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Regulation of expression of group IA capsular polysaccharides in *Escherichia coli* and related extracellular polysaccharides in other bacteria

C Whitfield and WJ Keenleyside

Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Bacterial surface polysaccharides fulfill a number of important roles in cell–cell interactions, survival in natural environments, and formation of biofilms. Consequently, the mechanisms involved in regulation of extracellular polysaccharides are predicted to have a significant impact on microbial adaptation. Strains of *Escherichia coli, Klebsiella* spp, and *Erwinia* spp produce extracellular polysaccharides which share structural features. There are also similarities in the organization of genes required for synthesis of these cell surface polymers and, in some cases, the mechanism of synthesis may be related. Despite the diverse habitats of these bacteria, the systems which regulate expression of their extracellular polysaccharides appear to share components and mechanisms. Understanding these regulatory processes may lead to novel therapeutic approaches for pathogens, or for control of unwanted biofilm formation in industrial settings.

Keywords: extracellular polysaccharides; capsules; regulation; synthesis; *rcs* genes

Introduction

Many bacteria are able to produce extracellular polysaccharides. The degree of association between these polymers and the cell surface is quite variable. Some are firmly (perhaps covalently) linked in the form of capsules (capsular polysaccharides; CPSs). Others are loosely associated with the cell surface or released from the surface completely, due to a lack of cell surface linkage. For this review these polymers will be referred to as slime extracellular polysaccharides (EPSs). The distinction between CPSs and EPSs is usually operationally defined, based on retention of the polymer during sedimentation of the cells by centrifugation. This distinction may have limited physiological relevance in many cases because, during growth in culture, cells with large amounts of CPS will slough a considerable amount of cell-free polymer, indistinguishable from EPS. Although some bacteria produce both EPS and CPS, for most there is a preference for one type. For a given organism, we will refer to CPS or EPS, depending on the most prevalent form.

CPS/EPS have a variety of potential biological roles. One is mediation of adherence to surfaces. It is now appreciated that in most natural ecosystems, the numbers of adherent bacteria exceed the free planktonic bacteria in the bulk fluid phase. Adherent bacteria are enmeshed in a fibrous, highly hydrated matrix comprised of CPS and/or EPS, forming a biofilm [15]. Biofilms are complex, highly specialized, and highly permeable structures, comprising one or more bacterial species with cooperative and interacting physiologies. Given the central role played by EPSs and CPSs in biofilm formation, the regulatory systems involved in controlling their synthesis in response to environmental cues are of prime importance. The description of the components involved in synthesis, together with an understanding of the mechanisms involved in environmental regulation of EPS and CPS expression, provide important steps towards understanding the formation and control of biofilms. In this review we will provide an overview of the synthesis and regulation of EPS and CPS in *Escherichia coli, Klebsiella pneumoniae*, and *Erwinia* spp. While this group of bacteria have different lifestyles, some of the EPSs and CPSs they produce are structurally similar, and the formation of these polymers is regulated by a common system. The information obtained from studies of the regulation of surface polymers in these bacteria provides a basis to examine other systems in bacteria occupying diverse ecological niches.

Correspondence: C Whitfield, Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada
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The group IA-family of cell surface polysaccharides

Strains of *E. coli* produce more than 70 distinct extracellular polysaccharides. The polymers are structurally diverse and each forms a surface K-(capsular)-antigen (CPS), which can be used to discriminate among isolates by serotyping. *E. coli* K-antigens are separated into two major distinct groups [35,79]. Group II capsular polysaccharides have serotypes K1 and K5 as their prototypes. Group II K-antigens are found in some of the most prominent pathogenic *E. coli* clones and are characteristically produced only at growth temperatures above 20° C [57]. These polymers form a cell-associated capsular structure. The precise nature of the surface linkage for group II K-antigens is unknown but many of the polymers terminate in a lipid moiety (diacylglycerol) and it is possible that these lipids are involved in interactions with the cell surface [82]. However, the linkage between polymer and lipid is labile and, in

culture, many polymer chains lack a lipid terminus. Related group II CPSs with similar structures and similar terminal lipid moieties are found in *E. coli*, *Neisseria meningitidis* and *Haemophilus influenzae*. These bacteria have CPS genetic and biosynthesis systems with conserved features [82].

Group I and group II K-antigens are quite different in terms of structure and surface linkage, and the mechanisms involved in their biosynthesis and regulation of expression are distinct [79]. Expression of group I K-antigens is not temperature-sensitive [57] and this reflects differences in synthesis and regulation of CPSs of groups I and II. Group I K-antigens are subdivided into group IA and group IB. Group IB K-antigens contain amino sugars, components which are absent in group IA CPSs [37]. Group IA CPSs form the topic for this review. *E. coli* group IA K-antigens are structurally similar to K-antigens of *K. pneumoniae*. For example, the *E. coli* K30 (group I) and *K. pneumoniae* K20 prototype CPSs studied in this laboratory are identical (Figure 1). Under certain growth conditions (see below), many members of the family *Enterobacteriaceae* produce a slime EPS known as colanic acid or M-antigen [47]. Examples include some strains and serovars of *E. coli*, *Salmonella enterica* and *Aerobacter (Klebsiella)*. Colanic acid is not serotype-specific, is not produced under routine growth conditions, and is a slime EPS rather than CPS. Consequently, it has traditionally been considered to be distinct from K-antigens. However, the composition and structure of colanic acid resemble group IA CPSs from *E. coli* and *Klebsiella* (Figure 1). In addition, the genetic loci involved in synthesis of the group IA K30 CPS and colanic acid appear to be allelic, and expression of these polymers involves a common regulatory system (see below). Consequently, colanic acid can be considered as a prevalent group IA-like polymer. *E. coli* strains with group IB [37] and group II K-antigens [40,64] can produce colanic acid simultaneously with their K-antigen, because the genetic loci involved in group IA and group II CPSs are not allelic, and the regulatory systems may be distinct.

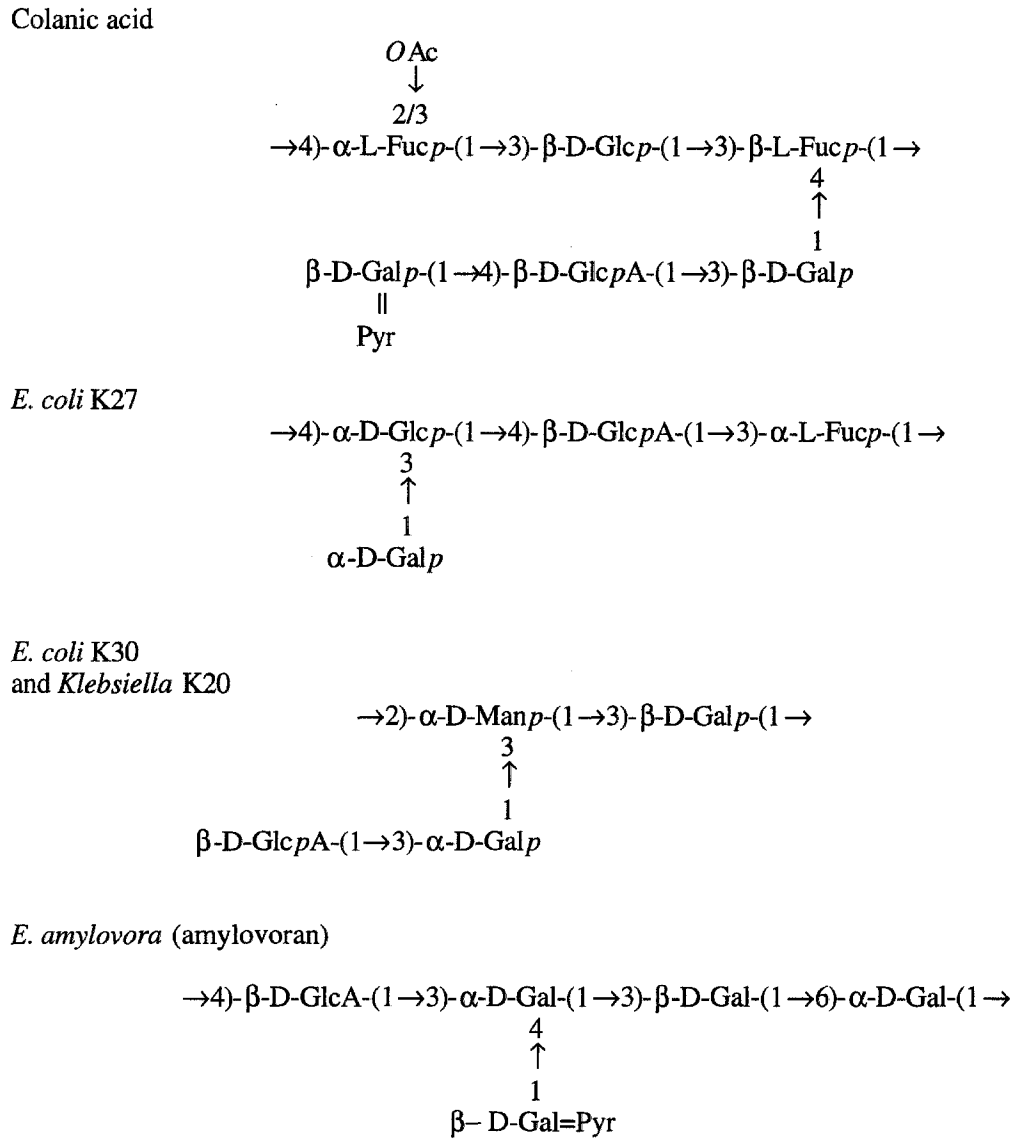

Figure 1 Repeating unit structures of extracellular polysaccharides belonging to the group IA family

