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The intersection of capsule gene expression, hypermucoviscosity and hypervirulence in *Klebsiella pneumoniae*

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Abstract

For ~30 years, two distinct groups of clinical isolates of *Klebsiella pneumoniae* have been recognized. Classical strains (cKp) are typically isolated from patients with some degree of immunocompromise and are not virulent in mouse models of infection whereas hypervirulent strains (hvKp) are associated with community acquired invasive infections and are highly virulent in mouse models of infection. Hyperproduction of capsule and a hypermucoviscous colony phenotype have been strongly associated with the hypervirulence of hvKp strains. Recent studies have begun to elucidate the relationship between capsule gene expression, hypermucoviscosity and hypervirulence. Additionally, genes associated with hyperproduction of capsule and hypermucoviscosity in hvKp strains have been identified in a few cKp isolates. However, it is not clear how the acquisition of these genes impacts the virulence of cKp isolates. A better understanding of the potential risks of these strains is particularly important given that many of them are resistant to multiple antibiotics, including carbapenems.

Introduction

For more than a century, *Klebsiella pneumoniae* has been recognized as a human pathogen responsible for a variety of different infections, yet bacterial factors contributing to these different disease presentations were not clear. However, two recent advances in the field have provided a path forward to formulate and focus questions for future mechanistic studies. The first is the differentiation of two groups of strains currently of clinical importance. The second is the application of whole genome sequencing and bioinformatic analysis of large groups of *K. pneumoniae* strains. Several excellent reviews of *K. pneumoniae* diseases, epidemiology, virulence factors and pathogenesis have been published recently [1,2,3], thus we will not go into the details of these aspects of *K. pneumoniae* biology here and instead will focus on the relationship between capsule, hypermucoviscosity and hypervirulence.

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Conflict of interest

Nothing declared.

For the past 30 years, two different groups of *K. pneumoniae* of particular concern have circulated — hypervirulent (hvKp) strains that are sensitive to carbapenem and classical strains (cKp) that frequently are carbapenem resistant (CR) [1•,3•,4]. The cKp strains are typically associated with nosocomial infections or infection in a long-term care setting, suggesting some degree of immunocompromise is necessary for these strains to cause disease. For these CR-cKp strains, few treatment options are available and, although colistin has been used, strains that are also resistant to colistin are emerging [5]. Bioinformatic analyses of large strain sets have been instrumental in helping to define genes linked to hypervirulence (hv-associated genes); these include *rmpA*, *rmpA2*, *iroBCDN*, *iutA*, *iucABCD* and *ybt* genes [6,7,8•,9,10•,11]. These hv-associated genes are often encoded on a large, highly conserved virulence plasmid such as pLVPK, but some strains have the hv-associated genes encoded on the chromosome as part of an ICE element [12–14]. The hv-associated genes listed above encode three different siderophore systems: salmochelin (*iroBCDN*), aerobactin (*iutA*, *iucABCD*) and yersiniabactin (*ybt* genes). cKp strains produce only enterobactin and occasionally yersiniabactin. However, production of multiple siderophores is strongly associated with hypervirulence and studies in mice have demonstrated a contribution of these siderophores to pathogenesis [14–20]. *rmpA* (regulator of mucoidy protein A) and *rmpA2* encode transcriptional regulators that seem to play similar roles enhancing production of capsule [7,8•,12,21–23] (see section on Rmp regulators for further discussion of *rmpA/A2*).

Strains of *K. pneumoniae* that are hypermucoviscous (HMV) were associated early on with hypervirulence; mutations that result in loss of this HMV phenotype also result in a reduction in virulence [8•,21,24•,25•,26,27]. The assay most often used to determine the HMV phenotype is a positive string test, where a loop touched to a colony will pull a ‘string’ of >5 mm (Box 1). However, the string test is not quantitative and can be influenced by culture conditions, age of the colony and user technique. The threshold to define a positive string test (5 mm) is quite low and known hvKp strains can yield ‘strings’ of >40 mm. In addition, a recent study aimed at identifying biomarkers that distinguish hvKp from cKp found that the string test was a less accurate predictor of hypervirulence than several genetic markers [28]. A more quantitative test sometimes used to assess the HMV phenotype is the sedimentation assay (Box 1). This assay has the potential to distinguish not only HMV from non-HMV positive strains, but also the potential to define HMV intermediate strains.

