Microbiology (1995), 141, 2023–2031 Printed in Great Britain

SGM SPECIAL LECTURE

Bacterial polysaccharides in sickness and in health

I. S. Roberts†

1995 Fleming Lecture

(Delivered at the 130th Meeting of the Society for General Microbiology, 5 January 1995)

School of Biological Sciences, Stopford Building, Oxford Road, University of Manchester, Manchester M13 9PT, UK

Keywords: polysaccharides, capsule, *Escherichia coli*

Overview

Polysaccharides are highly hydrated polymers composed of repeating single units (monosaccharides) joined by glycosidic linkages. They can be homo- or heteropolymers and may be substituted with both organic and inorganic molecules. Polysaccharides are an incredibly diverse range of molecules by virtue of not only the different possible monosaccharide units but also how these units are joined together. The presence of a number of hydroxyl groups that may be involved in the formation of a glycosidic bond means that any two monosaccharides may be joined in a number of ways. This is in contrast to any two amino acids which can be joined together to form only one of two possible dipeptides. Additional structural complexity may be achieved by the introduction of branches into the polysaccharide chain and the substitution with both organic and inorganic molecules. Therefore, polysaccharides represent a rich source of structurally diverse molecules, many of which may have unique chemical and physical properties which have been exploited by man in a number of industrial, biomedical and food processes.

Bacterial polysaccharides are usually associated with the outer surface of the bacterium. In crude terms, these molecules can be divided into two groups. Either they may form an amorphous layer of extracellular polysaccharide surrounding the cell which may be organized into a distinct structure termed a capsule, or alternatively, the polysaccharide molecule may be more intimately associated with the cell surface either through linkage to a lipid-A moiety, as in the case of the lipopolysaccharide (LPS) molecules in Gram-negative bacteria, or linked to cell-wall techoic acids as in Gram-positive bacteria. In this review I will be focusing on capsular and extracellular polysaccharides and how these polysaccharides are currently utilized in a number of everyday processes. In addition, I will describe the important roles played by capsules in invasive bacterial infections of man. Finally, as a paradigm, I will describe the genetics and biochemistry of capsular polysaccharide expression in *Escherichia coli* and outline the future possibilities for the engineering of polysaccharides of biomedical interest in *E. coli*.

The uses of bacterial polysaccharides

The food industry

In general terms, polysaccharides are either used as stabilizers to maintain emulsions of oil and water containing colloidal particles or as gelling agents to alter the texture of the food. As stabilizers, polysaccharides are widely used in dried products which are reconstituted by the addition of water. The presence of the polysaccharide prevents subsequent phase separation. Many of the polysaccharides used in these products are of algal or plant origin, but in products of low pH, such as salad dressings and yoghurts, microbial xanthan is used.

The use of polysaccharides to change the rheological properties of water within foods has been exploited to modify the texture of foods. Microbial polysaccharides such as xanthan and alginate are used as gel formers to change the texture of the food (Morris, 1990). The choice of polysaccharide or mixture of polysaccharides will depend on whether a thermoreversible or thermostable gel is required and the desired consistency of the final product. In the case of alginate-based gels, the consistency of the gel depends on the relative abundance of polyguluronic acid and polymannuronic acid stretches in the polysaccharide. When polymannuronic acids predominate, the gels are more elastic and less brittle, whilst a large proportion of polyguluronic acid leads to a rigid, brittle gel (Sutherland, 1991). Whilst much of the market is still currently taken up by plant- or animal-derived gelling agents, the susceptibility of the former to climatic influences and the unsuitability of the latter in non-meat products suggests a long-term future for microbially derived polysaccharides with the appropriate physicochemical properties.

Non-food industrial uses of bacterial polysaccharides

Bacterial polysaccharides have been exploited in a number of non-food industrial uses. The particular application of a specific polysaccharide is a reflection of its unique

†**Present address:** Until 1 October 1995: Department of Microbiology & Immunology, Medical Sciences Building, PO Box 138, University Road, Leicester, LE1 9HN, UK. Tel: +44 116 2522956. Fax: +44 116 2525030.

0002-0223 © 1995 SGM

physical properties. The widest non-food industrial application of bacterial derived polysaccharides is the use of xanthan in the oil industry (Moradi-Araghi *et al.*, 1988; Linton *et al.*, 1991). Xanthan has unique rheological properties demonstrating pseudo-plastic flow and visco-elasticity, retaining its physical properties over a broad temperature range (Morris *et al.*, 1983; Rinaudo & Milas, 1987). As such, xanthan has been exploited in drilling fluids to both lubricate the drill head and remove rock cuttings and in enhanced oil recovery. Other bacterial polysaccharides such as succinoglycan, which has a relatively low transition temperature have also been used in these processes (Clarke-Sturman *et al.*, 1989).

Polysaccharides are used as thickeners in the printing and textile industry. The rheological properties of the polysaccharide are important in restricting the flow of the dye and in maintaining the coating of dye prior to fixation. Whilst alginates are the principal polysaccharides used in these processes, both xanthan and succinoglycan are widely used particularly in the manufacture of paints and pigments (Sutherland, 1991).

The ability of polysaccharides to form hydrated gels has been exploited in the use of alginate-based gels for the immobilization of cells and enzymes in the biosynthesis of a number of industrial products (Skjaek-Braek & Martinsen, 1991). Immobilized cells have the advantage of allowing a continuous throughput of substrate at relatively high cell densities. The alginate gel has to be structurally robust whilst at the same time permitting access to the substrate molecules and exit to the product. Currently, algal alginates are primarily used to make gels (Martinsen *et al.*, 1989; Skjaek-Braek & Martinsen, 1991). The future use of bacterially derived alginates will depend on the relative cost of such products and the initial development costs.

Biomedical uses of bacterial polysaccharides

In economic terms, the most biomedically important polysaccharide is heparin with its widespread use as an effective anticoagulant (Kjellen & Lindahl, 1991). In addition, heparin and heparan sulphate may have important clinical applications in mediating inflammatory reactions (Lindahl *et al.*, 1994) and in reverting the transformed state of certain tumour cells (Mali *et al.*, 1994). Currently, heparin is principally derived from by-products of the meat processing industry although the similarity between heparin and certain microbial polysaccharides may permit the synthesis of heparin in bacteria (see below).