The EPSs of the plant pathogenic bacteria, *E. amylovora* and *E. stewartii* resemble group IA CPS from *E. coli*. The structure of amylovoran from *E. amylovora* is shown in Figure 1. The precise structure of the *E. stewartii* EPS has not been reported but preliminary results support a branched heptasaccharide repeating unit containing glucose, galactose, and glucuronic acid in a ratio of 4 : 2 : 1, in a group IA-like structure [45].

The *E. coli* group I K-antigens were initially thought to be a form of lipopolysaccharide O-antigen, attached to the cell surface via a typical lipid A-core molecule. While this appears to be the case for group IB K-antigens [34], recent work in our laboratory has shown that only short group IA oligosaccharides are attached to the surface via lipid A-core. Long chain group IA K-antigens are exported and assembled on the cell surface by a lipid A-core-independent pathway, to form the morphological structure termed the capsule [46]. Group IA K-antigens are firmly cell-associated, but the nature of a covalent linkage molecule (if any exists) is unclear. The nature and extent of the interactions of extracellular polysaccharides with the cell surfaces of *Klebsiella* and *Erwinia* strains also remain to be established.

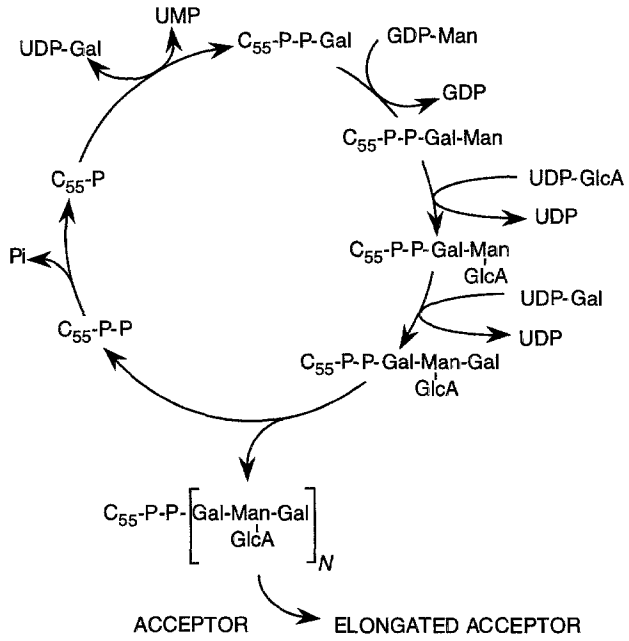

Figure 2 Biosynthesis of repeating units of group IA CPS in *Klebsiella* DD45 [75]

Biosynthesis of group IA extracellular polysaccharides

The pathway for biosynthesis of CPSs and EPSs involves a number of stages, beginning with formation of nucleotide diphosphate sugar precursors in the cytoplasm. This is followed by assembly and polymerization of repeating units in the cytoplasmic membrane, and export of the completed polymer from the periplasmic face of the cytoplasmic membrane, culminating in assembly on the cell surface [82]. Although early steps have been studied in a limited number of systems, the components and mechanisms involved in export are poorly understood. The reactions involved in biosynthesis of group IA and related polymers are best characterized for *Aerobacter (Klebsiella) aerogenes* strain DD45 [75]. Assembly of the DD45 repeating unit occurs by transfer of sugars from nucleotide diphospho precursors, to a lipid intermediate attached to undecaprenyl (Figure 2). The same lipid is used for assembly of peptidoglycan and lipopolysaccharide O-antigens, and the reaction sequence bears striking resemblance to the formation of the serogroup B and E O-antigens of *Salmonella enterica* (reviewed in [82]). The reaction sequence was established by *in vitro* synthesis with membranes as a source of enzyme. The requirement for undecaprenyl phosphate was clearly established by reconstitution of lipid-depleted membranes. Incomplete reaction sequences for the CPSs of *K. aerogenes* type 8 [70] and *E. coli* K30 (R Schulze and C Whitfield, unpublished results), and for colanic acid in *E. coli* K-12 [39], suggest that a similar reaction sequence exists for other group IA polymers. The isolation of lipid intermediates containing two repeating units of DD45 CPS [75] indicates polymerization occurs while attached to a lipid intermediate, rather than a mechanism where lipid-linked repeating units only donate the substrates for the polymerization reaction. Addition of modifications such as *O*-acetyl and ketal groups probably occurs at the level of the lipid intermediate, and in the case of the *K. pneumoniae* type 8 EPS these decorations are important for further polymerization [70].

The components and processes which occur between formation of lipid-linked intermediates and surface appearance of mature polymer are key unanswered questions. The topology of synthesis of group IA EPSs has not been established. If similarity to serogroup B and E O-antigen synthesis in *S. enterica* extends to transmembrane organization, lipid intermediates may be translocated across the cytoplasmic membrane and polymerized at the periplasmic face of the cytoplasmic membrane [82]. Assembly of the group I K29 CPS in *E. coli* occurs at discrete sites on the cell surface [5]. These sites overlay membrane adhesions where the cytoplasmic and outer membranes come into close apposition. The arguments for and against a physiological role in cell surface assembly for membrane adhesion sites are presented elsewhere [42,82].

Synthesis of group II CPSs in *E. coli*, *Haemophilus influenzae*, and *Neisseria meningitidis* follows a mechanism which is fundamentally different in terms of both components and membrane topology (reviewed in [82]). Group II CPSs appear to be synthesized and polymerized at the cytoplasmic face of the cytoplasmic membrane. They are then transported across the cytoplasmic membrane by a process which requires the action of an ATP-binding cassette transporter, known as an ABC transporter or traffic ATPase.

Synthesis of group IA-like polysaccharides is determined by polymorphic genetic loci

In *E. coli*, *his*-linked *cps* genes are required for synthesis of group IA CPSs [44,65,81]. These genes encode enzymes required for formation of sugar nucleotide precursors for monomers unique to the CPS, transferase enzymes for the

formation of repeating units, and polymerization functions. *cps* gene clusters are not sufficient for CPS synthesis since some sugar nucleotide precursors are formed by enzymes encoded by genes whose activity is not confined to CPS formation. Nevertheless, mutations in these genes do give CPS-deficient phenotypes. Examples include *galE* and *manA*, whose products are required for synthesis of UDP-Gal and GDP-Man, respectively [79]. Both of these genes are required for CPS synthesis in *E. coli* K30 (PR MacLachlan and C Whitfield, unpublished data). These genes are considered to encode 'housekeeping' functions because of additional roles in metabolism of galactose and mannose. In *E. coli* K30, later steps in GDP-Man formation are catalyzed by phosphomannomutase (RfbK) and GDP-mannose pyrophosphorylase (RfbM), respectively. These genes are found in the *rfb* gene cluster for biosynthesis of the lipopolysaccharide O9-antigen, which is located adjacent to *cps* ([81]; C Whitfield and C Dodgson, unpublished data). The requirement for these genes in synthesis of both O9 and K30 polysaccharides provides one possible explanation for the observation that group IA CPSs are predominantly found in strains with either the O8 or O9 polymannose O-antigens in *E. coli* [36]. In *E. coli* K27, an additional *trp*-linked genetic locus has been implicated in CPS expression [65]. This locus is not required for CPS expression in *E. coli* K30 [44,81].