All *K. pneumoniae* strains produce an extracellular polysaccharide capsule required for virulence [1•] and more than 130 different capsule types have been identified in *Klebsiella* [11]. The hvKp strains tend to produce more capsule (as defined using a uronic acid [UA] assay [Box 1]) than cKp strains and early studies associated this hypercapsule production with the hv-associated gene *rmpA* (or the closely related gene *rmpA2*) [7,8•,12,21,22]. HvKp with mutations in *rmpA*, or other genes that affect capsule production in hvKp, typically lose the HMV phenotype and show a strong reduction in virulence when tested in mice [8•,23,24•]. Thus, the HMV phenotype became strongly associated with the amount of capsule production despite an early report suggesting HMV does not require excess capsule production [8•]. The recent report of a mutation in a gene encoding a transcriptional regulator (RmpC) that reduces expression of capsule (*cps*) genes and production of capsule, but does not affect the HMV phenotype, provides the clearest indication to date that the

HMV phenotype does not require hyperproduction of capsule [25••]. However, mutations that prevent production of capsule also result in loss of HMV [21,24•,25••], underscoring a link between capsule and HMV.

In mouse models of infection (Box 1), hvKp strains are virulent even at low inoculation doses [24•,29–31] whereas cKp strains are not [32,33]. As mentioned above, hvKp strains typically have not been CR and CR-cKp have not been virulent in an immunocompetent host. However, in recent years CR-hvKp and CR-cKp that have acquired genes associated with hypervirulence (here designated as hv-CR-cKp) have been reported [34–40,41••,42••,43•]. In nearly all instances where tested, these isolates were considered to be HMV by the string test. To date, the majority of CR *K. pneumoniae* with hv-associated genes appear to be hvKp that have acquired a plasmid encoding a carbapenemase. Although virulence testing was not done in most of these reports, multi-locus sequence typing to determine strain sequence type (ST) and/or capsule type (K-type) indicated these strains come from ST or K-types strongly associated with hvKp. In reports of hv-CR-cKp, the hv-associated genes were encoded on pLVPK-like virulence plasmids that are typically found in hvKp. In most of these reports the function of the hv-associated genes or the effect of these genes on the virulence of these strains was not tested. However in cases where this was tested, acquisition of hv-associated genes was not always sufficient to confer virulence comparable to that of hvKp strains [41••,43•]. The strong association of HMV with capsule production and the association of HMV and capsule with virulence make understanding the regulation of *cps* expression and the link between *cps* expression and HMV important for determining what is required for a CR-cKp strain to become hypervirulent. Here we highlight what is currently known about HMV, *cps* expression and unanswered questions concerning HMV and virulence.

Strains used in *Klebsiella* capsule studies

In the field of *K. pneumoniae* capsule regulation, the majority of the research has been conducted using four hypervirulent strains. NTUH-K2044 [44] and CG43 [45] were both isolated from pyogenic liver abscesses. Strain Kp52145 is considered a reference strain for the K2 capsule type [46] and KPPR1S derivative of ATCC 43816 originated from a pneumonia patient [29,47]. Of these four strains, one (NTUH-K2044) is K1 capsule type while the other three are K2 capsule type, and they each are in different ST groups. CG43, Kp52145 and NTUH-K2044 each carry large virulence plasmids that confer hypervirulence, and NTUH-K2044 and KPPR1S carry a chromosomal ICE*Kp* element that is similar to a portion of the virulence plasmid and encodes genes associated with hypervirulence. Common methods used to characterize capsule-associated phenotypes are described in Box 1.

Global regulators

Rcs phosphorelay

The Rcs phosphorelay is a complex signal transduction system found in many Gram-negative bacteria. It is comprised of RcsC (sensor kinase), RcsD (histidine phosphotransferase), RcsB (response regulator), RcsA (auxiliary protein), and RcsF (outer