Hyaluronic acid is a polymer of alternating glucuronic acid and *N*-acetylglucosamine and is an important component of the extracellular matrix (Hardingham & Muir, 1972). In synovial fluid, hyaluronic acid is in solution with tissue-specific synovial glycoproteins which interact to provide the lubricating properties of the synovial fluid (Swann *et al.*, 1974). Hyaluronic acid is used in a number of surgical procedures including as a replacement for vitreous humour lost during corneal grafts (Balazs *et al.*, 1972; Miller *et al.*, 1977), in the treatment of osteoarthritis (Peyron & Balazs, 1974), and in drug delivery (Keller *et al.*, 1987; Ellwood, 1988). Hyaluronic acid is extracted from tissues, particularly rooster combs and umbilical cords (Jeanloz & Forchielli, 1950; Swann, 1968), for use in pharmaceutical products. The observation that group A and C streptococci produce hyaluronic acid capsules which are chemically identical to that found in mammals (Ruoff, 1991) has permitted the manufacture of hyaluronic acid by microbial fermentation (Chiba *et al.*, 1988). Considerable efforts have been expended on developing strains which result in high yields of hyaluronic acid (Hosoya *et al.*, 1989). The use of microbial fermentation offers considerable financial and ethical advantages over tissue extraction. The recent cloning and expression of the hyaluronic acid synthase gene from *Streptococcus pyogenes* (Dougherty & van de Rijn, 1994) may permit the expression of this polymer in other hosts (see below).

Cellulose, a homopolymer of (1–4)-β-linked glucose, is the most abundant biological polymer. Apart from being a major component of plant cell walls, cellulose is also synthesized by *Acetobacter* species. Bacterially derived cellulose is much purer than that extracted from plant tissue and as such it has been proposed for use in a number of medical applications including as an artificial skin, for topical drug delivery and in wound dressings (Byrom, 1991). However, due to problems with large-scale production the use of bacterially derived cellulose has remained rather limited (Byrom, 1991).

The most significant biomedical exploitation of bacterial polysaccharides has been in their use as vaccine agents. Capsular polysaccharides, with few exceptions, are immunogenic in non-infants, generally non-toxic and have none of the deleterious side-effects associated with whole-organism vaccines (Robbins, 1978; Jennings, 1990). In addition, the use of purified polysaccharides as vaccines allows the agent in question to be precisely physically and chemically defined. Vaccines based on purified capsular polysaccharides have been generated and used effectively in the prevention of a number of bacterial infections of man. In the case of *Streptococcus pneumoniae*, the vaccine consists of 23 polysaccharides based on epidemiological data to provide the maximum effective coverage (Robbins *et al.*, 1983). The situation is less complex in *Neisseria meningitidis* where serogroups A, B, C, W135 and Y are responsible for 90% of all infections (Jennings, 1990). A tetravalent vaccine consisting of purified polysaccharides from serogroups A, C, W135 and Y is currently used as the meningococcal vaccine (Cadoz *et al.*, 1985). However, despite the spectacular success of vaccines based on capsular polysaccharides there are still problems with this approach. The first is the poor immunogenicity of all polysaccharides in infants. The second is the poor immunogenicity of a few particular capsular polysaccharides in humans. Perhaps the best example of this is the group B polysaccharide of *N. meningitidis* which is a homopolymer of sialic acid (Bhattacharjee *et al.*, 1975). The presence of sialic acid residues in oligosaccharides on the surface of human cells means that the group B polysaccharide is poorly immunogenic (Wyle

et al., 1972). To overcome this problem, chemical modification of the group B polysaccharide has been undertaken to increase its immunogenicity (Jennings, 1990). Preliminary studies suggest that by chemical modification of the group B polysaccharide it is possible to generate antibodies specific to group B meningococci and as such this may offer a route for successful vaccine development (Jennings *et al.*, 1993).

To overcome the general poor immunogenicity of polysaccharides in infants, conjugate vaccines consisting of polysaccharides linked to protein carriers have been developed (Chu *et al.*, 1983; Beuvery *et al.*, 1983; Kuo *et al.*, 1995; Lett *et al.*, 1995). The conjugation of polysaccharides to proteins allows an immune response to be evoked in infants and importantly such polysaccharide–protein conjugates act as T-dependent antigens thereby allowing boosting to occur upon re-exposure (Robbins & Schneerson, 1990). A recent example of the success of such a strategy is the vaccine against *Haemophilus influenzae* type b, the major cause of meningitis in children under five (Broome, 1987). There are currently four vaccines available all of which consist of type b polysaccharide conjugated to a particular protein (Moxon & Rappouli, 1990). The results of field trials of these vaccines are promising with all of the vaccines capable of eliciting serum antibody to type b capsule and preventing more than 90% of all *H. influenzae* type b disease (Eskola *et al.*, 1990; Santosham *et al.*, 1991). These vaccines are now used routinely in many countries (Booy & Moxon, 1991).

Functions of bacterial capsules

A number of functions have been suggested for the roles of polysaccharide capsules (Table 1). These include protection against desiccation (Ophir & Gutnick, 1994) and mediating adhesion in biofilm formation (Costerton *et al.*, 1981). In invasive bacterial infections, interactions between the capsular polysaccharide and the host's immune system will be vital in deciding the outcome of the infection (Roberts *et al.*, 1989). The presence of a capsule is thought to confer resistance to non-specific host defence mechanisms in the absence of specific antibody. In particular, complement-mediated killing by the alternative pathway and C3b-mediated opsonophagocytosis by polymorphonuclear leukocytes (Cross, 1990). The capsule may act in concert with other cell-surface structures, such as O antigens, to confer resistance to complement-mediated killing (Kim *et al.*, 1986). Often, it is a particular combination of cell-surface structures that are responsible for conferring a high degree of resistance to complement-mediated killing (Kim *et al.*, 1986; Cross, 1990). By providing a permeability barrier to complement components, the capsule may mask underlying cell-surface structures which would otherwise be potent activators of the alternative pathway (Howard & Glynn, 1971).

Capsular polysaccharides may confer resistance to complement-mediated opsonophagocytosis both by steric effects in which the capsule masks the underlying C3b deposited on cell-surface structures from C3b receptors on the phagocyte cell surface and due to the net negative charge conveyed on the cell surface by the polysaccharide capsule (Brown *et al.*, 1983; Horwitz & Silverstein, 1980; Moxon & Kroll, 1990). In addition, certain capsular polysaccharides are themselves poor activators of the alternative pathway (Stevens *et al.*, 1978; Edwards *et al.*, 1982). Bacteria with capsular polysaccharides containing sialic acid bind factor H on their cell surface which interacts with C3b to form H-C3b thereby breaking the amplification loop of the alternative pathway (Moxon & Kroll, 1990). Beyond these direct interactions between the bacterial capsule and components of the host's non-specific immune response it has been suggested that certain capsular polysaccharides may modulate the ability of the host to mediate an immune response by effecting the release of cytokine molecules thereby disrupting the coordination of the host's cell-mediated immune response (Cross *et al.*, 1989).