Most genes for colanic acid biosynthesis in *E. coli* K-12 are also located near *his* and the cryptic *rfb* gene cluster, at approximately 44–45 min on the *E. coli* K-12 chromosome [3]. Genetic mapping studies defined a minimum of five genes, *cpsA–cpsE*, near *his* [74]. The *cps* genes in *E. coli* K-12 have not been characterized, although the *cpsB* and *cpsG* genes from the homologous and similarly located cluster for colanic acid synthesis in *Salmonella enterica* serovar Typhimurium have been cloned and sequenced [67]. *cpsB* and *cpsG* encode phosphomannomutase and GDP-mannose pyrophosphorylase enzymes, respectively. These are functional isozymes of RfbK and RfbM, encoded by the *rfb* lipopolysaccharide O-antigen biosynthesis gene cluster, and are thought to form part of the pathway leading to GDP-fucose synthesis via GDP-Man. *cpsB* and *cpsG* potentially allow co-expression of colanic acid with diverse O-antigens, many of which lack mannose or fucose in their repeating unit structure. This contrasts with the situation in *E. coli* O9:K30 described above. An additional locus, *cpsF*, maps at 90 min in *E. coli* K-12. *cpsF* mutants only show reduced mucoid phenotype so, unlike *cpsA–cpsE*, this gene is not essential for colanic acid synthesis [74]. The involvement of *cpsF* in colanic acid synthesis may be indirect, but it is regulated in a similar fashion to *his*-linked *cps* genes [74]. As is the case with *E. coli* K30, colanic acid synthesis is dependent on both *galE* and *manA* [47,74].

The structures of colanic acid and group IA CPSs are similar, and the *cps* genes required for their expression share a similar chromosomal map location. Transfer of the *E. coli* K30 *his-rfb-cps*$_{K30}$ region to *E. coli* K-12 results in replacement of the *cps*$_{K-12}$ genes and the transconjugant is able to synthesize K30 CPS, but not colanic acid [41,44]. Together these data indicate that K30 (and presumably other group IA CPSs) and colanic acid are members of a family of polysaccharides encoded by highly polymorphic allelic genetic loci.

The *cps* genes also map near *his* in *Klebsiella* [2,44,53]. Transfer of the *his-cps* region from *Klebsiella* K20 to *E. coli* K-12 was sufficient for K20 CPS synthesis [44], and a recombinant plasmid with a 23-kbp insert contains contiguous *cps* genes which direct formation of the *Klebsiella* K2 CPS in *E. coli* K-12 [2]. Using homologous recombination, Ofek *et al* [53] provided convincing evidence that *cps* genes are allelic in serotypes K2 and K21a. Although this is a small sample of the 82 different CPS types in *Klebsiella*, the available data are again consistent with the possibility that the diverse structures arise from polymorphism at a single genetic locus.

In *E. amylovora*, genes from the *ams* cluster encoded enzymes for amylovoran synthesis. The cluster minimally occupies 7 kbp. Five complementation groups, *amsA–amsE*, have been identified in three transcriptional units [6]. In *E. stewartii*, as with *E. coli* and *Klebsiella* spp, the *cps* locus maps near *his* [17]. Four *E. stewartii cps* regions, *cpsA–cpsD*, are contiguous and the cluster comprises at least 10 kbp [13]. In *Erwinia* spp, current estimates of the size of the clusters is based on available transposon insertions and additional DNA may be required. While it is not known whether the *ams* and *cps* loci of *Erwinia* spp are allelic, the genes are functionally equivalent and cross complementation of mutants in one species is possible with cloned genes from the other [6]. An interesting feature of the genetics of EPS formation of these bacteria, and a distinguishing feature from *E. coli* and *Klebsiella*, is the separation of *galE* from the other *gal* operon genes [6,17]. In *E. stewartii* [17], and probably in *E. amylovora* [6], the *galE* gene maps near *cps* but is not part of the *cps* cluster. It has been suggested that constitutive expression of a separated *galE* may reflect the requirement for large amounts of UDP-Gal for EPS biosynthesis [17]. However, some *Klebsiella* strains produce copious amounts of galactose-containing CPS and large amounts of galactose homopolymer lipopolysaccharide O-antigen [80], so the reasons for the separate *galE* in *Erwinia* may be more complicated.

The *rcs* regulatory system in *E. coli* K-12

In *E. coli* K-12 strains, the formation of colanic acid is not evident under routine growth conditions. Synthesis is activated by growth conditions including low temperature (eg 25° C), and growth in minimal media with high carbon : nitrogen ratios [47]. A variety of mutations also induce a mucoid phenotype, and these identify components which participate (directly or indirectly) in the regulation of colanic acid production. The proposed regulatory system is shown in Figure 3. There are three positive effectors for colanic acid synthesis, designated RcsA, RcsB, and RcsF (Rcs = Regulatory of Capsule Synthesis), and two negative regulatory elements (RcsC and Lon). The regulatory proteins control colanic acid synthesis at the level of transcription, by interaction with *cps* promoters [26]. Interpretation of genetic data has led to the proposal that the functional transcriptional activator is a heterodimer of RcsA-RcsB [69]. However it is still unknown whether colanic acid activation involves a single, or multiple, regulated promoters

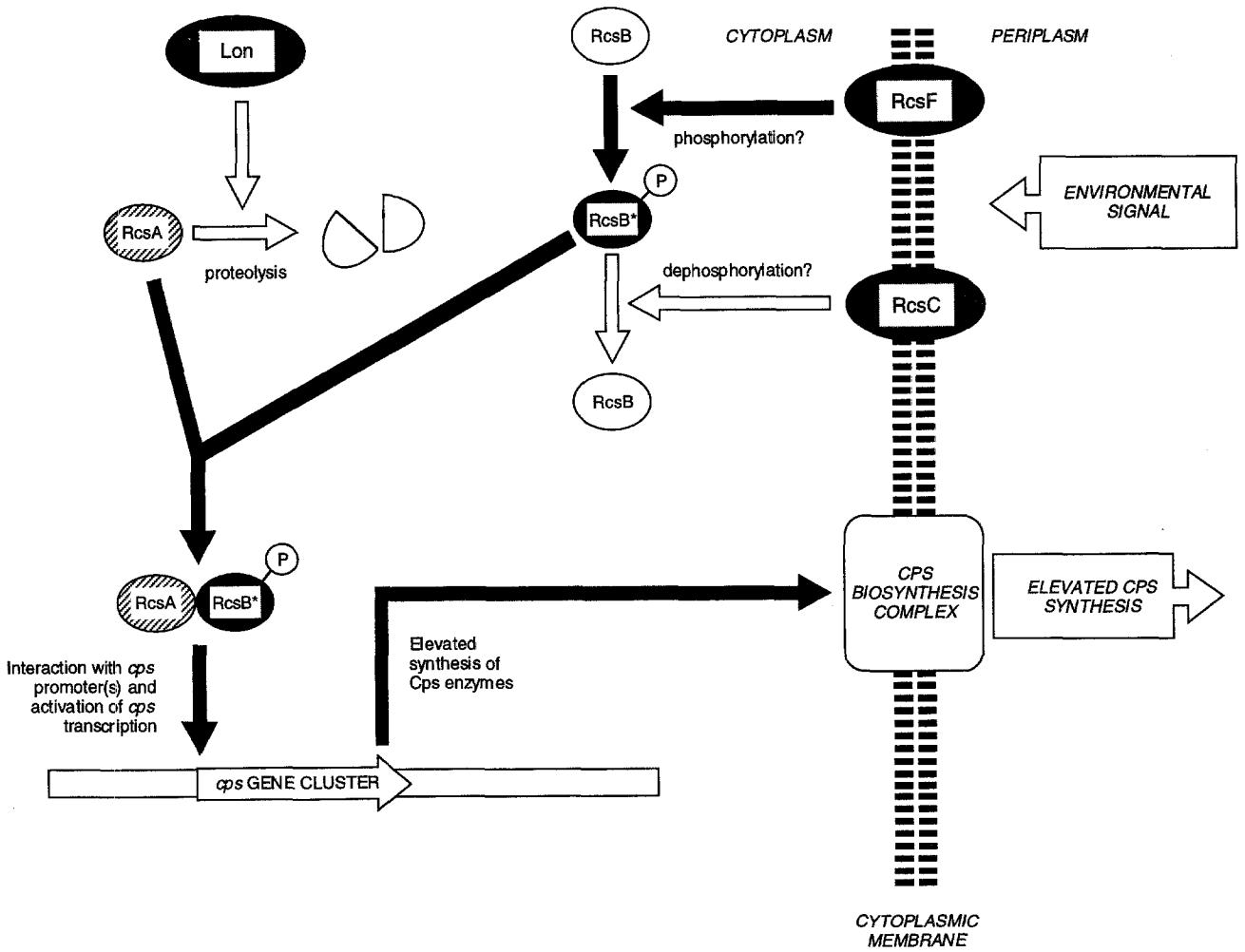

Figure 3 Regulation of group IA CPS and EPS by the *rcs* regulatory system. Modified from Gottesman and Stout [26]

within the *his*-linked *cps* gene cluster. The unlinked *cpsF* gene is regulated in the same manner as other *cps* genes, although it is apparently not essential for colanic acid synthesis [74]. There is no transcriptional regulation of either *manA* or *galE* [74]. The system for regulation of colanic acid is complex and involves both an environmentally responsive two-component system, which acts through RcsB, and a proteolytic system which limits availability of RcsA.

