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Carbon metabolism and synthesis of the polysaccharide capsule of *Streptococcus pneumoniae* and related pathogenic streptococci.

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Contents

Abstract

Streptococcus pneumoniae is a gram-positive humane pathogen which can cause respiratory and invasive diseases with a high morbidity and mortality. The polysaccharide synthesis and the carbon metabolism of *S. pneumonaie* are reviewed here.
The production of the capsule is mediated by the so-called Wzy-dependent pathway. Only in serotypes 3 and 37, the CPS synthesis is different.
Within the capsular polysaccharide biosynthesis locus there is a high diversity in 91 different serotypes. Only the first four genes, *cpsA-D*, are common to almost all of the serotypes, but following these are the serotype specific genes. The capsule synthesis seems to be transcriptional regulated upon adherence by down regulation of first gene, *cpsA.* In the nasopharynx and lungs, were glucose is low, capsule expression may well be regulated at a post-transcriptional level, which involves the auto-phosphorylation of CpsD.
For its survival, sugars are essential. By means of four exoglycosidases NanA, NanB, BgaA and StrH, it is capable of enzymatic degradation of host glycoproteins. A regulatory link between the carbon metabolism and capsule synthesis, maybe CcpA. This protein is known to be a regulator in the carbon control repression (CCR) and enables the bacteria to utilize the preferred sugars. This review is the first to suggest the regulator role of *cpsA* during adherence and thereby playing a role in the diminished capsule expression. The links between the capsule and the carbon metabolism are still unclear, but even though CcpA plays a role it is not the sole regulator.

Introduction

Streptococcus pneumoniae is a gram-positive humane pathogen which can cause respiratory and invasive diseases with a high morbidity and mortality.[1] Especially children, elderly, people with a compromised immune system and people with a chronic disease are vulnerable for these diseases of which pneumonia, sepsis and meningitis are the most common.[2,3] Worldwide it is a leading cause for morbidity and mortality among children, especially in developing countries.[4]

S. pneumoniae colonizes in the naso-oropharynx. Colonization in this place is asymptomatic and is used for the bacteria as a reservoir. It will be symptomatic as soon as it reaches the sterile part of the airway system. Here it can bind epithelial cells. This binding facilitates the infiltration of the epithelial cells.[5]

Like some other bacteria, *S. pneumoniae* is able to produce capsular polysaccharides. Between 200 and 400 nm thick[6], it's build from chains of sugar molecules. The capsule is one of the most important virulence factors of the bacterium, because it protects against phagocytosis from cells of the human immune system and increase the virulence of the bacteria.[2,7] In order to control this virulence factor, *S. pneumoniae* is capable of changing the thickness of its capsule (resulting in three different phases), although it is not known how.

The synthesis of the capsule is controlled in most serotypes by the Wzy-dependent pathway. Two types of proteins, a flippase (*wzx*) and a polymerase (*wzy*), play an important role in this pathway.[5] Also needed for the capsule production are the genes *cpsA, cpsB, cpsC* and *cpsD* and their respective protein products. These genes are highly conserved in all serotypes and are thought to be essential for the production. All these genes are located in the capsular polysaccharide (CPS) biosynthesis locus.[8]

The so important capsule needs carbon sources for its production. The carbon metabolism of *S. pneumoniae* plays therefore a significant role in the proliferation and invasiveness of the pathogen. And not only as a deliverer of carbon for the capsule, but also for nutrient acquisition and colonization. An important link between the carbon metabolism and the capsule production is thought to be the catobolite control protein A (CcpA), a transcriptional regulator of the capsule synthesis. [9,10]

Over the years there have been many studies regarding the capsule and the different aspects of it. However, there is still indistinctness. Mostly about the regulation of the phase variation and the role the carbon metabolism plays in the capsule production. In this review, I will give an overview of the knowledge available today on the capsule synthesis and the carbon metabolism of *Streptococcus pneumoniae* and where needed also of other pathogenic streptococci. It will give a broad view on the information know at this moment of these subjects, highlighting key studies done in the last years.

Capsule of the *Streptococcus pneumoniae*

Streptococcus pneumoniae is capable of producing a polysaccharide capsule, surrounding the cell wall of the cell which is between 200 and 400 nm thick.[6] The thickness of the capsule can differ. The thickness is negatively correlated to the virulence of *S. pneumoniae*.[7] The genes transcribing for the capsule are located in the capsular polysaccharide biosynthesis locus.