In contrast to the majority of capsular polysaccharides which are capable of eliciting an immune response, a small set of capsular polysaccharides are poorly immunogenic. These include polysaccharides containing sialic acid such as *E. coli* K1 or *N. meningitidis* serogroup B (Bhattacharjee *et al.*, 1975) and the *E. coli* K5 antigen which is similar to desulpho-heparin (Vann *et al.*, 1981). As a consequence of structural similarities between these capsular polysaccharides and polysaccharides encountered on host tissue (Finne, 1982; Lindahl *et al.*, 1994), these capsules are poorly immunogenic with infected individuals mounting a poor antibody response to the capsule (Wyle *et al.*, 1972; Roberts *et al.*, 1989). Therefore, the expression of these capsules confers some measure of resistance to the host's specific immune response.

Table 1. Functions of polysaccharide capsules

Function	Relevance
Prevention of desiccation	Transmission and survival
Adherence	Colonization of oral surfaces
	Colonization of indwelling catheters
Resistance to non-specific host immunity	Complement-mediated phagocytosis
	Complement-mediated killing
Resistance to specific host immunity	Poor antibody response to the capsule

The genetics and biochemistry of capsule production in *E. coli*

Capsule gene clusters have been cloned from a number of both Gram-negative and positive bacteria (Roberts *et al.*, 1986, 1988; Frosch *et al.*, 1989; Kroll *et al.*, 1989; Lee, 1992; De Angelis *et al.*, 1993; Garcia *et al.*, 1993; Arrecubieta *et al.*, 1994; Dillard & Yother, 1994). In this review, due to the constraints of space, I only intend to describe the group II capsule gene clusters of *E. coli* which in many ways can be regarded as a paradigm for other capsule gene clusters.

In excess of 70 capsular polysaccharides have been described in *E. coli* (Ørskov *et al.*, 1977). On the basis of a number of biochemical and genetic criteria, *E. coli* capsules were originally divided into two groups, I and II (Jann & Jann, 1987). Recently, this classification has been re-defined such that there are now at least three groups of capsule gene clusters (Pearce & Roberts, 1995). By far the best studied are the group II capsules. The gene clusters for a number of group II capsules have been cloned and subjected to a detailed molecular genetic analysis (Roberts *et al.*, 1986, 1988; Boulnois *et al.*, 1987; Vimr *et al.*, 1989). These studies have revealed that there is a common genetic organization consisting of three functional regions (Fig. 1) (Boulnois & Roberts, 1990). Two of these regions, 1 and 3, are common to all of the group II capsule gene clusters so far analysed and flank a central serotype-specific region 2 (Fig. 1). Regions 1 and 3 encode functions that are needed for the cell-surface expression of group II capsules albeit that these polysaccharide molecules are chemically distinct.

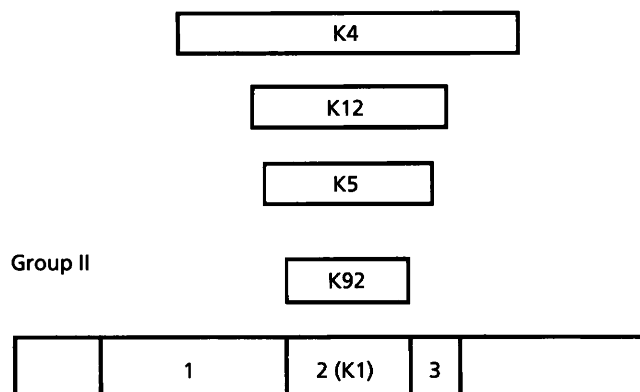

Fig. 1. **Schematic representation of the organization of group II capsule gene clusters. The K1 capsule gene cluster is shown with the three functional regions. Boxes labelled K92, K5, K12 and K4 represent the serotype-specific region 2s that are inserted between conserved regions 1 and 3.**

Region 3 contains two genes, *kpsM* and *kpsT*, organized in a single transcriptional unit (Fig. 2) (Smith *et al.*, 1990). Analysis of the predicted amino acid sequences of KpsM and KpsT indicated that they are members of the family of ATP-binding cassette (ABC)-type transporters and may comprise an inner-membrane polysaccharide-export system (Smith *et al.*, 1990; Pavelka *et al.*, 1991, 1994). Proteins homologous to both KpsM and KpsT have been identified in both *H. influenzae* and *N. meningitidis* and have been postulated to play an analogous role in the expression of capsules in these two species (Kroll *et al.*, 1990; Frosch *et al.*, 1991). This indicates commonality in the mechanism of capsule expression in these Gram-negative bacteria, and it has been suggested that these polysaccharide export systems may comprise a new subfamily of ABC transporters termed the ABC-2 subfamily (Reizer *et al.*, 1992).

Analysis of region 1 showed the presence of six genes, *kpsFEDUCS*, organized in a single transcriptional unit (Fig. 2) (Cieslewicz & Vimr, 1993; Pazzani *et al.*, 1993a, b). The precise functions of all of the proteins encoded within region 1 is not yet fully elucidated although

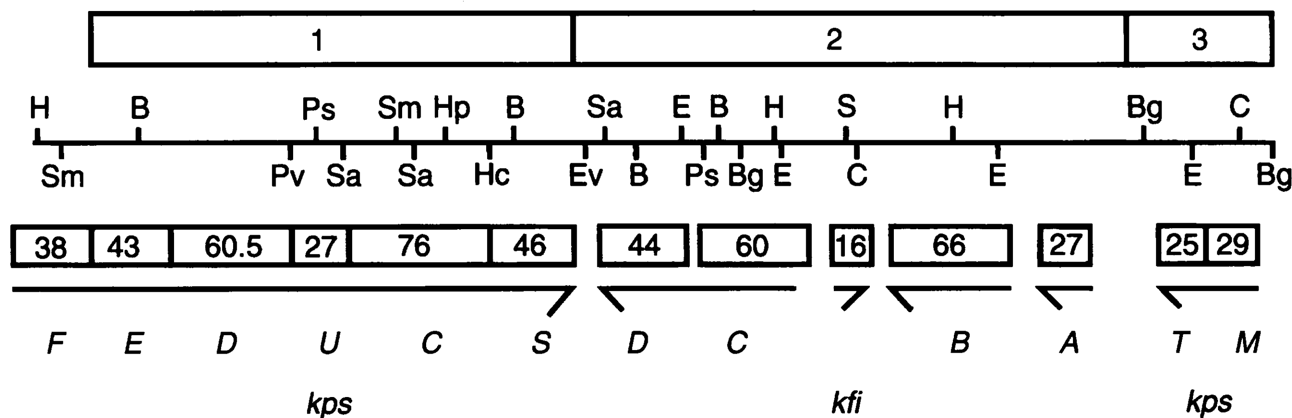

Fig. 2. Genetic organization of the K5 capsule gene cluster. Boxes 1–3 denote the three functional regions found in group II capsule gene clusters. The numbered boxes represent the genes and the size of the predicted proteins in kDa. The genes in regions 1 and 3 are referred to as *kps* whilst the K5-specific genes in region 2 are referred to as *kfi*. The arrows denote the likely major transcripts. Restriction sites: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; Ev, *Eco*RV; H, *Hin*dIII; Hc, *Hin*cII; Hp, *Hpa*I; Ps, *Pst*I; Pv, *Pvu*II; S, *Sst*I; Sa, *Sal*I; Sm, *Sma*I.