RcsA is an unstable regulatory protein, susceptible to degradation by the Lon protease

Early studies by Markovitz and coworkers [47] identified mutants with increased synthesis of colanic acid and elevated levels of activity for enzymes involved in colanic acid synthesis. One mutation, in a gene originally designated *capR*, is now known to be a *lon* defect. The Lon gene product is an ATP-dependent protease, with a variety of cellular targets, including RcsA [25]. Lon also degrades SulA, an inhibitor of cell division, providing an explanation for *lon* phenotypes including sensitivity to UV and SOS-inducing agents, and filamentation. *E. coli* K-12 *lon* strains are characteristically mucoid due to colanic acid overproduction, and show increased transcription of *cps* genes [74].

Mutations in positive regulators for colanic acid synthesis, including RcsA, were identified by decreased expression of *cps::lac* fusions in a *lon* background [27]. RcsA is an unstable protein in *E. coli* K-12, with a half-life of 5 min in wild type strains [72]. In *lon* mutants, the half-life is increased to 20 min. One possible explanation for the lack of mucoid phenotype in *E. coli* K-12 at 37° C is the limited availability of RcsA. It is suggested that RcsA is not fully functional at higher temperatures in *E. coli* K-12. Fluctuations in the amount of *rcsA* expression therefore have a profound effect on mucoid phenotype [58]. If the *rcsA* gene dosage is increased by using multicopy plasmids, a mucoid phenotype results and transcription of *cps::lac* fusions is increased [72].

Stability of RcsA is reduced in strains with *rcsB* mutations, and this is interpreted as protection from Lon of the RcsA component of RcsA-RcsB heterodimers [69]. Productive interactions between RcsA and RcsB can be reduced in strains which make C-terminal truncated RcsB proteins in addition to functional RcsB [69]. Together these observations provide strong evidence for the interaction between RcsA and RcsB. It has been suggested that the interaction between RcsA and RcsB is also affected by temperature [26].

The RscA sequence predicts a C-terminal helix-turn-helix sequence. This consensus sequence is found in mem-

bers of the LuxR family of transcriptional activators [31], which include UhpA, MalT, and FixJ. However, direct binding of RcsA to DNA (*cps* promoter) has not been demonstrated.

An environmentally regulated two-component regulatory system is involved in the control of colanic acid synthesis

Mutations in negatively acting effectors were initially identified by their ability to activate expression of a *cps::lac* fusion [27]. In addition to *lon* mutants, this strategy also identified the *rcsC* locus. *rcsC* maps at 48 min on the *E. coli* K-12 chromosome, adjacent to the structural gene for the positive regulator, *rcsB* [8]. The two genes are convergently transcribed. The N-terminal region of RcsB shares homology with a variety of effector proteins for diverse physiological processes [68]. Closest similarity is detected with FixJ, NarL, DegU, and UhpA, and most significantly, RcsA. RcsC is a trans-cytoplasmic membrane protein which shares significant C-terminal homology with regions conserved in a family of sensor proteins, including PhoR, EnvZ, NtrB, FixL, UhpB, DegS, and others [68]. Consequently, RcsB and RcsC belong to part of a family of environmentally responsive two-component regulatory systems. Based on systems which are better characterized at the biochemical level (primarily Ntr and Che), Stout and Gottesman [68] proposed that RcsC is the sensor which is receptive to environmental cues and transmits signals to the cytoplasmic C-terminal domain of RcsC. Usually, this domain is the site of an autophosphorylation reaction, at a conserved His residue. This region then transfers phosphate residues to the conserved N-terminal domain in the effector, in this case RcsB, through kinase activity. The phosphorylated effector then activates *cps* transcription (Figure 3). Phosphorylation of RcsB has now been detected *in vitro* (V Stout, personal communication). rcsC null mutations have no phenotype. In contrast, some point mutations (eg *rcsC137*) have a mucoid phenotype, consistent with constitutive activation of RcsB [26]. It has been suggested that RcsB transcriptional effector activity can be stimulated by cross-talk from other modified sensor-kinase proteins, such as EnvZ or PhoM [24]. Multicopy *rcsB* confers a mucoid phenotype and activates a *cps::lac* fusion [8]. This activation can occur in the absence of RcsA. This is therefore different from the RcsA activity, which has an absolute requirement for RcsB.

An additional component, RcsF, has also been described [23]. RcsF increases colanic acid expression, acts via the *rcsB* gene product, but is *rcsA*-independent. RcsF is likely a transmembrane protein but shares no significant homology with other known proteins. Significantly, RcsF can activate RcsB in the absence of RcsC. One explanation for these results is that RcsB is phosphorylated by RcsF and dephosphorylated by RcsC [23]. However, the absence of an obvious kinase motif in RcsF could reflect a less direct role. Recessive mutations with mucoid phenotypes, such as *rcsC137* [27], can then be explained by an inability to dephosphorylate RcsB, resulting in constitutive activation of *cps* transcription. It has been suggested that the interaction of RcsA with RcsB may help promote phosphorylation, or maintain RcsB in a phosphorylated state [69].

RcsB plays additional roles in the physiology of *E. coli* K-12. Multicopy RcsB is able to increase transcription of *ftsZ84*, a gene whose product plays a central role in cell division [24]. In keeping with the multiplicity of function, the regulation of *rcsB* is complex. RcsB also regulates its own expression [24]. Immediately upstream of *rcsB* is a σ^{54} (RpoN; NtrA) promoter and a σ^{70} promoter located in a LexA-binding region [24]. When tested in plasmid constructs, the role played by these promoters depends on the amount of upstream flanking sequence. Initial studies indicated that *rcsB* expression is drastically reduced (300-fold) in an *rpoN* background [68]. In a construct lacking further upstream sequences, *rcsB* expression was not dependent on either the σ^{54} or σ^{70} promoters. The physiological significance and precise role played by the upstream sequences, which are essential for *rcsB* expression, require further clarification.

Additional regulatory components in E. coli *K-12*

It is clear that there are additional genes whose products interact with the *rcs* regulatory system to stimulate colanic acid synthesis. Some of these cellular components are characterized, although the physiological significance of their interaction is not fully understood. For example, multicopy expression of one of the two subunits of the histone-like protein HU (HupA or HupB) activate transcription of a *cps::lacZ* fusion in an RcsA and RcsB-dependent process [58]. Available data favors a model where HupA or HupB cause overproduction of RcsA. As the authors indicate, other histone-like proteins (such as IHF) may have parallel effects. *ops* provides an example where the interacting component is not known. The *ops* gene and its product have not been identified but *ops* maps near *serA*. *ops* mutants are *o*verproducers of *p*oly*s*accharide [86], suggesting that *ops* might encode a negative regulator of colanic acid synthesis. Whether the primary role of the *ops* gene product is in *cps* expression, or whether it has additional cellular functions, remains to be established.

Role of *rcs* genes in *E. coli* strains other than K-12

The role played by the *rcs* regulatory system in *E. coli* depends on the strain studied. Since the *his*-linked genes required for production of colanic acid and group IA K-antigens of *E. coli* are allelic (see above), it is not surprising that the *rcs* system also regulates the group IA K-antigens. We have cloned *rcsA*, *rcsB* and *rcsC* genes from an *E. coli* strain with a prototype group IA CPS (serotype K30). The predicted RcsA (Figure 4), RcsB, and RcsC proteins are highly conserved in *E. coli* K-12 and K30 [37,41]. The putative regulatory sequences upstream of *rcsB* are also conserved.