Capsular biosynthetic locus

The capsular polysaccharide (CPS) biosynthesis locus is located between *dexB* and *aliA* (these two genes are not needed for capsule production) (Figure 1). This locus is on average 20,714 bp.[11] Four genes are conserved in nearly all known 91 serotypes of *Streptococcus pneumoniae*.[12,13] They are found in all but two serotypes at the 5' end of the CPS locus.[14,15] The *cpsA, cpsB, cpsC* and *cpsD* genes (Figure 1). The fact that these genes are so highly conserved in all of the other serotypes, suggest an important function or functions for these genes in the regulation of the biosynthesis of the capsule. Because of the importance of these genes, during the capsule synthesis, they will be discussed separately in this chapter.

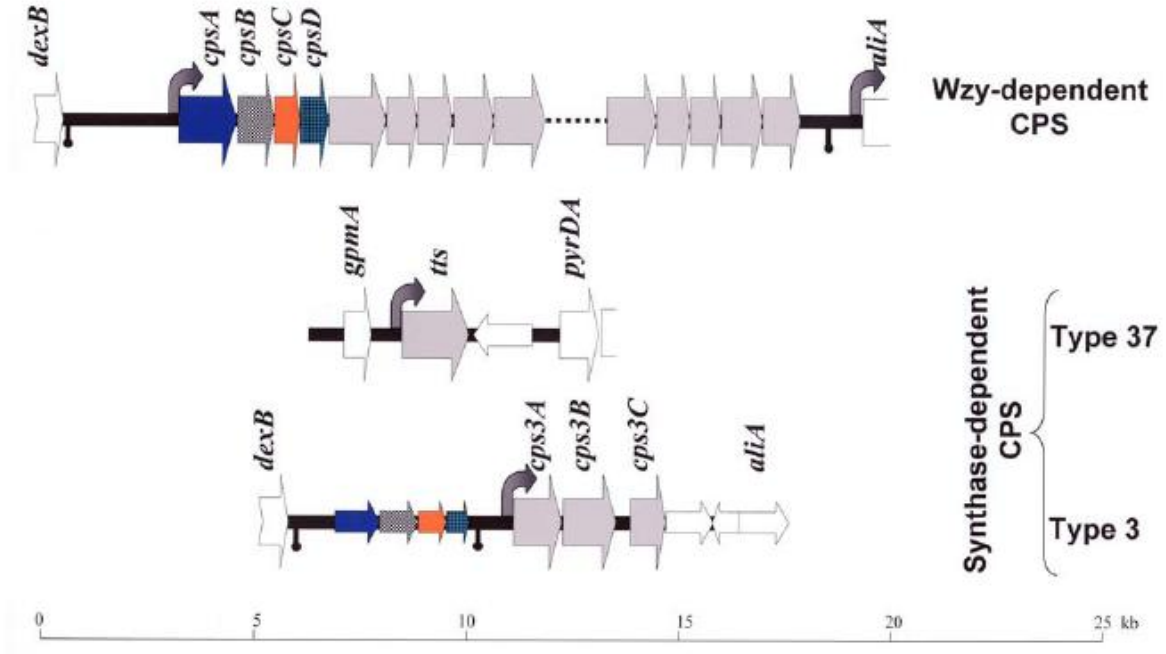

Figure 1: CPS loci of *Streptococcus pneumoniae*, showing the difference in loci between the Wzy-dependent, which has the *cpsA-D* genes and is the most common synthesis pathway for the capsule and Synthase-dependent pathway, which produces their capsule differently. *cpsA-D* are coloured and the serotype specific genes are in grey (modified from Moscosa et al., 2009).

Following the *cpsA-cpsD* genes are the genes that are serotype specific. Most genes can be subdivided in large categories, based on homology. 351 coding sequences are expected to be involved in glucosyl transferase, 74 in acetyl transferase and 71 in sugar phosphate transferase.[11] Also common to a large number of serotypes (23) are four ORFs involved in the synthesis of dTDP-Rha, one of the precursors for the synthesis of capsules containing rhamnose.[15,16] There are a number of these genes that show homology to other genes of different serotypes. The fifth gene for example, *cpsE,* is common to most serotypes and encodes the initial glucose phosphate transferase.[17] Also

present downstream in all serotypes, are the polysaccharide polymerase (*wzy*) gene and flippase (*wzx*) gene.