Table 2. Homology between proteins encoded by region 1 of the K5 capsule gene cluster and their cellular location

Protein	Cellular location	Homology*
KpsF	Cytoplasm	GutQ (72% over 314 aa)
KpsE	Inner membrane	BexC (73% over 359 aa)
		CtrB (73% over 355 aa)
KpsD	Periplasm	ExoF (67% over 100 aa)
KpsU	Cytoplasm	KdsB (70% over 246 aa)
		NeuA (66% over 246 aa)
KpsC	Cytoplasm	LpsZ (76% over 312 aa)
		LipA (70% over 550 aa)
KpsS	Cytoplasm	LipB (68% over 396 aa)

*GutQ, cytoplasmic protein of unknown function (Yamada *et al.*, 1990); BexC, inner-membrane protein encoded by the *H. influenzae* capsule gene cluster (Kroll *et al.*, 1990); CtrB, inner-membrane protein encoded by the *N. meningitidis* capsule gene cluster (Frosch *et al.*, 1991); ExoF, periplasmic protein involved in succinoglycan expression in *Rhizobium meliloti* (Muller *et al.*, 1993); KdsB-CMP-KDO synthetase involved in LPS expression in *E. coli* (Goldman & Kohlbrenner, 1985); NeuA, CMP-NeuNAc synthetase involved in K1 expression in *E. coli* (Zapata *et al.*, 1989); LpsZ, unknown role in LPS biosynthesis in *R. meliloti* (Brzoska & Signer, 1991); LipA/LipB, proteins encoded by the *N. meningitidis* capsule gene cluster (Frosch & Muller, 1993).

computer-aided database searches have revealed homologies to other proteins involved in the expression of polysaccharide capsules in other bacteria (Table 2). This indicates that there must be functional conservation in the export of capsular polysaccharides in these Gram-negative bacteria. The periplasmic location of polysaccharide in *kpsE* and *D* mutants suggests a role for these two proteins in the export of polysaccharide onto the cell surface (Bronner *et al.*, 1993a). The periplasmic location of the KpsD protein (Silver *et al.*, 1987; Pazzani *et al.*, 1993b) and the KpsE protein which is anchored to the inner membrane with a large periplasmic domain (Rosenow *et al.*, 1995; F. Esumeh & I. S. Roberts, unpublished results) are in keeping with this notion. The KpsC and S proteins are located in the cytoplasm associated with the inner face of the cytoplasmic membrane (G. Rigg & I. S. Roberts, unpublished results). Mutations in either the *kpsC* or *S* genes results in aggregates of cytoplasmic polysaccharide which lack any phosphatidic acid or 2-keto-3-deoxy-octonate (KDO) at its reducing end (Bronner *et al.*, 1993b). Therefore, it is tempting to speculate that the KpsC and S proteins may be involved in the attachment of KDO to phosphatidic acid and the subsequent ligation of the phosphatidyl-KDO to the reducing terminus of the polysaccharide prior to export across the cytoplasmic membrane by KpsM and T (Bronner *et al.*, 1993b). If this is the case, this would infer that the presence of phosphatidyl-KDO at the reducing terminus of group II *E. coli* polysaccharides is the motif recognized by the proteins involved in polysaccharide export. This is an appealing notion since it might explain how a conserved set of proteins could export chemically different polysaccharide molecules independent of the repeat structure of the polysaccharide. The *kpsU* gene within region 1 encodes a functional CMP-KDO transferase enzyme

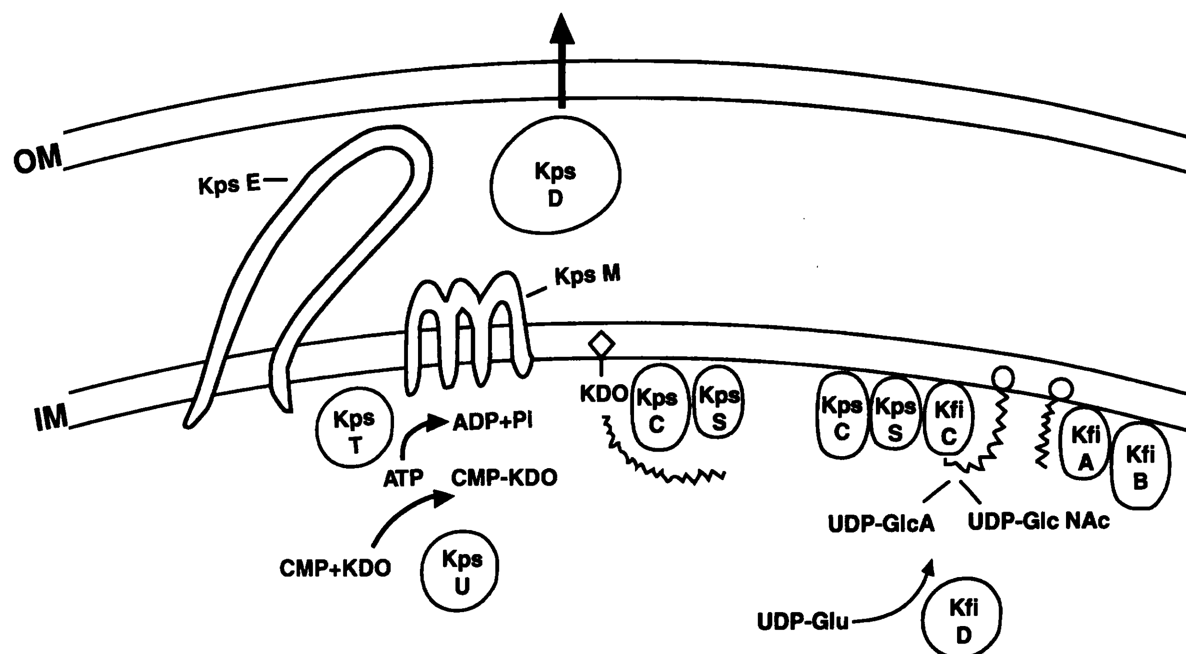

Fig. 3. A model for the biosynthesis and export of the K5 capsular polysaccharide. The proteins are denoted in accordance with the labelling in Fig. 2. IM and OM refer to the inner- and outer membranes, respectively. The polysaccharide is shown as a jagged line and the phosphatidic acid at the reducing terminus as a diamond in the inner membrane. The initial acceptor on which polysaccharide biosynthesis occurs is depicted by a circle in the inner membrane.