membrane lipoprotein) [48]. RcsB and RcsC were first identified by their role in colonic acid gene expression in *Escherichia coli* [49]. Soon thereafter, the *Klebsiella* homologs of RcsA and RcsB were found to function similarly when expressed in *E. coli* [50,51], and in *K. pneumoniae* [23,52]. It is now known that RcsB dimerizes with RcsA and binds a DNA sequence called the RcsAB box to activate expression of capsular gene expression in a number of bacterial organisms [48,53]. The direct regulation through binding of RcsAB to DNA has been shown for the upstream-most promoter of the three characterized capsule operons, located upstream of *galF*/ORF1 (Figure 1) [53]. In strains CG43 and KPPR1S, *rscB* mutants have reduced *cps* expression, reduced UA levels, and pellet tightly [21,25••]. Similar trends have been reported on *cps* expression and UA levels in *rsc* mutants of strain NTUH-K2044 [54]. In addition, the KPPR1S *rscB* strain is attenuated in a murine pneumonia model [25••].

cAMP Receptor Protein (CRP)

CRP has been shown to repress *cps* expression in CG43 and NTUH-K2044 [55,56]. In CG43, expression from all three *cps* promoters was increased in the *crp* strain, and CRP was shown to directly bind to the *wzi* and *manC* promoter regions. No CRP binding site was identified upstream of *galF* and binding of CRP to the *galF* promoter was not tested. CRP is known to repress *rscA* expression, so it was speculated that the increased transcription from the *galF* promoter in the *crp* strain was a consequence of increased levels of RcsA. Addition of glucose to the growth media resulted in increased *cps* expression whereas high cAMP levels (through mutation or direct addition to media) resulted in decreased *cps* expression. In NTUH-K2044, the *crp* mutant had increased mucoviscosity and produced more UA than the parental strain. Mice inoculated by intraperitoneal injection with the *crp* strain had an improved survival rate compared to those inoculated with the parental strain, suggesting this mutant is attenuated for virulence. However, the *crp* strain has a growth defect and the *crp* mutation likely affects expression of a large number of genes. As one might predict that increased capsule production would enhance virulence, the attenuation of the *crp* strain may, at least in part, be a consequence of the reduced growth rate and pleiotropic effects on gene expression.

Impact of iron levels on *cps* expression via Fur and IscR

The impact of iron homeostasis on *cps* gene expression has been investigated in strain CG43. Fur is an iron-responsive transcriptional regulator in the *Enterobacteriaceae* and was identified as playing a role in *cps* regulation when it was found to bind a region upstream of the *impA* promoter and repress its expression [21]. The *fur* strain was as resistant to sedimentation as the parental strain and produced more UA whereas a strain overproducing Fur formed tight pellets upon centrifugation and produced levels of UA comparable to the parental strain. A subsequent study showed that transcription of *rscA* increased in the *fur* strain, which might also contribute to elevated *cps* gene expression [57]. This study included an analysis of *cps* regulation and showed elevated levels of expression from all three promoters in the *fur* mutant and demonstrated that the elevated *cps* expression requires RmpA and RcsA. Thus, the impact of Fur on capsule levels is likely an indirect action mediated via RmpA and RcsA.

IscR is another iron-responsive transcriptional regulator found to control *cps* expression. IscR was identified as a repressor of genes that encode iron-sulfur cluster assembly proteins, and it binds a [2Fe-2S] cluster [58]. An *iscR* mutant in the *K. pneumoniae* strain CG43 had decreased UA levels, and slightly decreased *cps* expression from the *galF* and *manC* promoters [59]. An IscR box was identified upstream of *galF* and shown to be bound by IscR; this binding was dependent on the DNA sequence of the IscR box, and on the cysteine residues in IscR believed to coordinate the [2Fe-2S] cluster. These investigators further demonstrated that the *iscR* mutant was more susceptible to killing by normal human serum, a phenotype associated with reduced capsule.

IscR is active when bound by Fe, and therefore activates *cps* expression when iron levels are sufficient. In contrast, Fur exerts its repressive activity upon *cps* expression (indirectly) when iron levels are sufficient and relieves this repression when iron levels are low. Thus, it seems that *Klebsiella* has acquired mechanisms to ensure capsule production remains at least at a basal level independent of the availability of iron, while still allowing for alterations in capsule production in response to extreme iron concentrations.