Wzy-dependent pathway

As mentioned before, 88 of the serotypes produce their capsule through the Wzy-dependent pathway. This includes the products of the polymerase (*wzy*) and flippase (*wzx*) genes. Even though there is a big difference between the repeat unit polymerases, the protein products do have a best match in a BLAST search.[11,16]

Capsule synthesis starts with the transfer of a glucose phosphate onto a lipid acceptor in the cell membrane (Figure 2). This is catalyzed by CpsE or homologs in most serotypes.[18,19] Additional glycosyltransferases (GT's) catalyze transfer of the remaining sugars to the nonreducing end of the sub-unit.

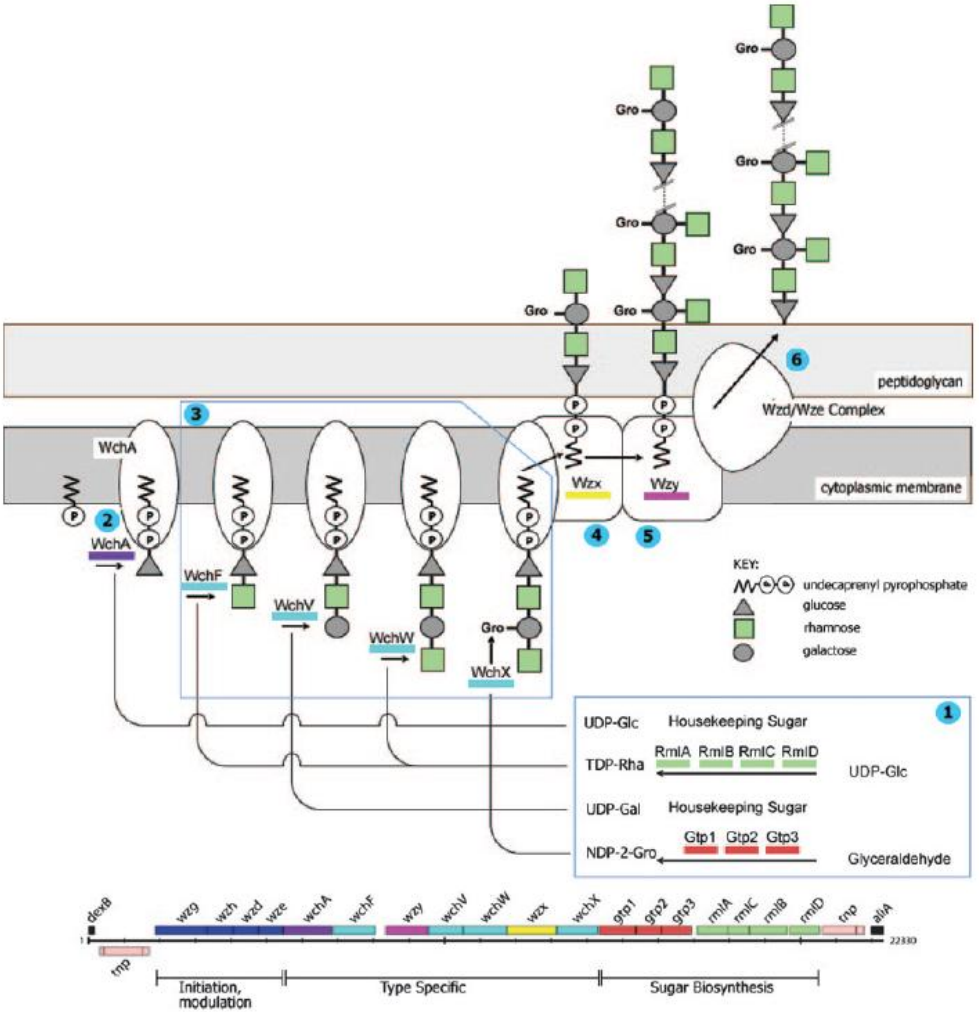

Figure 2: Schematic representation of the biosynthesis of CPS by the Wzy-dependent pathway. The biosynthesis of the CPS of serotype 23F is represented. UDP-linked components of the repeat CPS unit are synthesized by genes encoded within the cps locus or are available from central metabolism (1). Repeat unit biosynthesis is initiated by the transfer of glucose phosphate to the lipid carrier by the IT WchA (CpsE) (2), followed by sequential addition of the other components of the repeat unit, catalyzed by the GTs (3), and the lipid-linked repeat unit is transferred across the membrane by the Wzx flippase (4) and polymerized by Wzy to result in lipid-linked CPS (5). Finally, the lipid-linked CPS is linked to the cell wall by a poorly understood process involving the Wzd/Wze (CpsC/CpsD) complex, with release of the undecaprenyl phosphate carrier (6). The genes in the representation of the cps locus and their gene products are color coded; a key is given for the repeat unit constituents (Aanensen et al., 2007).