(Pazzani *et al.*, 1993a, b). This enzyme acts as a capsule-specific CMP-KDO synthetase enzyme to provide the CMP-KDO for the attachment of KDO to phosphatidic acid. The presence of this gene within region 1 of group II capsule gene clusters explains why strains expressing group II capsules have elevated levels of CMP-KDO synthetase activity (Finke *et al.*, 1990).

In contrast to the conservation of the regions 1 and 3 between different group II capsule gene clusters, the capsule-specific region 2s so far analysed appear unique for a particular K antigen (Boulnois & Roberts, 1990). To date the region 2 of the K5 capsule gene cluster has been subjected to the most detailed molecular genetic analysis (Petit *et al.*, 1995). It contains four genes encoding proteins for the biosynthesis of the K5 polysaccharide (Fig. 2). Computer-aided searches of the predicted amino acid sequence of these proteins has shed some light on their likely role in the biosynthesis of the K5 polysaccharide. By over-expression of the individual genes followed by enzyme assays it has been possible to confirm the function for two of the four proteins. The KfiC protein would appear to be the K5 transferase enzyme which adds the alternating sugar residues to the growing polysaccharide chain, whilst the KfiD protein is a UDP-glucose dehydrogenase enzyme which catalyses the production of UDP-glucuronic acid a component of the K5 polysaccharide (Petit *et al.*, 1995). The functions of the KfiA and B proteins are so far unknown, but it may be that they participate in the initial stages of polysaccharide biosynthesis (K. Jann & I. S. Roberts, unpublished results). By bringing together all of these data it has been possible to propose a model to explain K5 polysaccharide biosynthesis and how group II polysaccharides may be exported onto the cell surface in *E. coli* (Fig. 3). Clearly, there are still a lot of unanswered questions. What is the acceptor on which the K5 polysaccharide is synthesized? How is the export of polysaccharide molecules across two membranes achieved? Are sites of membrane adhesion, so-called Bayer junctions, important in this process? How is this large polysaccharide-synthesizing multiprotein complex maintained on the membrane? This model represents a start point from which a clearer picture can be further defined with increasing experimental evidence.

Polysaccharide engineering in bacteria

The widespread application of polysaccharides in a number of biomedical and industrial processes raises the question of whether it is feasible to attempt to synthesize these molecules in an heterologous host such as *E. coli*. The possibility of being able to generate polysaccharide molecules with desired physical and chemical properties is appealing. This is particularly so with polysaccharides of non-microbial origin which may have to be purified from animal tissue by lengthy extraction procedures. One such an example is heparin. The structural similarity between the *E. coli* K5 polysaccharide, which has the same sugar backbone as heparin but is neither *N*-deacetylated nor *N*-sulphated (Vann *et al.*, 1981), offers a possible route for the biosynthesis of this medically important molecule in *E. coli*. The recent cloning and expression in *E. coli* of the gene from *S. pyogenes* encoding the hyaluronic acid synthase (Dougherty & van de Rijn, 1994) may permit the expression of large amounts of hyaluronic acid in *E. coli*. Whether this will offer any commercial advantages over the current production methods using group A and C streptococci is not yet known.

The use of *E. coli* polysialyltransferase enzyme from the K1 capsule gene cluster to synthesize polysialylated neoglycosphingolipids of pharmacological importance (Cho & Troy, 1994) is another example of how polysaccharide engineering may be achieved using an *E. coli* glycosyltransferase. The diversity of capsular polysaccharides in *E. coli* means that there is a potential store of glycosyltransferases waiting to be exploited. By judicious choice of the glycosyltransferase genes that are cloned and co-expressed in the same cell it may be possible to construct novel polysaccharides with desired physical and chemical properties. Clearly, if these interesting ideas are to be pursued then a greater understanding of how polysaccharides are synthesized and exported in *E. coli* will be essential.

Concluding remarks

Polysaccharides are an incredibly diverse range of biologically important molecules. They play vital roles in mediating a broad range of biological processes. Clearly, a greater understanding of how polysaccharides are synthesized and exported in bacteria will be important in allowing us to synthesize in bacteria polysaccharides of industrial and pharmaceutical importance. Of equal significance is that an understanding of polysaccharide capsule production in pathogenic micro-organisms may permit the rational design of specific inhibitors of this process which may be used as chemotherapeutic agents. Hopefully over the next few years we can make significant progress in both these general areas.

Acknowledgements

I would like to thank Professor G. J. Boulnois in whose laboratory much of this work was initiated for his encouragement and support and Professors K. and B. Jann whose tireless collaboration has been invaluable. The progress made would have been impossible without the contribution of many research workers in the laboratory over the years to whom I am very grateful. Finally I would like to thank my many colleagues, particularly Dr D. Jones and Professor P. W. Andrew, in the Department of Microbiology at Leicester, who provided a stimulating and exciting environment in which to carry out research. The work in the laboratory was supported by the MRC, BBSERC and the Wellcome Trust. I thank the Lister Institute of Preventive Medicine for its financial support.

References

Arrecubieta, C., Lopez, R. & Garcia, E. (1994). Molecular characterization of *cap3A* gene from the operon required for the synthesis of the capsule of *Streptococcus pneumoniae* type 3a: sequencing of mutations responsible for the unencapsulated phenotype and localization of the capsule cluster on the pneumococcal chromosome. *J Bacteriol* **176**, 6375–6383.

Balazs, E. A., Freeman, M. I. & Kloti, R. (1972). Hyaluronic acid and replacement of vitreous and aqueous humor. *Mod Probl Ophthalmol* **10**, 3–21.

Beuvery, E. C., Miedema, F., van Delft, R. & Haverkamp, J. (1983). Preparation and immunochemical characterization of meningococcal group C polysaccharide-tetanus toxoid conjugates as a new generation of vaccines. *Infect Immun* **40**, 39–45.

Bhattacharjee, A. K., Jennings, H. J., Kenny, C. P., Martin, A. & Smith, I. C. P. (1975). Structural determination of the sialic acid polysaccharide antigens of *Neisseria menigitidis* serogroup B and serogroup C with carbon 13 nuclear magnetic resonance. *J Biol Chem* **250**, 1926–1932.

Booy, R. & Moxon, E. R. (1991). Immunisation of infants against *Haemophilus influenzae* type b in the UK. *Arch Dis Child* **66**, 1251–1254.

Boulnois, G. J. & Roberts, I. S. (1990). Genetics of capsular polysaccharide production in bacteria. *Curr Top Microbiol Immunol* **150**, 1–18.

Boulnois, G. J., Roberts, I. S., Hodge, R., Hardy, K., Jann, K. & Timmis, K. N. (1987). Further analysis of the cloned K1 capsule biosynthesis genes of *Escherichia coli*: definition of three functional regions for capsule production. *Mol & Gen Genet* **200**, 242–246.