The Rmp regulators

RmpA and RmpA2

RmpA was identified as a regulator of the mucoidy phenotype in 1989 [8•]. It has a LuxR-type DNA-binding domain, but the N-terminal portion is unique to *Klebsiella*. The *rmpA* gene is located either on the chromosome or on a large virulence plasmid and is predominantly found only in strains that possess an HMV phenotype. The correlation between *rmpA* and HMV/hv is very high, and the presence of *rmpA* is among a set of genes proposed as biomarkers to identify potential hvKp strains [28,60]. RmpA has been shown to activate *cps* gene expression in numerous strains [7,21,25••], and to activate its own expression [25••], but no evidence of direct regulation has been published. RmpA was shown to interact with RcsB, and it is postulated that RmpA can substitute for RcsA, leading to activation of capsular genes in the absence of RcsA [21].

RmpA2 was initially found to regulate *cps* expression in the *K. pneumoniae* strain Chedid, a K2 capsule type strain [23]. The *rmpA2* gene was given this name after sequencing revealed it to encode a longer protein than the originally cloned *rmpA* from Kp52145 [23]. Alignment of the coding sequences for RmpA and RmpA2 from several strains reveals that they share about 80% identity. Some strains, such as NTUH-K2044 and Kp52145, contain a truncated *rmpA2* gene but it is unknown if these encode functional proteins. In CG43, the *rmpA2* strain is non-HMV but does not show much change in *cps* expression [21,22]. However, when *rmpA2* is overexpressed, *cps* expression increased, so it may only impact transcription under certain conditions. To our knowledge, CG43 is the only strain known to have functional RmpA and RmpA2 proteins.

RmpC

In examining the role of RmpA in *cps* regulation in KPPR1S, it was noted that there was a neighboring gene predicted to encode another protein with a LuxR-type DNA-binding

domain. Deletion of this gene, *rmpC*, resulted in reduced expression from *cps* promoters and reduced production of capsule similar to that observed for the *rmpA* mutant. However, unlike the *rmpA* mutant, this strain remains HMV [25••]. This is the first report of a mutant with reduced capsule that is still HMV. It was also determined to be co-transcribed with *rmpA*. The *rmpC* strain is attenuated in the pneumonia model, but the phenotype is not as severe as mutants such as *rmpA* and *kvrA* that are non-HMV but have similar decreases in *cps* expression as the *rmpC* mutant [25••]. Thus, it appears that HMV may be a more important virulence attribute than production of high levels of capsule.

MarR-family regulators

KvrA

KvrA is the *K. pneumoniae* homolog of SlyA, a regulator of virulence in *Salmonella*. KvrA, along with KvrB, was identified as contributing to virulence through a screen that specifically targeted genes predicted to encode MarR-family regulators [24•]. The *kvrA* mutant is essentially avirulent in the mouse pneumonia model, and was found to be non-HMV and to have reduced *cps* expression from the *manC* and *galF* promoters. Subsequently, KvrA was also shown to regulate expression from the *rmpA* promoter [25••], but *cps* gene expression in the *kvrA* strain could not be restored by expression of *rmpA* on a plasmid. This suggests that KvrA may impact expression from the *galF* and *manC* promoters independently of its effect on *rmpA*. KvrA is found in both cKp and hvKp strains, but it did not appear to control *cps* expression in the classical strain KPNIH1, an ST258 isolate [24•]. However, a KPNIH1 *kvrA* mutant does have a colonization defect, suggesting there are other genes regulated by KvrA that potentially contribute to pathogenesis.

It is not yet known if KvrA directly binds to the *rmpA* or *cps* promoters. The function of this class of MarR-like regulators that includes SlyA and RovA involves derepressing H-NS-mediated gene silencing (reviewed in [61]). There is one report on the role of H-NS in a clinical isolate (K39, non-HMV) where *cps* expression, UA levels and mucoviscosity were elevated in an *hns* mutant [62]. It is therefore tempting to speculate that the role of KvrA in *cps* transcription is as a derepressor of H-NS-mediated silencing.

KvrB

KvrB is the *K. pneumoniae* homolog of EmrR. In *E. coli*, EmrR represses the EmrAB drug efflux pump [63]. The genes encoding EmrAB homologs are encoded downstream of KvrB, and the genetic context is the same as in *E. coli*, suggesting this locus functions similarly in *K. pneumoniae*. In addition, the EmrR homolog in an uropathogenic strain of *E. coli*, called MprA, is required for capsule expression [64]. A *kvrB* mutant was attenuated in the mouse pneumonia model, but the colonization levels in the lungs and spleens were much higher than for other mutants such as *kvrA* and *rcsB* [24•,25••]. This *kvrB* mutant is non-HMV and has reduced *cps* expression, both of which likely contribute to its virulence defect. In addition, like KvrA, KvrB was found to regulate the *rmpA* promoter. But unlike KvrA, the effect of KvrB on *cps* expression is likely to be solely through its action at the *rmpA* promoter as *cps* expression was restored in the *kvrB* strain carrying *rmpA* on a plasmid [25••].