The sugars in the polysaccharide chains used for the capsule are often serotype specific and show a high divergence. One example is capsules containing rhamnose, which is, as mentioned before, specific for 23 of the 91 serotypes. This oligosaccharide is transferred across the membrane using the Wzx flippases, which have 12 membrane-spanning domains. The unit is now on the outer face of the membrane and can be polymerized by Wzy. This involves the transfer of the reducing end of chain onto the nonreducing end of a new repeat unit. The final step is the attachment of the chains to the cell wall, involving the CpsC-CpsD-ATP complex (as mentioned above) (Figure 2).[16,20]

Only in serotypes 3 and 37, the synthesis is different. These types both produce their capsule via the synthase-dependent pathway. Type 3 has the *cpsA-D* genes, but these are mutated is such a way that they are no longer transcribed. In serotype 37 the genes are no longer present (Figure 1). Here is CPS production started by the *tts* gene, located outside the CPS locus.[21,22]

Chain length regulation

The regulation of the chain length of the capsule is regulated by different mechanisms. The majority of the serotypes has, as mentioned, the Wzy-dependent pathway for the biosynthesis of the capsule. In these serotypes regulation of the chain length is partly affected by the actions of the tyrosine phosphoregulatory system. In serotype 2, Cps2C and Cps2D play a role in this chain length regulation[32]; this is in concurrence with the CpsC and CpsD homologs found in *S. agalactiae*. Here resulted a deletion in about one-half of the chain lengths of that produced of the wild type (and a > 90% reduction in total amount of cell-associated polysaccharide).[26]

In the synthase-dependent pathway of serotypes 3 and 37, chain regulation is thought to be regulated by cellular uridine diphosphate-glucuronic acid (UDP-GlcUA) concentrations in serotype 3.[23,24] A reduction in UDP-GlcUA results in a increased frequency of chain termination and increase in the number of chains that remain cell-associated (phosphatidylglycerol-linked), probably because a minimum of chain length should be achieved.[24]

The mechanisms involved in the chain length regulation however are poorly understood. Physiological consequences for the bacterium are not reported, but it is suggested that the regulation of the chain length is one of the ways for *S. pneumoniae* to control the amount of capsule.[24]

cpsA

During the process of the capsule synthesis, there is still a lot unknown, but it is clear that the first four genes of the CPS locus play an important role during this production, in all serotypes. It is connected to the wzy-dependent pathway and the chain length regulation.

The *cpsA* gene (or *wzg* gene or *cap1* gene) is the first gene in the CPS locus (Figure) and the most conserved of all the four genes. There have been many studies, looking at *cpsA* of *S. pneumoniae* and its expression at different levels or the effects of its protein. Nevertheless there is still much unclear. It is suggested by different groups that *cpsA* could play a role in the regulation of the capsule expression. One of the reasons being, that it is so highly conserved.[8,25]Although Morona et al did found that while CpsB, C and D are essential for encapsulation, CpsA is not.[8]In *Streptococcus agalactiae* it is found that CpsA is a transcriptional regulator of the CPS locus[26]

Over the years, different studies were preformed to examine the expression of *cpsA*. Interestingly the results found were different in all studies.

