in capsular polysaccharides both between different bacterial species but also within the same bacterial species. This diversity is a consequence of not only differences in the repeat monosaccharide components but also differences in linkage between the different repeating monosaccharide units. The selective pressure that has driven this diversity is unclear as are the mechanisms by which it has been achieved. However, a consequence of this structural diversity is that there exists a library of diverse polysaccharide structures within the microbiome that may be exploited to engineer novel polysaccharide molecules with particular biochemical, pharmacological, or immunological properties.

II. FUNCTIONS OF BACTERIAL CAPSULES

A number of functions have been assigned to bacterial capsules in different bacteria including adhesion, transmission, resistance to innate host defenses, resistance to the host's adaptive immune response, and intracellular survival (Roberts, 1996). In certain cases it is possible to directly correlate the function of the bacterial capsule with the chemical structure of the capsular polysaccharide. For instance, the adhesion of Group A Streptococci to pharyngeal cells mediated via the interaction between the hyaluoronic acid capsule and CD44, the hyaluronic acid binding protein (Cywes and Wessels, 2001). In the case of invasive pathogens an ability to survive innate host defenses is essential. It has been known for a long time that the expression of a polysaccharide capsule confers some measure of resistance to complement-mediated killing (Roberts, 1996) even though mechanistically the basis for this is not always clear. In the case of capsules that contain sialic acid, binding of factor H and the inhibition of the complement activation cascade can explain this resistance, but with

other capsules it may be due to steric effects and masking the cell surface form the membrane-attack complex. What is clear is that complement mediated resistance is likely to involve a number of cell surface structures which contribute to the overall effect (Burns and Hull, 1998, 1999). The ability of capsules to confer resistance to phagocytosis by polymorphonuclear (PMNL) cells has long been assigned to the negatively charged polysaccharide capsule and the repulsive effect on the negatively charged cell surface of the PMNL (Roberts, 1996). However, it is likely that poor opsonization with complement of encapsulated bacteria will also play a role in this protection (Roberts, 1996). The *Escherichia coli* K1 capsule is vital for intracellular survival and crossing the blood brain barrier (Kim *et al.*, 2003). Specifically, the K1 capsule moderates the maturation process of *E. coli* containing vacuoles inside endothelial cells preventing fusion with lysosomes (Kim *et al.*, 2003). As such, expression of the K1 capsule is critical to the pathology of the disease.

III. CAPSULAR POLYSACCHARIDES IN *E. coli*

E. coli is a facultatively anaerobic, Gram-negative bacillus that forms part of the commensal human bowel flora, but in the environment can be found in soil and water, usually as the result of fecal contamination. Although considered a commensal organism and widely used as a workhorse in molecular biology research, *E. coli* is capable of causing a range of diseases in humans and animals, including gastro-intestinal and urinary tract infections, meningitis, and septicemia. A common feature of *E. coli* isolates responsible for extraintestinal infections is the expression of a polysaccharide capsule or K antigen. The expression of certain K antigens is strongly associated with particular infections. For example, the K1 capsule is the most common capsule type found in isolates of *E. coli* causing neonatal meningitis and urinary tract infection. The K5 polysaccharide is associated both with urinary tract infection and sepsis, but not with meningitis. In both cases, these capsules are more often found associated with infection than in the normal intestinal flora of healthy individuals (Kaijser and Jodal, 1984).

There are more than 80 different K antigens in *E. coli*, and originally, they were divided into Groups I and II on the basis of serological, biosynthetic and genetic data (Jann and Jann, 1997). The system has since been restructured to take account solely of biochemical and genetic data, comprising four groups: Group 1 (Ia), Group 2 (II), Group 3 (III), and Group 4 (Ib) (Whitfield and Roberts, 1999). The following sections briefly consider the genetics, biosynthesis and evolution of the capsular polysaccharides of the related Groups 1 and 4, then Group 3, followed by a detailed analysis of the Group 2 capsular polysaccharides.

IV. *E. coli* GROUP I CAPSULES

Group 1 capsules, encoded by the *cps* locus located near *his*, are typified by *E. coli* K30, which is a polymer of galactose, mannose, and glucuronic acid. They are similar to those expressed by *Klebsiella* strains, although in *E. coli* the capsular polysaccharide is expressed in two forms. The first comprises one to several repeat units of the K polysaccharide linked to lipid A-core, and is termed K_{LPS} (Dodgson *et al.*, 1996). This is not synthesized by *Klebsiella* spp. (Whitfield and Roberts, 1999). Lipid A and core are two conserved constituent domains of LPS, the third being the highly variable O antigen. All three components are synthesized separately and ligated together later. Lipid A is formed from UDP-GlcNAc and fatty acids that are transferred to a Kdo disaccharide. The core is an oligosaccharide linker that is formed on lipid A by the sequential transfer of glucose, galactose, and GlcNAc from their nucleotide precursors. The second higher molecular weight polysaccharide forms the capsule proper. In each case the repeating unit of the polysaccharide is identical.

