Regulation of capsule biosynthesis in *Streptococcus pneumoniae* (and related pathogenic streptococci) by environmental factors and transcription regulators

Auteur: Arjan Kol (1646753)
Begeleider: Jetta J.E. Bijlsma
Afdeling Medische microbiologie, laboratorium Moleculaire Bacteriologie, Universitair Medisch Centrum Groningen (UMCG), Groningen, Nederland. 13-07-2009

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Summary

Streptococcus pneumoniae (also known as pneumococcus) is a gram positive human pathogen that causes local infections and serious life-threatening diseases. *S. pneumoniae* produces capsular polysaccharides that are crucial for systemic virulence. The capsule also protects the bacteria from opsonophagocytosis after invasion. The biosynthesis of the capsule is mainly regulated by the *cps* locus. Two-component systems (TCS), other transcriptional regulators and environmental factors play roles in the regulation of the capsule expression.

The orphan two-component signal transduction response regulator RitR plays a very important role in the regulation of capsule biosynthesis. RitR downregulates the *ugd* gene which is required for the synthesis of the *S. pneumoniae* type 3 capsule. The environmental factors Mg^{2+} and antimicrobial peptide LL-37 could have an effect on this system. Another two-component system involved in the capsule biosynthesis is SivS/R. A Δ*siv* mutant of *Streptococcus iniae* transcribed two-thirds less *cpsA* (the first gene of the capsule operon of S. iniae). There is also a transcription factor that has an effect on capsule biosynthesis, called RegM. A *regM* mutant showed a significantly decreased expression of the *cps* locus. Besides the environmental factors Mg^{2+} and human antimicrobial peptide LL-37, environmental factors such as sugar source and availability, oxygen, iron and environmental conditions in biofilms also play important roles.

Most of the environmental factors and transcriptional regulators described had a significant and strong effect on the expression of capsule genes and capsule. The underlying mechanisms are very complex and there is still little known about these mechanisms. Further studies are still needed to find out if all of the environmental factors mentioned above have an effect on the capsule biosynthesis in *S. pneumoniae*.

Introduction

Streptococcus pneumoniae (also known as pneumococcus) is a gram positive human pathogen that causes local infections and serious life-threatening diseases, such as meningitis, sepsis, pneumonia and acute otitis media, which involves infection of the middle-ear space. *S. pneumoniae* colonizes mostly the nasopharynx of young healthy children without causing harm and is often asymptomatic, but when they do invade they cause serious diseases (1). Worldwide, pneumococcal septicaemia is a major cause of infant mortality in developing countries, where it causes more than 1.2 million infant deaths per year (2).

S. pneumoniae is able to produce capsular polysaccharides. The capsule protects the bacteria from opsonophagocytosis after invasion (1). Maximal expression of capsular polysaccharides is crucial for systemic virulence. The expression of the capsule also reduces entrapment in the mucus in the nasal cavity (3). However, the capsule hinders colonization, because it reduces bacterial attachment to respiratory epithelial cells. Therefore, the bacteria need to down-regulate capsule expression in order to enhance their adherence to epithelial cells (4). Hammerschmidt et al. (2005) have shown that the bacteria have a thinner capsule layer during the interaction with epithelial cells. The different levels of capsule expression have been associated with different phenotypic appearances (5).

Studies have shown that *Streptococcus pneumoniae* displays phase variation between opaque and transparent (6). Phase variation is the molecular mechanism that leads to the reversible switching of the gene expression state (7). Opaque variants produce more capsular polysaccharide than transparent variants. In order to survive in diverse host environments (blood, nasopharynx, middle-ear space etc.), the bacteria undergo phase variation between these two phenotypes (8).

There are 91 known pneumococcal capsular serotypes, which express structurally and antigenetically different capsular polysaccharides (7). The polysaccharide capsule of 89 of the 91 serotypes of *S. pneumoniae* is produced by a Wzy-dependent pathway and is a major virulence factor of *S. pneumoniae*. The biosynthesis of the capsule is regulated by the *cps* locus, but there are also genes outside of this region that regulate capsule biosynthesis (7, 9). The capsule operon appears to be arranged as a single transcriptional unit. The unit is located between the genes *dexB* and *aliA*. These two genes are not involved in the capsule synthesis in *S. pneumoniae* (10).