In 2002, Ogunniyi et al demonstrated that *cpsA* expression levels were higher in mice blood (after 24 hours) in comparison with *S. pneumoniae* grown in serum broth.[27] This is probably a result of different factors present in the blood, which could trigger *cpsA* expression. A study in 2006 looked at some virulence factors and there expression *in vivo* in blood, lungs and nasopharynx. One of the factors looked at was *cps2A*. They found no differences in expression of *cps2A*, suggesting that regulation of the capsule takes place at post-transcriptional level.[28] They suggest a role for *cpsD* in this regulation and more studies have suggested that.[8] Hathaway et al, found an increase in *cpsA* expression, *in vitro* conditions, in serotypes associated with invasiveness in comparison with serotypes associated with colonization. They also show that there is an ambient oxygen level needed for this difference in expression.[29]
One of the last studies done shows a decrease in *cpsA* expression levels, during adherent growth. After which they suggest a link between *cpsA* levels and capsule expression.[30] But this is something which is still debated, mainly because of the different results of different studies. There might be more systems in which *cpsA* is involved. Hathaway et al, suggest an involvement in metabolism, based on results showing an increase in lag phase at higher expression of capsule genes expression.[29,31]

cpsB, *cpsC, cpsD* and interactions of CpsB, CpsC and CpsD

The *cpsB* gene (or *wzh* gene or *cap2* gene) is, as shown in Figure 1, located at the second position. It transcribes for the CpsB protein. As mentioned before, the gene is essential for the encapsulation of *S. pneumoniae*.[8] Over the years it has been shown by different studies that CpsB plays an important role in the phosphotyrosine regulatory system, which will be discussed in more detail later on.[8,32] And it is suggested that CpsB has an even bigger role than just phosphorylation, because a CpsBΔ mutant had a bigger, negative effect on the capsule production than the CpsDΔ mutant in Group B streptococcus.[26]

Morona et al were the first to suggest that phosphorylation of tyrosine in CpsD negatively regulates the CPS synthesis. They proposed a role for CpsB in this phosphorylation, either by direct dephosphorylation of CpsD or by means of an indirect role in phosphatase activity. This conclusion was based on results showing an inactive tyrosine-phosphorylated form of CpsD in a CpsBΔ mutant.[8] This was confirmed *in vitro* by Bender et al, who showed that Cps2B is a phosphatase enzyme on Cps2D and an inhibitor of phosphorylation of Cps2D.With purified proteins which were brought to expression in *E. coli*, phosphatase activity for Cps2B was shown, using Cps2D-P as a substrate. By means of co-expression in *E. coli* and in a second experiment using ELISA, the inhibition of Cps2D phosphorylation by Cps2B was shown.[32] Finally, in 2002 it was confirmed that CpsB is a phosphotyrosine-protein phosphatase, by measuring free phosphate levels in different *S. pneumoniae* strains (normal, ΔB, ΔD). By means of a Conserved Domain search in the NCBI database they found CpsB to be a member of the PHP-family of phosphoesterases. They also concluded that the presence of manganese is required for optimal activity, after testing the phosphatase activity under the presence of different metal ions.[33] The importance of CpsB is shown by the fact that a loss of the protein leads to the inability to attach the CPS to the cell wall via the CpsD phosphorylation and that this leads to the inability to cause invasive disease in serotype 2.[34]

The last two genes are *cpsC* and *cpsD* (or *wzd* or *cap3* and *wze* or *cap4* respectively). The genes transcribe the proteins CpsC and CpsD. Both of these genes are essential for the encapsulation, just as *cpsB* is. The proteins also play an important role in the phosphotyrosine regulatory system. CpsD is an autophosphorylating protein-tyrosine kinase and CpsC is required for tyrosine phosphorylation of CpsD.[8]

Morona et al hypothesize that CpsC and CpsD need to interact in such a way that ATP can bind to the CpsC-CpsD complex forming a new CpsC-CpsD-ATP complex (Figure 3). This complex subsequently should function in the regulation of CPS production, enabling the production of CPS to go up. Autophosphorylation of CpsD results in an interaction change between CpsD-P and CpsC, which results in a slower production of CPS. CpsB can, as mentioned before, dephosphorylate CpsD-P and so reactivate CpsD to form again the complex with CpsC and ATP.[8]
It seems that an increase in CpsD-P leads to an increase of CPS that is attached to cell wall, which is a necessity for survival of the bacterium in the blood of mice. Loss of cell wall associated CPS results in lower invasiveness in mice, however not all the CPS is attached to the cell well in normal conditions. Significant loss of CPS that is not attached, results in an even lower invasiveness. Because the total amount of produced CPS is down regulated if CpsD is phosphorylated, the survival rate of *S. pneumoniae* in mice is low.[34,35] This is even clearer, with a CpsC mutant (CpsC knock-out) which leads to decreased attachment to the cell wall, and thereby completely preventing entering of the blood stream, in a mice disease model.[34]
Bender and Yother suggest a slightly different complex in their research. They hypothesize that CpsB also takes part of the complex. This could explain the diminished encapsulation of CpsB mutants as they point out. Different environmental signals could dissociate this complex and thereby exert an influence on the CPS production. CpsA and/or CpsC, which are both membrane bound, could act as sensors for these signals.[32] Weiser et al suggest oxygen as one of those environmental signals. They found a lower CPS production during ambient oxygen levels and higher CPS production at diminished oxygen levels.[36]