Other regulators

KvgAS and KvhAS two-component systems

Both cKp and hvKp strains encode homologs of the Evg/BvgAS two-component system, designated KvhAS [65]. The hvKp strains have a second pair of Evg/BvgAS homologs, designated KvgAS, and a neighboring gene encoding another response regulator named KvhR. In strain CG43, KvhR appears to play a role in both *cps* expression and HMV, and KvgA appears only to impact HMV. Loss of *kvhA* did not appear to affect either HMV or *cps* expression, but overexpressing *kvhA* did result in a reduction of both phenotypes. The exact nature of how these systems are involved in capsule and HMV has yet to be explored. The fact that KvhR and KvgA both impact HMV and are only found in hvKp suggests further investigation is warranted to understand their potential contribution to hypervirulence.

TraDIS screen

In a recent study, Dorman *et al.* utilized sedimentation properties to screen a transposon library for genes that contribute to altered capsule phenotypes [66•]. They found 19 transcriptional regulators, 13 of which were proposed to increase capsule production. Among the 19 were RmpA and RcsB, and they also identified H-NS, KvrA and KvrB as well as a number of other regulators with no previously known role in capsule synthesis. With all but RcsB, it remains to be determined which of these regulators acts directly at the capsule promoters.

Concluding remarks

So many regulators of capsule ...

Summation of the literature on transcriptional regulation of *K. pneumoniae* capsule genes reveals there are numerous regulators that likely function to ensure *cps* expression is activated when needed (Figure 2). While most of these regulators have not yet been demonstrated to act directly at *cps* promoters, the abundance of regulators that contribute to *cps* regulation is an indicator of how critical capsule is to *K. pneumoniae* survival. Complicating analyses of capsule regulation is that it has been difficult to extrapolate results from one strain to another due to the variability in methods used and differences in regulatory gene content between strains (see Box 2). In addition, the role(s) of these regulators need to be evaluated in the context of other regulatory mutants to ascertain a number of critical factors, such as direct versus indirect regulation, whether they participate in a regulatory cascade, and whether there is cooperative regulation.

But what really causes HMV and what is the link between HMV and hypervirulence?

The data generated on HMV have been from hvKp strains, but the primary focus has been on expression of capsule with less emphasis on the role of the transcriptional regulators in conferring HMV. This is in large part due to the assumption that the HMV phenotype is a consequence of capsule overproduction. However, we recently reported on a regulator that impacts *cps* expression but not HMV, indicating overproduction of capsule is not a requirement for HMV [25••]. Mutants that have a complete loss of capsule appear to be

incapable of attaining HMV as a *manC* mutant overproducing RmpA remains non-HMV (personal observations). Thus, the HMV phenotype is likely to be due to a combination of capsule and something else. Although HMV is dependent on the presence of capsule, HMV and capsule need to be considered independently in addition to investigating the link that connects them.

Finally, more emphasis needs to be placed on quantifying HMV and assessing virulence of hv-CR-cKp clinical isolates (see Box 2). As more hv-CR-cKp strains are isolated, characterized and sequenced, the genetic data can be used to identify genes that correlate with HMV and hypervirulence. Genomic and phenotypic analyses will facilitate an understanding of what causes a strain to become HMV and hypervirulent. This information also will be essential in determining if the capacity to acquire HMV and/or hypervirulence is capsule type-dependent, and will be important for assessing the risks associated with cKp strains that acquire genes associated with HMV and hypervirulence in hvKp strains.

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Box 1**Assays for capsule production and capsule-dependent virulence phenotypes****Uronic acid (UA) content.**

UA is a component of many capsules often in the form of glucuronic acid or galactouronic acid. It is most frequently determined by a colorometric assay.

Capsule (cps) gene expression.