There is evidence that the regulation of capsule expression takes place on transcriptional level. Ogunniyi et al. (2002) have shown by RT-PCR that during invasive disease, the first gene of the capsular polysaccharide biosynthesis locus (*cps2A*) in *Streptococcus pneumoniae* serotype 2 D39 was upregulated approximately fourfold at 12 and 24 h after intraperitoneal infection compared to expression in vitro (11). Mahdi et al. (2007) have shown by RT-PCR that the gene *cpsA* is expressed differently in different environmental niches (12). Furthermore, Δ*cpsA* mutants produced reduced levels of CPS (13). However, some of the results are contradictory. A microarry analysis performed by Orihuela et al. (2004) revealed that there was no increase in expression of *cpsA* in *S. pneumoniae* D39 from the blood of mice compared to in vitro (14). Because the synthesis of the capsule also takes place on transcriptional level, transcriptional regulators might have an effect on the capsule synthesis.

Streptococcus pneumoniae encounter different environments during their infectious cycle. Their ability to adapt to these changes is mediated by global regulatory networks. One of these adaptations is the up- or down-regulation of the capsule expression. Two-component systems (TCS) and other transcriptional regulators seem to play roles in the regulation of the capsule expression (15). Furthermore, environmental factors seem to have an effect on this regulation. But what are the transcription regulators and environmental factors that play roles in the regulation of the capsule biosynthesis? And what are the effects of those environmental factors on opaque and transparent phenotypes and phase variation? In this paper I will try to

answer these questions by looking at *Streptococcus pneumoniae* and related pathogenic streptococci. I will discuss related pathogenic streptococci as well, because some environmental factors that have an effect on the capsule biosynthesis in these bacteria could also have an effect on the capsule biosynthesis in *Streptococcus pneumoniae*. Furthermore, some transcription regulators in related pathogenic streptococci show similarities to transcription regulators in *S. pneumoniae*. The related pathogenic streptococci discussed in this paper are group A streptococcus (GAS or *Streptococcus pyogenes*), *Streptococcus iniae*, *Streptococcus suis* and group B Streptococcus (*Streptococcus agalactiae*).

Transcription factors and two-component sensor signal transduction systems in *Streptococcus pneumoniae* and related pathogenic streptococci

In *Streptococcus pneumoniae*, transcription factors are involved in the regulation of capsule synthesis. (16) Transcription factors are proteins that bind to DNA binding sites and thereby regulate transcription. Besides transcription factors, two-component signal transduction systems also play important roles in the regulation of capsule synthesis. These systems respond to external (environmental) stimuli and for the adaptation of bacteria to the environment, a number virulence genes (including the capsule genes) is controlled via these two-component sensor signal transduction systems (17). The basic model of this system consists of a membrane-associated sensor histidine kinase (S) and a cytoplasmatic response regulator (R) (see FIG. 1). An external stimulus will cause an activation of the sensor histidine kinase. The activated sensor histidine kinase then uses ATP to autoposphorylates a histidine residue. The phosphate group is subsequently transferred by the sensor histidine kinase to an aspartate residue in its cytoplasmatic response regulator, and this will lead to a conformational alteration that allows the response regulator to bind to promoter regions upstream of its target genes and thereby regulate gene expression of many genes under which capsule genes. The response regulator is mostly a DNA-binding transcriptional regulator (15, 18, 19).
The transcription factors and two-component sensor signal transduction systems in *S. pneumoniae* described in this paper are shown in figure 2.

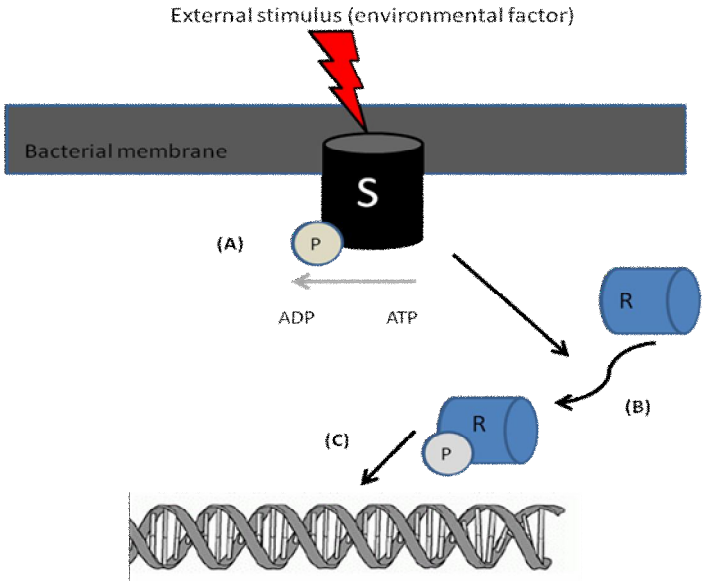

Figure 1. Basic model of a two-component sensor signal transduction system. An external stimulus causes an activation of the sensor protein. The activated sensor protein (S) then uses ATP to autoposphorylate a histidine residue **(A)**.The phosphate group is subsequently transferred from the sensor protein to an aspartate residue on a cytoplasmatic response regulator (R) **(B)**. The RR then binds to promoter regions to control gene expression **(C)**.