Given the strong conservation in the regulatory components, it is assumed that the essential features of the regulatory process are also shared. A number of experimental observations support this hypothesis. The $rcsA_{K30}$ and $rcsB_{K30}$ genes were originally identified by their ability to complement the corresponding mutations in *E. coli* K-12. In multicopy, both $rcsA_{K30}$ and $rcsB_{K30}$ increase the formation of K30 CPS by a wild type strain [37,41], and the

effect of $rcsA_{K30}$ is $rcsB_{K30}$-dependent. Mutants with putative defects in *lon* [41] and $rcsC_{K30}$ (analogous to *rcsC137* in *E. coli* K-12) [37] both increase the formation of K30 CPS. The effect of the $rcsC_{K30}$ defect is abrogated by a $rcsB_{K30}$ mutation. However, despite the similarities in the effects of gene dosage and mutations on the expression of colanic acid and K30 CPS, there still remain some subtle differences between the two systems. In *E. coli* K30, CPS is produced under routine growth conditions at 30–37° C, resulting in formation of a capsular structure [81]. In contrast, under similar growth conditions $cps_{K\text{-}12}$ transcription is low in *E. coli* K-12 [27]. Colanic acid production in wild type *E. coli* K-12 strains is dependent on both growth temperature and composition of the culture medium [47]. The basal level of colanic acid formation is therefore much lower than that of K30 CPS. In *E. coli* K-12, the *rcs* system is required for colanic acid synthesis, but *rcs* activity in *E. coli* K30 seems to be confined to high levels of CPS production. Colanic acid may be characterized as EPS rather than CPS. However, overproduction of *E. coli* K30 CPS leads to a substantial increase in the amount of cell-free polymer [37,41].

E. coli strains with group IB and group II K-antigens contain a *cps* locus and can synthesize colanic acid [37,40,41,64]. These strains produce undetectable amounts of colanic acid at 37° C on rich growth media, and a regulatory mutation or introduction of multicopy *rcsA* or *rcsB* is required before the levels of colanic acid formation become obvious. This situation therefore resembles colanic acid formation in *E. coli* K-12. There is no evidence for a dramatic increase in group IB CPS expression under circumstances where colanic acid synthesis is activated [37], suggesting that regulation of group IA and IB CPSs is different. Similarly, in strains which produce group II CPSs, conditions which activate expression of colanic acid have no effect on the synthesis of the group II CPS at 37° C [40]. Studies on the regulation of *E. coli* K54 CPS indicate that conditions which elevate the amounts of RcsA in the cell (multicopy *rcsA* or *lon* mutation) activate colanic acid production but actually reduce the expression of two Tn*phoA* fusions in the K54 capsule biosynthesis genes [64]. However, the reduction is quite small and is only detected at low growth temperatures. The K54 CPS is an atypical group II K-antigen. Although the genes are found near *serA*, as are other group II capsule biosynthesis gene clusters, genes which are conserved in most group II clusters are absent in *E. coli* K54 [20]. The precise status of these strains remains to be established.

rcs genes in *Klebsiella*

Many of the CPSs of *Klebsiella* spp are structurally similar to colanic acid and the group IA CPSs of *E. coli*, and they share biosynthetic (see above) and regulatory features. *rcsA* has been cloned from *Klebsiella* serotypes K21 [1] and K20 [48], and hybridization experiments indicate that *rcsA* sequences are widespread in *Klebsiella*. The predicted $RcsA_{Kp}$ protein shows a high degree of conservation when compared to the *E. coli* K-12 RcsA (Figure 4), and cloned *rcsA* from *Klebsiella* complements an *rcsA* defect in *E. coli* K-12 [11,48], suggesting conservation of function. However, the only direct indication of RcsA function in CPS formation in *Klebsiella* is the ability of a multicopy plasmid containing *rcsA* to restore wild type levels of CPS formation in a mutant expressing reduced amounts of polysaccharide [48]. No chromosomal *rcsA* mutations have been constructed to verify these findings. *rcsB* and *rcsC* have not been identified in *Klebsiella*. Transfer of the *his-cps* region from *Klebsiella* K20 to *E. coli* K-12 resulted in expression of K20 CPS in *E. coli*, although the amount synthesized was reduced when compared to the wild type *Klebsiella* strain [44]. However, when *Klebsiella* K2 *cps* genes are expressed in *E. coli* K-12, formation of K2 CPS is dependent on RcsA and RcsB functions supplied by the host [77]. In the absence of RcsA and RcsB activities no K2 CPS is detected on the cell surface. Northern blot data supports the conclusion that host RcsB is required for transcription of *Klebsiella cps* in *E. coli* K-12 [77]. The observation that no K2 CPS is produced in *rcsA* and *rcsB* hosts, rather than a reduction in synthesis, may reflect either the sensitivity of the detection methods or an altered efficiency in the interaction of various components in this heterologous system.

Regulation of CPS expression in *Klebsiella* involves an additional level not seen in other *rcs*-regulated systems. The highly virulent K1 and K2 serotypes are particularly mucoid. High level CPS expression requires a 180-kbp plasmid which carries the genes for the aerobactin iron acquisition system, in addition to genes *rmpA* and *rmpB*, which regulate CPS expression [51]. *rmpA* mutants lack high level CPS synthesis [51] and subsequent research indicates that *rmpA* activates synthesis of CPS in K2 and other serotypes [78]. Cloned *rmpA-rmpB* caused a mucoid phenotype in lon^+ *E. coli* K-12 due to the formation of colanic acid. The activity of RmpA is related to that of RcsA and multicopy *rmpA* can complement an *rcsA* mutation in *E. coli* K-12 *lon*. As with RcsA, the action of RmpA is dependent on RcsB [53], so RmpA augments, rather than overrides, the 'normal' *rcs* regulatory system. In the absence of *rmpB*, *rmpA* can only activate expression of colanic acid in lon^- hosts. However, the role played by RmpB is not understood [76]. Two reports of *rmpA* sequence showed some discrepancies [52,78] but available data indicate that RmpA contains regions at the N- and C-termini which are similar to RcsA. In addition, RmpA has a C-terminal helix-turn-helix motif resembling LuxR and FixJ, and a central domain with similarity to the nitrogen regulator NtrC [78]. RmpA is required for synthesis of K2 CPS in *E. coli* K-12 strains containing cloned *cps* genes [2,78]. Again, the absolute requirement for RmpA over and above RcsA and RcsB in this heterologous system, may reflect inefficient interaction between the components. In *Klebsiella*, as in *E. coli* K30, CPS is produced under all growth conditions and RmpA is only required for exceptionally high level CPS synthesis [51,78].

rcs genes in *Erwinia*

Highly conserved *rcsA* genes have been identified in both *E. stewartii* [60] and *E. amylovora* [7,10,11] (Figure 4). RcsA is a versatile regulator in *E. amylovora* since *rcsA* mutants are unable to synthesize both amylovoran and the

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1     MSTIIMDLCSYTRLGLTGYLLSRGVKKREINDIETVDDLAIACDSQRPSVVFINEDCFIH   RcsA K-12
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::   RcsA K30
      :::M:::::::::::::::T:::I::Q::VEVNSAA::QKH:T:CC:A:::L:::::V:   RcsA Kp
      :P:::::S:N:::::::E:MTVK::::KN:SL:NDIAQIQNK:QQLK:G::L::::::::   RcsA Ea
      :P:::::S:N::::::SD:MS:K::::KN:TSVSDIEQ:QQR:EQLKPG:::::::::::   RcsA Es

61    DASNSQRIKLIINQHPNTLFIVFMAIANVHFDEYLLVRKNLLISSKSIKPESLDDILGDI
      ::::::H::H::::::::::::::::::::::::::::::::::::::::::::::::::
      :DESNGI:RQ::T:N:A:::VI::SL::I:::R::R::::::::::::T:KD::V::VNY
      ESDA:E::RK::L:::D:::FI::::S:I::E:::Y::N::I:T::A::IST::SL:NGY
      ETDS:E::RS::::::E:::FI::::S:I::E:::Y:::::I:T::A:::ST::SL:STY

121   LKKETTITSFLNMPTLSLSRTESSMLRMWMAGQGTIQISDQMNIKAKTVSSHKGNIKRKI
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      ::YKN:SVGQ:TL::::::K:::N::Q:::::H::S:::T:::::::::::::::::K::
   FQKK:NLSVRHGTHSEVHP:T::Q:::N::K:::S:HD::::::K:Q:::::::::::::::
   LQKK:NMSPR:S:G:DVHP:T::Q:::N::K:::S:HD::::::K:Q:::::::::::::::