Frequently monitored using *lacZ* or *gfp* transcriptional reporters to the three defined promoters in the *cps* locus (Figure 1). qRT-PCR is also used and is gaining popularity.

Mucoidy. A quick test for mucoidy is the *string test*, where a loop or stick is used to touch a colony on a plate and, as it is lifted away, a string connects the stick and the colony. To be considered hypermucoviscous (HMV), the string must reach at least 5 mm, however these strings can stretch for several cm! Another test is the *sedimentation assay*. HMV strains do not pellet well upon centrifugation. This is a visually satisfying assay as the resulting supernatant remains turbid, whereas the non-mucoid strains form tight pellets with cleared supernatants. This can be a somewhat quantifiable assay if the OD₆₀₀ of the supernatant is measured and compared to the OD₆₀₀ of the starting culture.

Serum resistance.

Capsule confers protection against complement-mediated killing. Viable bacteria are determined following exposure and compared to the input to assess the ability of a strain to resist killing. This assay is a convenient way to assess capsule-dependent virulence attributes *in vitro*.

Tissue culture models.

Capsule blocks adherence to cells in culture and prevents phagocytosis. Tissue culture assays are used to measure adherent and intracellular bacteria and can be performed with transformed cell lines or primary cells. Like the serum resistance assay, these assays are a relatively fast and inexpensive ways to assess capsule-dependent virulence properties *in vitro*.

Animal models.

There are several mouse models of *Klebsiella* infection used to study capsule mutants, including sepsis (intraperitoneal injection), pneumonia (intranasal or intratracheal inoculation), and gastrointestinal colonization (oral gavage). These models have been widely applied to assess mouse survival (single dose over several days), LD₅₀ (multiple doses over several days), or bacterial burden (CFU/organ).

Box 2**A proposal to the *Klebsiella* community**

The methodology used to define capsule production, HMV and hypervirulence has varied significantly from one study to another, making direct comparisons between strains a challenge. In studies examining *cps* transcriptional regulation, some forms of capsule regulation (reporter or qRT-PCR) and quantification of UA levels are fairly constant between studies, but growth medium and conditions vary. In reports of newly isolated hv-CR-cKp strains, HMV is primarily assessed by the string test, and capsule levels and virulence are rarely examined. Assessments of HMV have ranged between qualitative (string test or images of centrifuged cultures) to quantitative (sedimentation assay). Virulence studies in mice have also been variable (see Box 1). While these types of variations are not unusual, as labs often favor a particular set of assays, this does make it difficult to extrapolate analyses between strains. This is further complicated by the recently recognized degree of genomic variation between strains of *K. pneumoniae*. As more strains are isolated that have both CR and hv-associated genes, there is a greater need for understanding the mechanisms that confer hypervirulence. Development and application of a consistent set of assays will expedite advancements and make it easier to assess the potential risks associated with these new isolates. Thus, we propose, at a minimum, the following assays for evaluating capsule production, hypermucoviscosity and hypervirulence:

- Capsule should be quantified using the UA assay
- HMV should be quantified using the sedimentation assay
- Virulence should be measured in mice using a mouse model consistent with the origin of the strain (i.e., sepsis model for a blood isolate, pneumonia model for lung isolate, etc.)

In assessing potentially hypervirulent cKp isolates, some consideration should be given to determining the scale of virulence (i.e., is the strain more virulent than typical classical isolates? Has it become hypervirulent or does it have an intermediate virulence phenotype?).

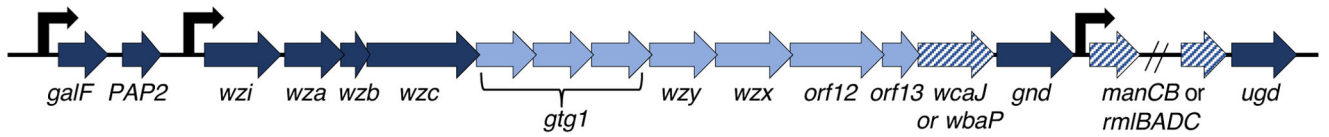


Figure 1.

Schematic of the capsule locus in KPPR1S. The three characterized promoters are indicated upstream of *galF* (also referred to as *orf 1-2*), *wzi* (*orf 3-15*) and *manC* (*orf 16-17*). Genes in dark blue are highly conserved among different capsule types; those in light blue are capsule type-specific, and those in dark blue hatches can vary depending on the sugar precursors that comprise the capsule. Orfs 12 and 13 appear to be unique to *Klebsiella*.