Bronner, D., Sieberth, V., Pazzani, C., Roberts, I. S., Boulnois, G. J., Jann, B. & Jann, K. (1993a). Expression of the capsular K5 polysaccharide of *Escherichia coli*: biochemical and electron microscopic analyses of mutants with defects in region 1 of the K5 gene cluster. *J Bacteriol* **175**, 5984–5992.

Bronner, D., Sieberth, V., Pazzani, C., Smith, A. N., Boulnois, G. J. Roberts, I. S., Jann, B. & Jann, K. (1993b). Synthesis of the K5 (group II) capsular polysaccharide in transport-deficient recombinant *Escherichia coli. FEMS Microbiol Lett* **113**, 279–284.

Broome, C. V. (1987). Epidemiology of *Haemophilus influenzae* type b infections in the United States. *Pediatr Infect Dis J* **6**, 779–782.

Brown, E. J., Joiner, K. A., Gaither, T. A., Hammer, C. H. & Frank, M. M. (1983). The interaction of C3b bound tp pneumococci with factor H (beta 1H globulin), factor I (C3b/C4b inactivator) and properdin factor B of the human complement system. *J Immunol* **131**, 409–415.

Brzoska, P. & Signer, E. R. (1991). *lpsZ*, a lipopolysaccharide gene involved in the symbiosis of *Rhizobium meliloti. J Bacteriol* **173**, 3235–3237.

Byrom, D. (1991). Microbial cellulose. In *Biomaterials: Novel Materials from Biological Sources*, pp. 263–284. Edited by D. Byrom. Basingstoke: Macmillan Publishers.

Cadoz, M., Armand, J., Arminjon, F., Gire, R. & Lafaix, C. (1985). Tetravalent (A, C, Y, W135) meningococcal vaccine in children: immunogenicity and safety. *Vaccine* **3**, 340–342.

Chiba, S., Kitagawa, H. & Myoshi, T. (1988). Fermentative manufacture of high molecular weight hyaluronic acid. Patent JP 63129991.

Cho, J.-W. & Troy, F. A. (1994). Polysialic acid engineering: synthesis of polysialylated neoglycosphingolipids by using polysialyltransferase from neuroinvasive *Escherichia coli* K1. *Proc Natl Acad Sci USA* **91**, 11427–11431.

Chu, C., Schneerson, R., Robbins, J. B. & Rastogi, S. C. (1983). Further studies on the immunogenicity of *Haemophilus influenzae* type b and pneumococcal 6A polysaccharide–protein conjugates. *Infect Immun* **40**, 245–256.

Cieslewicz, M., Steenbergen, S. M. & Vimr, E. R. (1993). Cloning, sequencing, expression and complementation analysis of the *Escherichia coli* K1 *kps* region 1 gene *kpsE* and identification of an upstream open reading frame encoding a protein with homology to GutQ. *J Bacteriol* **175**, 8018–8023.

Clarke-Sturman, A. J., den Ottelander, D. & Sturla, P. L. (1989). Succinoglycan. A new biopolymer for the oilfield. In *Oil-field Chemistry: Enhanced Recovery and Production Stimulation*, pp. 157–168. Edited by J. K. Borchardt & T. F. Yen. Washington, DC: American Chemical Society.

Costerton, J. W., Irvin, R. T. & Cheng, K.-J. (1981). The bacterial glycocalyx in nature and disease. *Annu Rev Microbiol* **35**, 299–324.

Cross, A. S. (1990). The biologic significance of bacterial encapsulation. *Curr Top Microbiol Immunol* **150**, 87–98.

Cross, A. S., Sadoff, J. C., Kelly, N., Bernton, E. & Gemski, P. (1989). Pre-treatment with recombinant murine necrosis factor and murine interleukin-1 protects mice from lethal bacterial infection. *J Exp Med* **169**, 2021–2027.

De Angelis, P. L., Papaconstantinou, J. & Wegel, P. H. (1993). Isolation of a *Streptococcus pyogenes* gene locus that directs Hyaluran biosynthesis in acapsular mutants and in heterologous bacteria. *J Biol Chem* **268**, 14568–14571.

Dillard, J. P. & Yother, J. (1994). Genetic and molecular characterisation of capsular polysaccharide biosynthesis in *Streptococcus pneumoniae* type 3. *Mol Microbiol* **12**, 959–972.

Dougherty, B. & van de Rijn, I. (1994). Molecular characterization of *hasA* from an operon required for hyaluronic acid synthesis in group A Streptococci. *J Biol Chem* **269**, 169–175.

Edwards, M. S., Kasper, D. L., Jennings, H. J., Baker, C. J. & Nicholson-Weller, A. (1982). Capsular sialic acid prevents activation of the alternative pathway by type III group B streptococci. *J Immunol* **128**, 1278–1283.

Ellwood, D. C. (1988). Hyaluronic acid derivatives as sustained-release pharmaceuticals. Patent EP 296740.

Eskola, J., Kayhty, H. & Takala, A. K. (1990). A randomised, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* type b disease. *N Engl J Med* **323**, 1381–1387.

Finke, A., Jann, B. & Jann, K. (1990). CMP-KDO synthetase activity in *Escherichia coli* expressing different capsular polysaccharides. *FEMS Microbiol Lett* **69**, 129–134.

Finne, J. (1982). Occurrence of unique polysialosyl carbohydrate units in glycoproteins of developing brain. *J Biol Chem* **257**, 11966–11970.

Frosch, M. & Muller, A. (1993). Phospholipid substitution of capsular polysaccharides and mechanisms of capsule formation in *Neisseria meningitidis. Mol Microbiol* **8**, 483–493.

Frosch, M., Weisgerber, C. & Meyer, T. F. (1989). Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B. *Proc Natl Acad Sci USA* **86**, 1669–1673.

Frosch, M., Edwards, U., Bousset, K., Krauße, B. & Weisgerber, C. (1991). Evidence for a common molecular origin of capsular gene loci in gram-negative bacteria expressing group II capsular polysaccharides. *Mol Microbiol* **5**, 1251–1263.

Garcia, E., Garcia, P. & Lopez, R. (1993). Cloning and sequencing of a gene involved in the synthesis of the capsular polysaccharide of *Streptococcus pneumoniae* type 3. *Mol & Gen Genet* **239**, 188–195.

Goldman, R. C. & Kohlbrenner, W. E. (1985). Molecular cloning of the structural gene coding for CTP:CMP-3-deoxy-D-*manno*-octulosonate cytidyltransferase from *Escherichia coli. J Bacteriol* **163**, 256–261.

Hardingham, T. & Muir, H. (1972). The specific interaction of

hyaluronic acid with cartilage proteoglycans. *Biochim Biophys Acta* **279**, 401–405.

Horwitz, M. A. & Silverstein S. C. (1980). Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis. *J Clin Invest* **65**, 82–94.