181   KTHNKQVIYHVVRLTDNVTNGIFVNMR   207
      :::::::::::::::::::::::::::
      Q:::::::::I::::E:I:S::Q::::
      ::::::::::::::::::::S::Y::E:
      ::::::::::::::::::::S::Y::V:
```

Figure 4 Alignment of predicted RcsA proteins from bacteria with group IA extracellular polysaccharides. The proteins are from *E. coli* K-12 [69], *E. coli* K30 [41], *Klebsiella pneumoniae* [1,48], *Erwinia amylovora* [11], and *Erwinia stewartii* [60]. The sources are indicated by subscripts. The protein from *E. coli* K-12 is the best characterized and is therefore used as the prototype. Identical amino acid residues are indicated by colons and the putative helix-turn-helix DNA binding region is underlined. The alignment accommodates four additional amino acid residues unique to the *Erwinia* homologs

polyfructose polymer, levan [7]. In *E stewartii*, RcsA transcriptionally regulates expression of *cps::lacZ* fusions [73]. As with *E. coli* K-12, *E. stewartii galE::lacZ* fusions are not regulated by RcsA. *ams::lacZ* fusions are not expressed in *E. stewartii rcsA* mutants [45], suggesting a conserved regulatory process in *Erwinia* species. These observations are consistent with RcsA regulating EPS expression in *Erwinia* in a manner similar to the parallel system in *E. coli* K-12. Further confirmation is provided by cross complementation with *rcsA* homologs from *E. coli* K-12 and *E. stewartii* [73]. In *E. coli* K-12, the *E. amylovora* RcsA protein activates colanic acid in an RcsB-dependent process [10,11] and *E. stewartii* RcsA is Lon-sensitive [73]. Preliminary reports of *rcsB* and *rcsC* genes in *Erwinia* indicate similarities in structure and function to the *E. coli* K-12 homologs [14,62]. It therefore appears that the complete *rcs* regulatory system is functional in *Erwinia*.

Is the *rcs* system confined to cell surface polymers formed by a common biosynthetic mechanism?

In addition to regulating EPS production in *Erwinia amylovora*, RcsA plays a role in regulation of levansucrase [7]. This extracellular enzyme synthesizes levan (polyfructose) homopolymer at, or outside, the cell surface using sucrose as a substrate [45]. Synthesis of levans therefore represents a fundamentally different process to that involved in group IA CPS/EPS. *Salmonella enterica* serovars produce a variety of cell surface polysaccharides. These include a vast array of lipopolysaccharide O-antigens, colanic acid [47], and in serovars Typhi and Paratyphi, the Vi antigen. Vi antigen is a homopolymer of galactosaminuronic acid and is synthesized by all strains of *S. enterica* serovars Typhi and Paratyphi, as well as by some strains of *Citrobacter*. Two genetic loci, *viaA* and *viaB* are required for expression of the Vi antigen. *viaB* encodes the enzymes involved in biosynthesis. Genetic analysis of *viaB* supports a biosynthetic mechanism resembling *E. coli* group II CPSs [29,30,43]. The approximately 14-kbp *viaB* region is unique to strains which produce Vi. In contrast, functions encoded by *viaA* are detected in *E. coli* K-12 and *S. enterica* serovar Typhimurium, despite the fact that these bacteria do not produce Vi antigen [38]. It is now known that *rcsB* can functionally replace *viaA*, and that both RcsA and RcsB are required for Vi expression in *E. coli* K-12 strains harboring cloned *viaB* on a plasmid [33]. The precise roles played by RcsA and RcsB have not been defined experimentally, but by analogy to their functions in other systems, they are presumed to be transcriptional activators of Vi expression. These limited examples indicate that *rcs*-mediated regulation is not confined to expression of surface polysaccharides produced by a single biosynthetic mechanism.

Biological significance of *rcs* regulatory systems: functions of *rcs*-regulated polysaccharides

Although very high levels of CPS/EPS expression can be obtained in regulatory mutants, it is not established that these high levels are seen *in vivo*. The primary roles

described so far for group IA CPSs/EPSs in animal pathogens are ones involving protection. The group IA CPSs of *E. coli* are virulence determinants frequently found in enterotoxigenic and enteropathogenic strains [56]. These CPSs have a primary role in evasion of phagocytosis [32] and unencapsulated mutants are readily ingested by phagocytes. The K-antigens of *Klebsiella* are also anti-phagocytic [83]. Several studies have shown the importance of both abundance of CPS and rate of synthesis in virulence of *Klebsiella* in pulmonary [18,19], intraperitoneal [22,71], and burn [16] infection models. One consequence of higher levels of expression in both *E. coli* [37,41] and *Klebsiella* [18,21] is the release of larger amounts of cell-free polysaccharide. In the case of *K. pneumoniae*, cell-free EPS has been shown to neutralize circulating antibody [61] and mediate effects including immune tolerance [4,50,55], and impairment of macrophage induction and function [84,85]. At this level, the ability to up-regulate polymer synthesis and release cell-free EPS seems to be a valuable trait.

When examined *in vitro*, the group IA CPS of *E. coli* K30 has a detrimental effect on adhesion mediated by the K99 pili [63]. However, K30 CPS enhances colonization of epithelia of piglets, lambs and calves when studied *in vivo* [28,49,66]. Electron microscopic examination has revealed heavily encapsulated *E. coli* colonizing epithelia in a calf model [9]. Unencapsulated bacteria are unable to colonize [63].

High levels of EPS expression are equally important for virulence of the plant pathogenic *Erwinia* strains, and the amounts of polymer produced are increased *in planta*. *Erwinia amylovora* causes fire-blight disease of members of the Pomoideae and other plants. The disease progresses until extensive cell leakage and necrosis occurs, and the infected tissues become withered [62]. *E. stewartii* is a pathogen of corn seedlings, and infection causes water-soaking and leaf blight. Bacterial colonization of xylem vessels leads to blocking and ultimately to wilting, and EPS is implicated in the blocking phenomenon [45]. EPS-deficient *ams* [6] and *cps* [12,17] mutants are non-pathogenic. An *rcsA* mutant of *E. stewartii* is both non-mucoid and avirulent [73].

In most strains of *E. coli*, the formation of colanic acid is minimal at 37° C, making a virulence role for this particular polymer unlikely. However, this assumes that there is no activation of the biosynthetic system *in vivo*. Other than regulatory mutants, only two situations have been reported which involve *rcs*-mediated activation of *E. coli* K-12 *cps* expression and both require RcsC. The first is in deep rough mutants [59]. However, such mutants exhibit drastic and pleiotropic cell surface effects, and this may not reflect sensing of stimuli for which the *rcs* system is designed. Desiccation conditions activate colanic acid synthesis in *E. coli* K-12 and EPS synthesis in *E. stewartii* [54]. Colanic acid (and presumably other group IA CPS/EPS) binds water and therefore during dehydration, the polymer maintains the cell surface in a hydrated state longer than that of unencapsulated bacteria. It has been suggested that the effects of desiccation and deep-rough LPS mutations may be related [54]. The bacteria which possess an *rcs* regulatory system occupy diverse ecological niches and face different environmental pressures. As indicated above, protection from desiccation is clearly not the only role played by these polysaccharides.

The fact that these diverse bacteria use a conserved regulatory system to control key defenses is intriguing. For all of the bacteria discussed in this review, the central and unanswered question is exactly which environmental conditions activate polymer synthesis through the *rcs*-pathway *in vivo*.

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References

1 Allen P, CA Hart and JR Saunders. 1987. Isolation from *Klebsiella* and characterization of two *rcs* genes that activate colanic acid capsular biosynthesis in *Escherichia coli*. J Gen Microbiol 133: 331–340.