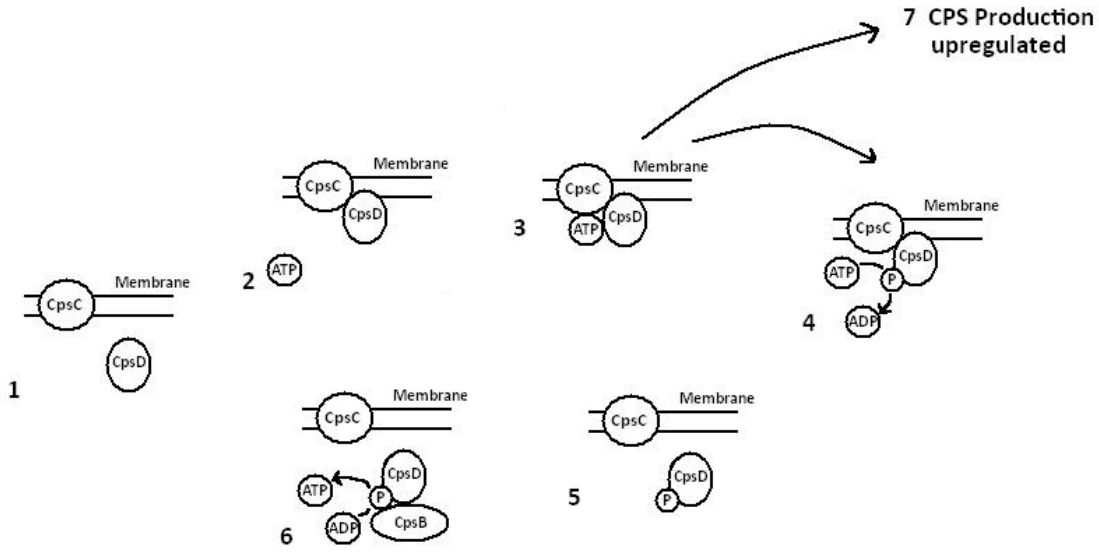

Figure 3: The different (schematic) steps involving CpsB, CpsC and CpsD. CpsD can bind to membrane-bound CpsC (2). ATP can bind this complex (3). Either this complex upregulates CPS production (7) or CpsD autophosphorylates using the bound ATP (4), consequently leading to disassociation of CpsC and CpsD-P (5). CpsB can dephosphorylate CpsD-P (6) resulting again in the CpsC-CpsD complex (2), on which ATP can bind again (3).

At the C-terminus of CpsD is a structural $[YGX]_4$ motif located. Due to the high conservation among other species, this is a likely target sequence for autophosphorylation of the tyrosine residues. Mutants with a altered $[YGX]_4$ motif (into $[FGX]_4$), show no change in CPS production, although the morphology is changed somewhat.[8] A mutant in which the $[YGX]_4$ domain was removed, showed no

differences with a mutant without the *cpsD* gene, demonstrating the necessity of the repeat domain for the function of the protein. Other mutants having a single tyrosine residue in the $[YGX]_4$ domain (at different positions) and lacking CpsB, exhibited less capsule, resulting in a rough colony morphology, indicating that in the absence of an active protein-tyrosine phosphatase, phosphorylation of just one of the tyrosine residues in the $[YGX]_4$ domain was sufficient to inactivate CpsD.[8,33]