Hosoya, H., Kimura, M. & Endo, H. (1989). Hyaluronic acid and its manufacture with hyaluronidase-deficient *Streptococcus* mutant. Patent JP 01067196.

Howard, C. J. & Glynn, A. A. (1971). The virulence for mice of strains of *Escherichia coli* related to the effects of K antigens on their resistance to phagocytosis and killing by complement. *Immunology* **20**, 767–777.

Jann, K. & Jann, B. (1987). Polysaccharide antigens of *Escherichia coli. Rev Infect Dis* **9**, 517–526.

Jeanloz, R. W. & Forchielli, E. (1950). Studies on hyaluronic acid and related substances. I. Preparation of hyaluronic acid and derivatives from human umbilical cord. *J Biol Chem* **186**, 495–511.

Jennings, H. J. (1990). Capsular polysaccharides as vaccine candidates. *Curr Top Microbiol Immunol* **150**, 97–128.

Jennings, H. J., Brisson, J.-P., Kulakowska, M. & Michon, F. (1993). Polysialic acid vaccines against meningitis caused by *Neisseria meningitidis* and *Escherichia coli*. In *Polysialic Acid from Microbes to Man*, pp. 25–38. Edited by J. Roth, U. Rutishauser & F. A. Troy. Basel: Birkhauser-Verlag.

Keller, N., Olejnk, O. & Abelson, M. B. (1987). Sustained release compositions containing polysaccharides especially hyaluronic acid for injection into the eye. Patent EP 244178.

Kim, K. S., Kang, J. H. & Cross, A. S. (1986). The role of capsular antigens in serum resistance and *in vivo* virulence of *Escherichia coli*. *FEMS Microbiol Lett* **35**, 275–278.

Kjellen, L. & Lindahl, U. (1991). Proteoglycans: structure and interactions. *Annu Rev Biochem* **60**, 443–475.

Kroll, J. S., Zamze, S., Loynds, B. & Moxon, E. R. (1989). Common organization of chromosomal loci for the production of different capsular polysaccharides in *Haemophilus influenzae*. *J Bacteriol* **171**, 3343–3347.

Kroll, J. S., Loynds, B., Brophy, L. N. & Moxon, E. R. (1990). The *bex* locus in encapsulated *Haemophilus influenzae*: a chromosomal region involved in capsule polysaccharide export. *Mol Microbiol* **4**, 1853–1862.

Kuo, J., Douglas, M., Ree, H. K. & Lindberg, A. A. (1995). Characterization of a recombinant pneumolysin and its use as a protein carrier for pneumococcal type 18C conjugate vaccines. *Infect Immun* **63**, 2706–2713.

Lee, C. Y. (1992). Cloning of genes affecting capsule expression in *Staphylococcus aureus* strain M. *Mol Microbiol* **6**, 1515–1522.

Lett, E., Klopfenstein, C., Klein, J.-P., Scholler, M. & Wachsmann, D. (1995). Mucosal immunogenicity of polysaccharides conjugated to a peptide or multiple-antigen peptide containing T- and B-cell epitopes. *Infect Immun* **63**, 2645–2651.

Lindahl, U., Lidholt, K., Spillmann, D. & Kjellen, L. (1994). More to heparin than anticoagulation. *Thromb Res* **75**, 1–32.

Linton, J. D., Ash, S. G. & Huybrechts, L. (1991). Microbial polysaccharides. In *Biomaterials: Novel Materials from Biological Sources*, pp. 215–262. Edited by D. Byrom. Basingstoke: MacMillan Publishers.

Mali, M., Andtfolk, H., Miettinen, H. & Jalkanen, M. (1994). Suppression of tumor cell growth by syndecan-1 ectodomain. *J Biol Chem* **269**, 27795–27798.

Martinsen, A., Skjaek-Braek, G. & Smidsrod, O. (1989). Alginate as immobilisation material. I. Correlation between chemical and physical properties of alginate gel beads. *Biotechnol Bioeng* **33**, 79–89.

Miller, D., O'Connor, P. & Williams, J. (1977). Use of Na-hyaluronate during intraocular lens implantation in rabbits. *Ophthalmic Surg* **8**, 58–61.

Moradi-Araghi, A., Beardmore, D. H. & Stahl, G. A. (1988). The application of gels in enhanced oil recovery: theory, polymers and crosslinker systems. In *Water-Soluble Polymers for Petroleum Recovery*, pp. 299–312. Edited by G. A. Stahl & D. N. Schulz. New York: Plenum Press.

Morris, V. J. (1990). Science, structure and applications of microbial polysaccharides. In *Gums and Stabilisers for the Food Industry*, pp. 315–328. Edited by G. O. Phillips. Oxford: IRL.

Morris, V. J., Franklin, D. & I'Anson, K. (1983). Rheology and microstructure of dispersions and solutions of the microbial polysaccharide from *Xanthomonas campestris* (xanthan gum). *Carbohydr Res* **121**, 13–30.

Moxon, E. R. & Kroll, J. S. (1990). The role of bacterial polysaccharide capsules as virulence factors. *Curr Top Microbiol Immunol* **150**, 65–86.

Moxon, E. R. & Rappuoli, R. (1990). Modern vaccines: *Haemophilus influenzae* and whooping cough. *Lancet* **335**, 1324–1329.

Muller, P., Keller, M., Weng, M., Quandt, J., Arnold, W. & Puhler, A. (1993). Genetic analysis of the *Rhizobium meliloti exo* YFQ operon: ExoY is homologous to sugar transferases and ExoQ represents a transmembrane protein. *Mol Plant–Microbe Interact* **6**, 55–65.

Ophir, T. & Gutnick, D. L. (1994). A role for exopolysaccharides in the protection of micro-organisms from desiccation. *Appl Environ Microbiol* **60**, 740–745.

Ørskov, I., Ørskov, F., Jann, B. & Jann, K. (1977). Serology, chemistry and genetics of O and K antigens of *Escherichia coli*. *Bacteriol Rev* **41**, 667–710.

Pavelka, M. S., Wright, L. & Silver, R. P. (1991). Identification of two genes, *kpsM* and *kpsT*, in region 3 of the polysialic gene cluster of *Escherichia coli* K1. *J Bacteriol* **173**, 4603–4610.

Pavelka, M. S., Hayes, S. F. & Silver, R. P. (1994). Characterisation of KpsT, the ATP-binding component of the ABC-transporter involved in the export of capsular polysialic acid in *Escherichia coli* K1. *J Biol Chem* **269**, 20149–20158.

Pazzani, C., Rosenow, C., Boulnois, G. J., Bronner, D., Jann, K. & Roberts, I. S. (1993a). Molecular analysis of region 1 of the *Escherichia coli* K5 antigen gene cluster: a region encoding proteins involved in cell surface expression of capsular polysaccharide. *J Bacteriol* **175**, 5978–5983.