2 Arakawa Y, M Ohta, R Wacharotayankun, M Mori, N Kido, H Ito, T Komatsu, T Sugiyama and N Kato. 1991. Biosynthesis of *Klebsiella* K2 capsular polysaccharide in *Escherichia coli* HB101 requires the function of *rmpA* and the chromosomal *cps* gene cluster of the virulent strain *Klebsiella pneumoniae* Chedid (O1:K2). Infect Immun 59: 2043–2050.
3 Bachmann BJ. 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol Rev 54: 130–197.
4 Batshon BA, H Baer and MF Schaffer. 1963. Immunologic paralysis in mice by *Klebsiella pneumoniae* type 2 polysaccharide. J Immunol 90: 121–126.
5 Bayer ME and H Thurow. 1977. Polysaccharide capsule of *Escherichia coli*: microscope study of its size, structure, and sites of synthesis. J Bacteriol 130: 911–936.
6 Bernhard F, DL Coplin and K Geider. 1993. A gene cluster for amylovoran synthesis in *Erwinia amylovora*: characterization and relationship of *cps* genes in *Erwinia stewartii*. Mol Gen Genet 239: 158–168.
7 Bernhard F, K Poetter, K Geider and DL Coplin. 1990. The *rcsA* gene from *Erwinia amylovora*: identification, nucleotide sequence, and regulation of exopolysaccharide biosynthesis. Molec Plant–Microbe Interact 3: 429–437.
8 Brill JA, C Quinlan-Walshe and S Gottesman. 1988. Fine-structure mapping and identification of two regulators of capsule synthesis in *Escherichia coli* K-12. J Bacteriol 170: 2599–2611.
9 Chan R, SD Acres and JW Costerton. 1982. Use of specific antibody to demonstrate glycocalyx, K99 pili, and the spatial relationships of $K99^+$ enterotoxigenic *Escherichia coli* in the ileum of colostrum-fed calves. Infect Immun 37: 1170–1180.
10 Chatterjee A, W Chun and AK Chatterjee. 1990. Isolation and characterization of an *rcsA*-like gene of *Erwinia amylovora* that activates extracellular polysaccharide production in *Erwinia* species, *Escherichia coli*, and *Salmonella typhimurium*. Molec Plant–Microbe Interact 3: 144–148.
11 Coleman M, R Pearce, E Hitchin, F Busfield, JW Mansfield and IS Roberts. 1990. Molecular cloning, expression and nucleotide sequence of the *rcsA* gene of *Erwinia amylovora*, encoding a positive regulator of capsule expression: evidence for a family of related capsule activator proteins. J Gen Microbiol 136: 1799–1806.
12 Coplin DL, RD Frederick, DR Majerczak and ES Haas. 1986. Molecular cloning of virulence genes from *Erwinia stewartii*. J Bacteriol 168: 619–623.
13 Coplin DL and DR Majerczak. 1990. Extracellular polysaccharide genes in *Erwinia stewartii*: directed mutagenesis and complementation analysis. Molec Plant–Microbe Interact 3: 286–292.
14 Coplin D, K Poetter and DR Majerczak. 1991. Regulation of exopolysaccharide synthesis in *Erwinia stewartii* by *rcsB* and *rcsC*. Phytopathology 81: 1220 (abstract).
15 Costerton JW, Z Lewandowski, D DeBeer, D Caldwell, D Korber and G James. 1994. Biofilms, the customized microniche. J Bacteriol 176: 2137–2142.
16 Cryz SJJ, E Fürer and R Germanier. 1984. Experimental *Klebsiella*

pneumoniae burn wound sepsis: role of capsular polysaccharide. Infect Immun 43: 440–441.

17 Dolph PJ, DR Majeczak and DL Coplin. 1988. Characterization of a gene cluster for exopolysaccharide biosynthesis and virulence in *Erwinia stewartii*. J Bacteriol 170: 865–871.

18 Domenico P, DL Diedrich and DC Straus. 1985. Extracellular polysaccharide production by *Klebsiella pneumoniae* and its relationship to virulence. Can J Microbiol 31: 472–478.

19 Domenico P, WGJ Johnson and DC Straus. 1982. Lobar pneumonia in rats produced by clinical isolates of *Klebsiella pneumoniae*. Infect Immun 37: 327–335.

20 Drake C, G Boulnois and I Roberts. 1993. The *Escherichia coli serA*-linked capsule locus and its flanking sequences are polymorphic, genetic evidence for the existence of more than two groups of capsule gene clusters. J Gen Microbiol 139: 1707–1714.

21 Duguid JP and JF Wikinson. 1953. The influence of cultural conditions on polysaccharide production by *Aerobacter aerogenes*. J Gen Microbiol 9: 174–189.

22 Ehrenworth L and H Baer. 1956. The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. J Bacteriol 72: 713–717.

23 Gervais FG and GR Drapeau. 1992. Identification, cloning, and characterization of *rcsF*, a new regulator gene for exopolysaccharide synthesis that suppresses the division mutation *ftsZ84* in *Escherichia coli* K-12. J Bacteriol 174: 8016–8022.

24 Gervais FG, P Phoenix and GR Drapeau. 1992. The *rcsB* gene, a positive regulator of colanic acid biosynthesis in *Escherichia coli*, is also an activator of *ftsZ* expression. J Bacteriol 174: 3964–3971.

25 Gottesman S and MR Maurizi. 1992. Regulation by proteolysis: energy-dependent proteases and their targets. Microbiol Rev 56: 592–621.

26 Gottesman S and V Stout. 1991. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12. Molec Microbiol 5: 1599–1606.

27 Gottesman S, P Trisler and A Torres-Cabassa. 1985. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. J Bacteriol 162: 1111–1119.

28 Hadad JJ and CL Gyles. 1982. The role of K antigens of enteropathogenic *Escherichia coli* in colonization of the small intestine of calves. Can J Comp Med 46: 21–26.

29 Hashimoto Y, T Ezaki, N Li and H Yamamoto. 1991. Molecular cloning of the *viaB* region of *Salmonella typhi*. FEMS Microbiol Lett 90: 53–56.

30 Hashimoto Y, N Li, H Yokoyama and T Ezaki. 1993. Complete nucleotide sequence and molecular characterization of ViaB region encoding Vi antigen in *Salmonella typhi*. J Bacteriol 175: 4456–4465.

31 Henikoff J, C Wallace and JP Brown. 1990. Finding protein similarities with nucleotide sequence databases. Meths Enzymol 183: 111–132.

32 Horwitz MA. 1982. Phagocytosis of microorganisms. Rev Infect Dis 4: 104–123.

33 Houng H-SH, KF Noon, JT Ou and LS Baron. 1992. Expression of Vi antigen in *Escherichia coli* K-12: characterization of ViaB from *Citrobacter freundii* and identity of ViaA with RcsB. J Bacteriol 174: 5910–5915.

34 Jann K, T Dengler and B Jann. 1992. Core-lipid A on the K40 polysaccharide of *Escherichia coli* O8:K40:H9, a representative of group I capsular polysaccharides. Zentrablatt fur Bakteriologie 276: 196–204.

35 Jann B and K Jann. 1990. Structure and biosynthesis of the capsular antigens of *Escherichia coli*. In: Bacterial Capsules (K Jann and B Jann, eds), pp 19–42, Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong.

36 Jayaratne P, D Bronner, R MacLachlan, C Dodgson, N Kido and C Whitfield. 1994. Cloning and analysis of duplicated *rfbK* and *rfbM* genes involved in formation of GDP-mannose in *Escherichia coli* O9:K30 and participation of *rfb* genes in the synthesis of the group I K30 capsular polysaccharide. J Bacteriol 176: 3126–3139.

37 Jayaratne P, WJ Keenleyside, PR MacLachlan, C Dodgson and C Whitfield. 1993. Characterization of *rcsB* and *rcsC* from *Escherichia coli* O9:K30:H12 and examination of the role of the *rcs* regulatory system in expression of group I capsular polysaccharides. J Bacteriol 175: 5384–5394.

38 Johnson EM and LS Baron. 1969. Genetic transfer of the Vi antigen from *Salmonella typhosa* to *Escherichia coli*. J Bacteriol 99: 358–359.

39 Johnson JG and DB Wilson. 1977. Role of sugar-lipid intermediate in colanic acid synthesis by *Escherichia coli*. J Bacteriol 129: 225–236.

40 Keenleyside WJ, D Bronner, K Jann, B Jann and C Whitfield. 1993. Co-expression of colanic acid and serotype-specific capsular polysaccharides in *Escherichia coli* strains with group II K-antigens. J Bacteriol 175: 6725–6730.

41 Keenleyside WJ, P Jayaratne, PR MacLachlan and C Whitfield. 1992. The *rcsA* gene of *Escherichia coli* O9:K30:H12 is involved in the expression of the serotype-specific group I K (capsular) antigen. J Bacteriol 174: 8–16.

42 Kellenberger E. 1990. The 'Bayer bridges' confronted with results from improved electron microscopy methods. Molec Microbiol 4: 697–705.

43 Kolyva S, H Waxin and MY Popoff. 1992. The Vi antigen of *Salmonella typhi*: molecular analysis of the *viaB* locus. J Gen Microbiol 138: 297–304.

44 Laakso DH, MK Homonylo, SJ Wilmot and C Whitfield. 1988. Transfer and expression of the genetic determinants for O and K antigen synthesis in *Escherichia coli* O9:K(A)30:H12 and *Klebsiella* sp O1:K20, in *Escherichia coli* K-12. Can J Microbiol 34: 987–992.