Carbon metabolism of *Streptococcus pneumoniae*

Nutrient acquisition

Sugars are essential for *Streptococcus pneumoniae* to survive. Of all the transporters on the membrane, it is predicted that over 30% of them are sugar transporters, more than any other related streptococcus.[37] This means that it is probably capable of utilizing a large number of different sugars. But the glucose concentration in the upper airway of the human is low in comparison with the blood or the lungs, even though this is the place that *S. pneumoniae* likes to colonize.[38] In order to survive in this region, it needs to be capable of exploiting different carbon sources. Two of those are O- and N-linked glycans (mostly monosaccharides attached to a protein via the O- or N-site of an amino acid), which are available on the surface of epithelial cells, host defense cells and other bacteria. By means of the four exoglycosidases neuraminidase A (nanA), neuraminidase B (nanB), beta-galactosidase (bgaA) and beta-N-acetylhexosaminidase (strH), it is capable of enzymatic degradation host glycoproteins, where it can break down the glycosidic bonds at the terminal residue, in order to use these as a carbon source for their metabolism. The sequential deglycosylation of host glycoconjugates is needed for efficient growth and to sustain this growth.[39] Additional advantage is the change in clearance function of the host defense molecules if their molecules are deglycosylated by *S. pneumoniae.* It is thought that this will help in such a way that the bacterium can survive in the host.[40]

Two different systems are also discovered to play a role in nutrient acquisition and at the same time are thought to be necessary in the virulence of the bacteria. *Sus* and *scr* are both sucrose transport and metabolic systems. A hydrolase needed in both systems are transcribed from the respective genes *susH* and *scrH*. A loss of these genes results in a disability to utilize sucrose and similar disaccharides. The systems are sucrose inducible and are regulated by the SusR and ScrR regulators. *Sus* plays a role in the fitness in the lung and possible co-operate with CcpA (this protein will be discussed in more detail in the following chapter) in a system which could link carbon metabolism and *in vivo* fitness of *S. pneumoniae* in the lungs. S*cr* plays a role in the colonization of the nasopharynx. Both assumptions are made based upon knock-out strains tested in mouse models. [41,42]

CcpA

Since a couple of years, there have been a number of studies involving the catabolite control protein A (CcpA). This protein was known to be a regulator in the carbon catabolite repression (CCR). This system enables the bacteria to utilize the preferred sugars. The CCR makes it possible to use the preferred sugars first, by silencing the genes specific for the use of nonpreferred sugars.[43,44,45] The main regulator of the CCR in *Bacillus subtilis*, were the CCR is studied extensively, is CcpA.[44]

In *Streptococcus pneumonia*e they identified a homolog of the CcpA from the *Staphylococcus aureus*. Firstly designated as RegM, later on as CcpA as well (for the sake of clarity, I will use the names CcpA and *ccpA* even though it may be named differently in the original study). The protein product is transcribed from the *ccpA* gene, located outside the CPS locus of *S. pneumoniae* (approximately 1,5 million nucleotides away from *cpsA* (NCBI Entrez Gene)). Normally when there is enough glucose for the bacteria to use, CcpA represses genes involved alternative carbon sources. Glucose will enter the glycolytic pathway and generate intermediates, which will trigger an ATP-dependent protein kinase. The phosphocarrier Hpr from the phosphotransferase system (PTS) will be phosphorylated by this kinase. P-Hpr enhances binding of CcpA to different catabolite-responsive elements (*cre*).[46,47] By binding to the *cre* box, CcpA will repress the genes involved with the alternative sugars, by inhibiting the binding of RNA-polymerase and will activate genes whose products are involved in excretion of

excess carbon.[48,49] The CcpA homolog of *S. pneumoniae* is found to be just as involved in the sugar pathways control as the 'original' CcpA.[9] It acts as a repressor of α-glucosidase and β-galactosidase in the presence of glucose, by means of regulation of PTS-encoding operon, together with a second repressor, which is unidentified. [50] However, in the absence of glucose or when glucose levels are diminished, which is the case in the lungs, the bacteria has to use different source of sugars. The low glucose levels will relieve the *pts-bgaA* repression. This will mean a release of CcpA from the *cre* site, which consequently will lead to a diminished repression of BgaA and thus β-galactosidase, so the bacteria will be able to cleave glycoproteins and use these as a carbon source.[50]

A CcpAΔ mutant showed to be insufficient to produce enough capsule to survive in a host and showed a decrease in expression of the CPS locus and was severely compromised in virulence and colonization of the nasopharynx. Thus being the first ever found transcriptional regulator of the capsule expression of *S. pneumoniae,* which is located outside the CPS locus.[10]
Even so, CcpA is not the sole regulator of the CCR. The mutant only affected catabolite repression of one of four sugar-metabolizing enzymes that were tested in a study. The role of CcpA is therefore thought to be much smaller than in other related streptococci and this suggests an other regulator/regulators besides CcpA. These might be homologs of CcpB and CcpC from *Bacillis subtilis*, where these proteins show regulator functions, although this is not yet tested.[9,10,51]