Pazzani, C., Smith, A., Jann, K., Boulnois, G. J. & Roberts, I. S. (1993b). The export of capsular polysaccharides in *Escherichia coli*. In *Polysialic Acid from Microbes to Man*, pp. 113–125. Edited by J. Roth, U. Rutishauser & F. A. Troy. Basel: Birkhauser-Verlag.

Pearce, R. & Roberts, I. S. (1995). Cloning and analysis of the gene clusters for production of the *Escherichia coli* K10 and K54 antigens: identification of a new group of *serA*-linked capsule gene clusters. *J Bacteriol* **177**, 3992–3997.

Petit, C., Rigg, G., Pazzani, C., Smith, A., Sieberth, V., Stevens, M., Boulnois, G. J., Jann, K. & Roberts, I. S. (1995). Analysis of region 2 of the *Escherichia coli* K5 capsule gene cluster: a region encoding proteins for the biosynthesis of the K5 polysaccharide. *Mol Microbiol* (in press).

Peyron, J. G. & Balazs, E. A. (1974). Preliminary clinical assessment of Na-hyaluronate injection into human arthritic joints. *Pathol Biol* **22**, 731–736.

Reizer, J., Reizer, A. & Saier, M. H. (1992). A new subfamily of bacterial ABC-type transport systems catalysing export of drugs and carbohydrates. *Protein Sci* **1**, 1232–1236.

Rinaudo, M. & Milas, M. (1987). On the properties of polysaccharides. Relation between chemical structure and physical properties. In *Industrial Polysaccharides. Progress in Biotechnology*, pp. 217–223. Edited by M. Yalpani. Amsterdam: Elsevier.

Robbins, J. B. (1978). Vaccines for the prevention of encapsulated bacterial diseases: current status, problems and prospects for the future. *Immunochemistry* **15**, 839–854.

Robbins, J. B. & Schneerson, R. (1990). Polysaccharide-protein conjugates: a new generation of vaccines. *J Infect Dis* **161**, 821–832.

Robbins, J. R., Austrian, R., Lee, C.-J., Rastogi, S. C., Schiffman, G., Henrichsen, J., Makela, P. H., Broome, C. V., Facklam, R. R., Tiesjema, R. H. & Parke, J. C. (1983). Considerations for formulating the second-generation pneumococcal polysaccharide vaccine with emphasis on cross-reactive types within groups. *J Infect Dis* **148**, 1136–1159.

Roberts, I. S., Mountford, R., High, N., Bitter-Suermann, D., Jann, K., Timmis, K. N. & Boulnois, G. J. (1986). Molecular cloning and analysis of genes for the production of the K5, K7, K12 and K92 capsular polysaccharides in *Escherichia coli*. *J Bacteriol* **168**, 1228–1233.

Roberts, I. S., Mountford, R., Hodge, R., Jann, K. & Boulnois, G. J. (1988). Common organization of gene clusters for production of different capsular polysaccharides (K antigens) in *Escherichia coli*. *J Bacteriol* **170**, 1305–1310.

Roberts, I. S., Saunders, F. K. & Boulnois, G. J. (1989). Bacterial capsules and interactions with complement and phagocytes. *Biochem Soc Trans* **17**, 462–464.

Rosenow, C., Esumeh, F., Roberts, I. S. & Jann, K. (1995). Characterization and localization of the KpsE protein of *Escherichia coli* K5, which is involved in polysaccharide export. *J Bacteriol* **177**, 1137–1143.

Ruoff, K. L. (1991). The genus *Streptococcus*– Medical. In *The Prokaryotes*, pp. 1450–1464. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer-Verlag.

Santosham, M., Wolff, M., Reid, R. & Hohenboken, M. (1991). The efficacy in Navajo infants of a conjugate vaccine consisting of *Haemophilus influenzae* type b polysaccharide and *Neisseria meningitidis* outer-membrane protein complex. *New Engl J Med* **324**, 1767–1772.

Silver, R. P., Vann, W. F. & Aaronson, W. (1987). Translocation of capsular polysaccharide in pathogenic strains of *Escherichia coli* requires a 60-kilodalton periplasmic protein. *J Bacteriol* **169**, 5489–5495.

Skjaek-Braek, G. & Martinsen, A. (1991). Applications of some algal polysaccharides in biotechnology. In *Seaweed Resources in Europe: Uses and Potential*, pp. 219–257. Edited by M. D. Guiry & G. Blunden. London: Wiley.

Smith, A. N., Boulnois, G. J. & Roberts, I. S. (1990). Molecular analysis of the *Escherichia coli* K5 *kps* locus: identification and characterization of an inner-membrane capsular polysaccharide transport system. *Mol Microbiol* **4**, 1863–1869.

Stevens, P., Huang, S. N. H., Welch, W. D. & Young, L. S. (1978). Restricted complement activation by *Escherichia coli* with the K1 capsular serotype: a possible role in pathogenicity. *J Immunol* **121**, 2171–2180.

Sutherland, I. W. (1991). *Biotechnology of Microbial Exopolysaccharides*. Cambridge: Cambridge University Press.

Swann, D. A. (1968). Studies on hyaluronic acid II. The protein components of rooster comb hyaluronic acid. *Biochim Biophys Acta* **160**, 96–105.

Swann, D. A., Radin, E. L., Nazimiec, M., Weisser, P. A., Curran, N. & Lewinnek, G. (1974). Role of hyaluronic acid in joint lubrication. *Ann Rheum Dis* **33**, 318–326.

Vann, W. F., Schmidt, M. A., Jann, B. & Jann, K. (1981). The structure of the capsular polysaccharide (K5 antigen) of urinary tract infective *Escherichia coli* O10:K5:H4. A polymer similar to desulfo-heparin. *Eur J Biochem* **116**, 359–364.

Vimr, E. R., Aaronson, W. & Silver, R. P. (1989). Genetic analysis of chromosomal mutations in the polysialic gene cluster of *Escherichia coli* K1. *J Bacteriol* **171**, 1106–1117.

Wyle, F. A., Artenstein, M. S., Brandt, B. L., Tramont, E. C., Kapser, D. L., Altieri, P. L., Berman, S. L. & Lowenthal, J. P. (1972). Immunologic response of man to group B meningococcal polysaccharide vaccines. *J Infect Dis* **126**, 514–521.

Yamada, M., Yamada, Y. & Saier, M. H. (1990). Nucleotide sequence and expression of the *gutQ* gene within the glucitol operon of *Escherichia coli*. *J DNA Seq Map* **1**, 141–145.

Zapata, G., Vann, W. F., Aaronson, W., Lewis, M. S. & Moos, M. (1989). Sequence of the cloned *Escherichia coli* K1 CMP-*N*-acetyl neuraminic acid synthetase gene. *J Biol Chem* **264**, 14769–14774.