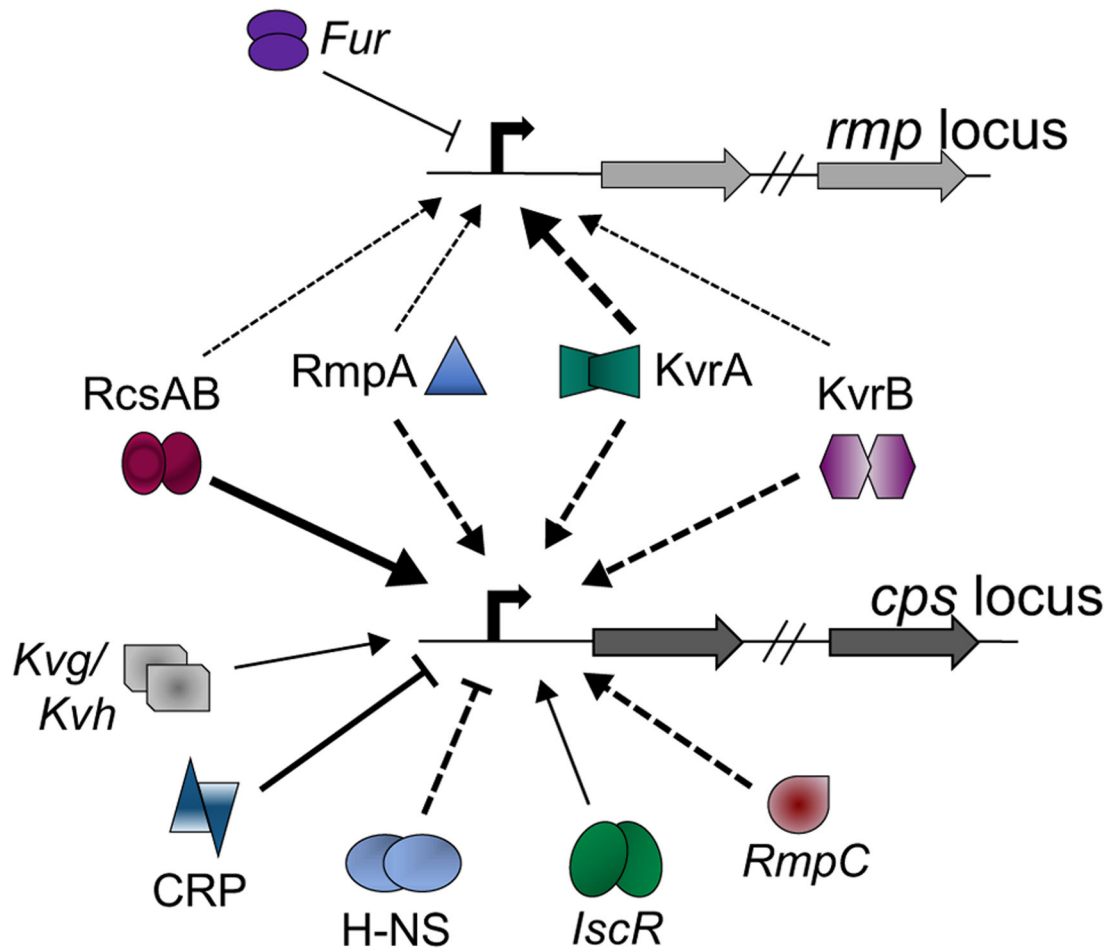


Figure 2. Complex matrix of capsule regulation. Each shape represents a different transcriptional regulator known to contribute in some way to the expression of capsule biosynthetic genes. If known or logically presumed, dimerization is indicated by doublets. Arrow thickness indicates the relative impact of the regulator: thin, small fold-change in mutant strain; thicker, larger fold-change in mutant strain. Arrows indicate activators, and T-shaped lines indicate repressors. Solid lines indicate that direct binding has been demonstrated for at least one of the *cps* promoters, and dashed lines indicate direct binding has not been tested. Italicized proteins have been characterized in only a single *K. pneumoniae* strain whereas the others have been characterized in at least 2 strains.