Discussion

The capsular polysaccharide biosynthesis locus shows high diversity in the 91 different serotypes. Only the first four genes, *cpsA-D*, are common to almost all of the serotypes, but following these genes are the serotype specific genes. Even though these are specific for different serotype(s), there is homology between the genes. Worth mentioning are the *wzy* and *wzx* genes, involved in polysaccharide polymerase and flippase respectively, which are involved in the synthesis of the capsule of all but two serotypes.

From the first four highly conserved genes, there is the most indistinctness and disagreement over *cpsA* and its role in the regulation of the capsule expression. In the lung and nasopharynx the capsule is lower than in the blood.[52] However, the *cps2A* mRNA expression did not differ *in vivo* between the lung, nasopharynx and the blood. This leads LeMessurier et al. to conclude a post-transcriptional regulation of the capsule, possibly via the auto-phosphorylating of CpsD.[28] CcpA could also be involved here, even though capsule levels are lower, there is still some needed to survive. The CcpA mutant of Iyer et al. showed compromised virulence and low capsule expression levels.[10] Thus, CcpA can be the regulatory link between the carbon metabolism and polysaccharide capsule production, as Giammarinaro and Paton suggest.[9]
In 2008 Hall-Stoodly et al. did a study of *S. pneumoniae* on biofilm matrix. *CpsA* expression was down regulated compared to the control.[30] It is known that the bacteria phenotypically vary capsule production upon adherence to epithelial cells.[53] Hall-Stoodly hypothesized that the capsule expression should also be down, when growing on a biofilm.[30] The lower expression of *cpsA* thus shows indirect a transcriptional regulation of the capsule upon adherence, something which was also found in our own experiment (Dik and Kol, unpublished study). Overall it can be hypothesized that different mechanism are involved in the regulation of the polysaccharide capsule of *S. pneumoniae*. In the nasopharynx and lungs, were glucose is low, capsule expression may well be regulated at a post-transcriptional level, which involves the auto-phosphorylation of CpsD. But as soon as the bacterium adheres, it is important to lose as much of the capsule in order to infiltrate the host cell. To do so efficiently, it seems that transcriptional regulation is also needed. *CpsA,* and possibly other CPS genes, are down regulated in order to change to the transparent phenotype.

The capsule of *S. pneumoniae* consists of sugar molecules and is the most important virulence factor. So it can be assumed that the carbon metabolism plays an important role in synthesis of the capsule and thus in the survival of the pathogen and the invasiveness. However to this date there are just a number of proteins/systems found, which link the carbon metabolism and the virulence of the bacterium. Most notably are the catabolite control protein A (CcpA) and the *sus* and *scr* systems.[10,41,50] Especially CcpA was thought to be a mayor regulator. Iyer et al., concluded nonetheless that the role of CcpA could not be as big as in other related streptococci.[10] The *sus* and *scr* systems do seem to play an interlinking role between the carbon metabolism and the virulence of *S. pneumoniae*. But the precise role of the systems is not clear. Therefore they make important research subjects, because they are one of the few known links between the carbon metabolism and the virulence of *S. pneumoniae*. Because of the smaller role CcpA seems to have, research could focus on finding the expected other regulators. Possible by doing a new homology search between *Bascillus subtilis* and *S. pneumoniae* and do a knock-out study with the deleted homologs.

Concluding, this review is the first to suggest the regulator role of *cpsA* during adherence and thereby playing a role in the diminished capsule expression. The links between the capsule and the carbon metabolism are still unclear and more research is needed to unravel the mechanism.

Search method

For this review, articles were searched in the PubMed database of the NCBI. Searches were done, using the following keywords (in italic): *capsule/cpsa/cpsb/cpsc/cpsd/cpse/wzy/wzx/polysaccharide* together with either *pneumoniae* or *pneumococcus* (or related streptococci as GAS or GBS); *carbon metabolism/carbohydrate/ccpa/regm/chain length/udp/sugars* together with either *pneumoniae* or *pneumococcus* (or related streptococci as GAS or GBS). More articles were found by using the 'related articles' given by PubMed. Finally articles were found using the reference lists of already found articles.

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