The Group 1 capsule biosynthetic locus is a 16 kb region of DNA encoding 12 ORFs located in the same region of DNA as the typical O antigen biosynthetic locus in *E. coli* K-12 and strains bearing capsules from Group 2, 3, or 4 (Drummelsmith and Whitfield, 1999; Rahn *et al.*, 1999). The Group 1 gene cluster is distinguished by the presence of an essential polymerization and translocation region dedicated to capsule expression (*wzi–wzc)* that is conserved between different strains of *E. coli* expressing Group 1 capsules and *K. pneumoniae* (Whitfield, 2006). Strains bearing Group 1 capsules are unable to co-express colanic acid, the first evidence for which emerged when it was found that multicopy RcsB in *E. coli* K30, K1, K5, and K-12 resulted in a mucoid phenotype at 37 °C, but only in serotype K30 was mucoidy the result of serotype-specific capsular polysaccharide expression: in all of the other strains this was due to colanic acid expression (Drummelsmith and Whitfield, 1999; Keenleyside *et al.*, 1992). Unlike bacteria belonging to Groups 2, 3, and 4, the genes responsible for synthesis of this EPS have been lost from Group 1 strains, probably through extensive DNA re-arrangements involving replacement of the O-antigen synthesis region by a large segment of DNA laterally transferred from *K. pneumoniae* (Rahn *et al.*, 1999; Whitfield, 2006).

It is not known how Group 1 capsules are linked to the bacterial cell surface, but unlike K_{LPS}, it does not involve LPS (Whitfield, 2006). The repeat units of these capsules are formed on the cytoplasmic face of the bacterial inner membrane followed by export across the inner membrane and polymerization to form the capsular polysaccharide. The precursor monosaccharides are first synthesized by the appropriate enzymes (e.g., ManB and ManC are responsible for generating UDP-mannose).

The glycosyltransferase enzyme WbaP then transfers galactose from free UDP-galactose in the cytoplasm to undecaprenyl phosphate, a lipid carrier molecule (Drummelsmith and Whitfield, 1999; Roberts, 1996). A further glycosyl transferase, namely WbaZ, then completes the formation of the repeating unit backbone, -2)-α-Man-(1–3)-β-Gal-(1-. A side-branch also exists, formed from repeating glucuronic acid and galactose residues by the glycosyltransferase WcaN, which is linked to the main polysaccharide chain by WcaO (Drummelsmith and Whitfield, 1999). The repeat units are flipped across the bacterial inner membrane by an unknown process involving Wzx before being attached to the reducing terminus of the nascent undecaprenyl phosphate-linked polysaccharide at its reducing terminus by Wzy on the periplasmic face of the inner membrane. The Wzy protein is believed to function as a polymerase, although this role has not been directly demonstrated (Whitfield, 2006). Mutations in Wzy abolish capsule expression and reduce the length of K_{LPS} to one repeat unit (Drummelsmith and Whitfield, 1999). At some point the length of the nascent polymer must trigger export, and either Wzy or WaaL may play a role in determining the chain length (Whitfield, 2006). Strains carrying mutations in *wzy* are acapsular and add only one repeat unit onto K_{LPS} (Drummelsmith and Whitfield, 1999). Polymerization is terminated for K_{LPS} by WaaL-mediated transfer of the polymer to lipid A-core.

Translocation of the finished polymer involves the products of the genes *wza*, *wzb*, and *wzc*, encoded within a polymerization and translocation locus located upstream of the serotype-specific biosynthetic loci. Wzi (formerly *orf3* or *orfX*) is not essential for capsule expression (Drummelsmith and Whitfield, 1999), but *wzi* mutants show a significant reduction in cell associated and cell-free polymer (Rahn *et al.*, 2003). Wza is a surface-exposed outer membrane lipoprotein that forms octameric structures in the outer membrane, the bulk of which are exposed in the periplasm, and is essential for surface presentation of capsule (Collins *et al.*, 2007; Dong *et al.*, 2006; Drummelsmith and Whitfield, 2000; Nesper *et al.*, 2003). It represents the outer membrane accessory (OMA) protein of Group 1 capsules. OMAs carry a conserved signal peptidase motif that, after cleavage, is modified at a conserved cysteine residue to yield a lipoprotein (Paulsen *et al.*, 1997). Failure to acetylate Wza results in a failure of capsule export and intracellular accumulation of capsule polymer (Nesper *et al.*, 2003). Wza is found associated with Wzc, interacting via their periplasmic domains (Collins *et al.*, 2007). Wza-Wzc interaction is believed to hold Wza in an open conformation conducive to capsule export, as the Wza octomer encloses a large central cavity with a 22Å pore (Collins *et al.*, 2007). However, in the absence of Wzc, the Wza ring is closed at both the periplasmic and external faces (Beis *et al.*, 2004; Dong *et al.*, 2006). Wzc is a tyrosine autokinase protein similar to the chain-length regulating protein Wzz found in strains from other capsule groups.