45 Leigh JA and DL Coplin. 1992. Exopolysaccharides in plant–bacterial interactions. Ann Rev Microbiol 46: 307–346.

46 MacLachlan PR, WJ Keenleyside, C Dodgson and C Whitfield. 1993. Formation of the K30 (group I) capsule in *Escherichia coli* O9:K30 does not require attachment to lipopolysaccharide lipid A-core. J Bacteriol 175: 7515–7522.

47 Markovitz A. 1977. Genetics and regulation of bacterial capsular polysaccharide biosynthesis and radiation sensitivity. In: Surface Carbohydrates of the Prokaryotic Cell (IW Sutherland, ed), pp 415–462, Academic Press, New York.

48 McCallum KL and C Whitfield. 1991. The *rcsA* gene of *Klebsiella pneumoniae* O1:K20 is involved in expression of the serotype-specific K (capsular) antigen. Infect Immun 59: 494–502.

49 Nagy B, HW Moon and RE Issacson. 1976. Colonization of porcine small intestine by *Escherichia coli*: ileal colonization and adhesion by pig enteropathogens that lack K88 antigen and by some acapsular mutants. Infect Immun 13: 1214–1220.

50 Nakashima I, T Kobayashi and N Kato. 1971. Alteration in the antibody response to bovine serum albumin by capsular polysaccharide of *Klebsiella pneumoniae*. J Immunol 107: 1112–1121.

51 Nassif X, J-M Fournier, J Arondel and PJ Sansonetti. 1989. Mucoid phenotype of *Klebsiella pneumoniae* is a plasmid-encoded virulence factor. Infect Immun 57: 546–552.

52 Nassif X, N Honoré, T Vasselon, ST Cole and PJ Sansonetti. 1989. Positive control of colanic acid synthesis in *Escherichia coli* by *rmpA* and *rmpB*, two virulence-plasmid genes of *Klebsiella pneumoniae*. Molec Microbiol 3: 1349–1359.

53 Ofek I, K Kabha, A Athamna, G Frankel, DJ Wozniak, DL Hasty and DE Ohman. 1993. Genetic exchange of determinants for capsular polysaccharide biosynthesis between *Klebsiella pneumoniae* strains expressing serotypes K2 and K21a. Infect Immun 61: 4208–4216.

54 Ophir T and DL Gutnick. 1994. A role for exopolysaccharides in the protection of microrganisms from dessication. Appl Environ Microbiol 60: 740–745.

55 Ørskov I. 1956. 'Immunological paralysis' induced in rabbits by a heavily capsulated *Klebsiella* strain. Acta Pathol Microbiol Scand 38B: 375–384.

56 Ørskov F, I Ørskov, B Jann and K Jann. 1971. Immunoelectrophoretic patterns of extracts from all *Escherichia coli* O and K antigen test strains: correlation with pathogenicity. Acta Pathol Microbiol Scand 79B: 142–152.

57 Ørskov F, V Sharma and I Ørskov. 1984. Influence of growth temperature on the development of *Escherichia coli* polysaccharide K-antigens. J Gen Microbiol 130: 2681–2684.

58 Painbéni E, E Mouray, S Gottesman and J Rouvière-Yaniv. 1993. An imbalance in HU synthesis induces mucoidy in *Escherichia coli*. J Mol Biol 234: 1021–1037.

59 Parker CT, AW Kloser, CA Schnaitman, MA Stein, S Gottesman and BW Gibson. 1992. Role of the *rfaG* and *rfaP* genes in determining the lipopolysaccharide core structure and cell surface properties of *Escherichia coli* K-12. J Bacteriol 174: 2525–2538.

60 Poetter K and DL Coplin. 1991. Structural and functional analysis of the *rcsA* gene from *Erwinia stewartii*. Mol Gen Genet 229: 155–160.

61 Pollack M. 1976. Significance of circulating capsular antigens in *Klebsiella* infections. Infect Immun 13: 1543–1548.
62 Roberts IS and MJ Coleman. 1991. The virulence of *Erwinia amylovora*: molecular genetic perspectives. J Gen Microbiol 137: 1453–1457.
63 Runnels PL and PL Moon. 1984. Capsule reduces adherence of enterotoxigenic *Escherichia coli* to isolated intestinal epithelial cells of pigs. Infect Immun 45: 737–740.
64 Russo TA and G Singh. 1993. An extraintestinal, pathogenic isolate of *Escherichia coli* (O4/K54/H5) can produce a group 1 capsule which is divergently regulated from its constitutively produced group 2 K54 capsular polysaccharide. J Bacteriol 175: 7617–7623.
65 Schmidt G, B Jann, K Jann, I Ørskov and F Ørskov. 1977. Genetic determinants of the synthesis of the polysaccharide capsular antigen K27(A) of *Escherichia coli*. J Gen Microbiol 100: 355–361.
66 Smith HW and MB Huggins. 1978. The influence of plasmid-determined and other characteristics of enteropathogenic *Escherichia coli* on their ability to proliferate in the alimentary tracts of piglets, calves and lambs. J Med Microbiol 11: 471–492.
67 Stevenson G, SJ Lee, LK Romana and PR Reeves. 1991. The *cps* gene cluster of *Salmonella* strain LT2 includes a second mannose pathway: sequence of two genes and relationship to genes in the *rfb* gene cluster. Mol Gen Genet 227: 173–180.
68 Stout V and S Gottesman. 1990. RcsB and RcsC: a two-component regulator of capsule synthesis in *Escherichia coli* K-12. J Bacteriol 172: 659–669.
69 Stout V, A Torres-Cabassa, MR Maurizi, D Gutnick and S Gottesman. 1991. RcsA, an unstable positive regulator of capsular polysaccharide synthesis. J Bacteriol 173: 1738–1747.
70 Sutherland IW and M Norval. 1970. The synthesis of exopolysaccharide by *Klebsiella aerogenes* membrane preparations and the involvement of lipid intermediates. Biochem J 120: 567–576.
71 Takahashi M, K Yoshida and CL San Clemente. 1977. Relation of colonial morphologies in soft agar to morphological and biological properties of the K-9 strain of *Klebsiella pneumoniae* and its virulence. Can J Microbiol 23: 448–451.
72 Torres-Cabassa A and S Gottesman. 1987. Capsule synthesis in *Escherichia coli* K-12 is regulated by proteolysis. J Bacteriol 169: 981–989.
73 Torres-Cabassa A, S Gottesman, RD Frederick, PJ Dolph and DL Coplin. 1987. Control of extracellular polysaccharide synthesis in *Erwinia stewartii* and *Escherichia coli* K-12: a common regulatory function. J Bacteriol 169: 4525–4531.
74 Trisler P and S Gottesman. 1984. *lon* transcriptional regulation of genes necessary for capsular polysaccharide synthesis in *Escherichia coli* K-12. J Bacteriol 160: 184–191.
75 Troy FA, FE Frerman and EC Heath. 1971. The biosynthesis of capsular polysaccharide in *Klebsiella aerogenes*. J Biol Chem 246: 118–133.
76 Vasselon T, PJ Sansonetti and X Nassif. 1991. Nucleotide sequence of *rmpB*, a *Klebsiella pneumoniae* gene that positively controls colanic acid biosynthesis in *Escherichia coli*. Res Microbiol 142: 47–54.
77 Wacharotayankun R, Y Arakawa, M Ohta, T Hasegawa, M Mori, T Horii and N Kato. 1992. Involvement of *rcsB* in *Klebsiella* K2 capsule synthesis in *Escherichia coli* K-12. J Bacteriol 174: 1063–1067.
78 Wacharotayankun R, Y Arakawa, M Ohta, K Tanaka, T Akashi, M Mori and N Kato. 1993. Enhancement of extracapsular polysaccharide synthesis in *Klebsiella pneumoniae* by RmpA2, which shows homology to NtrC and FixJ. Infect Immun 61: 3164–3174.
79 Whitfield C, WJ Keenleyside and BR Clarke. 1994. Structure, function and synthesis of cell surface polysaccharides in *Escherichia coli*. In: *Escherichia coli* in Domestic Animals and Man (CL Gyles, ed), pp 437–494, CAB International, Wallingford, UK.
80 Whitfield C, JC Richards, MB Perry, BR Clarke and LL MacLean. 1991. Expression of two structurally distinct D-galactan O-antigens in the lipopolysaccharide of *Klebsiella pneumoniae* serotype O1. J Bacteriol 173: 1420–1431.
81 Whitfield C, G Schoenhals and L Graham. 1989. Mutants of *Escherichia coli* O9:K30 with altered synthesis and expression of the capsular K antigen. J Gen Microbiol 135: 2589–2599.
82 Whitfield C and MA Valvano. 1993. Biosynthesis and expression of cell surface polysaccharides in gram-negative bacteria. Adv Micro Physiol 35: 135–246.
83 Williams P and JM Tomás. 1990. The pathogenicity of *Klebsiella pneumoniae*. Rev Med Microbiol 1: 196–204.
84 Yokochi T, I Nakashima and N Kato. 1977. Effect of capsular polysaccharide of *Klebsiella pneumoniae* on the differentiation and functional capacity of macrophages cultured *in vitro*. Microbiol Immunol 21: 601–610.
85 Yokochi T, I Nakashima and N Kato. 1979. Further studies on generation of macrophages in *in vitro* cultures of mouse spleen cells and its inhibition by the capsular polysaccharide of *Klebsiella pneumoniae*. Microbiol Immunol 23: 487–499.
86 Zinkewich-Péotti K and JM Fraser. 1988. New locus for exopolysaccharide, overproduction in *Escherichia coli* K-12. J Bacteriol 170: 1405–1407.