The orphan two-component signal transduction response regulator RitR

One of the transcription regulators regulating the capsular polysaccharide biosynthesis locus is RitR (for Repressor of iron transport). RitR is an orphan two-component signal transduction response regulator, which is in involved in iron uptake regulation. The primary role is to maintain iron homeostasis in *S. pneumoniae* (20). Ulijasz et al. (2004) have shown that the response regulator RitR represses iron uptake by negatively regulating the Piu hemin-iron transport system (20). The response regulator doesn't have a cognate histidine kinase as a neighbouring locus in the genome. However, Ulijasz et al. (2009) have recently found that the RitR DNA-binding domain is phosphorylated by a Ser-Thr phosphokinase (21).

Binding sites for RitR were found in the *cpsp* (the *cps* promoter) region and in the promoter region of *ugd* in *S. pneumoniae* (22). A microarray analysis found that RitR down-regulates the expression of the *ugd* gene, which encodes for Ugd (UDP glucose dehydrogenase). UDP glucose dehydrogenase is required for the synthesis of the *S. pneumoniae* type 3 capsule from UDPglucose and UDP-glucuronic acid by the type 3 synthase (which is encoded by the *cps3S* gene) (20). Different *S. pneumoniae* strains containing mutant UDP-Glucose dehydrogenases expressed reduced levels of UDP-glucuronic acid and a reduction in total capsule amount (23).

The involvement of RitR in iron uptake regulation means that the iron concentration could have an effect on RitR. However this seems contradictory, because the high iron concentrations in blood would lead to down-regulation of the *ugd* gene and thereby reducing the amount of capsule. This is unfavourable for *S. pneumoniae*, because in the blood stream it needs maximum CPS biosynthesis to protect itself against opsonophagocytosis.

RitR is very similar to the streptococcal global response regulator CovR (for control of virulence regulator, also known as CsrR, for capsule synthesis regulator). This regulator is present in *Streptococcus pyogenes, Sreptococcus agalactiae and Streptococcus suis* and binding sites for CovR were also found in *S. pneumoniae* (22). CovR is the regulator of the two-component signal transduction system CovR/CovS and regulates about 15% of the genes, including several important virulence factors (24). This system consists of a response regulator and a sensor (see also FIG. 1.). The response regulator is usually a DNA-binding protein that modulates the expression of target genes.

The global response regulator CovR downregulates the CPS biosynthesis in *Streptococcus suis* serotype 2. A microarray analysis showed that the expression of *cps2C (*an open reading frame of the CPS biosynthesis operon) was upregulated in a *Streptococcus suis* serotype 2 Δ*covR* mutant (25). *Cps2C* might possibly be involved in export of polysaccharide and in chain length determination of the *S. suis* serotype 2 capsule (26). Furthermore, Lamy et al. (2004) have shown by macroarray analysis that the expression of *cpsE* and *cpsG* was downregulated in a *Streptococcus agalactiae* Δ*covR* mutant (27).

Because of the similarities between RitR and CovR, RitR may also play an important role in the expression of other genes involved in the capsule biosynthesis of S. *pneumoniae*.

RegM

The biosynthesis of the capsule of *S. pneumoniae* is regulated by the *cps* locus. There are also genes outside of the *cps* locus that regulate capsule biosynthesis. One of these genes is *regM* a homologue of *ccpA,* which encodes for the catabolite repressor protein CcpA (28). The transcription factor CcpA contributes to virulence and regulation of sugar metabolism and plays a direct role in carbon catabolite repression. Carbon catabolite repression is a complex of regulatory mechanisms that silence or activate certain genes (including virulence genes) in response to a carbon source and availability (29). The *ccpA* homologue *regM* is the first gene found outside of the *cps* locus that regulates capsule biosynthesis.

One study has shown that RegM is probably involved in the transcriptional activation of the *cps* operon in the serotype 2 strain D39 (28). A *regM* mutant in this study showed a significantly decreased expression of the *cps* locus in the presence of either glucose or sucrose compared to the wild type ($p<0,001$ and $p<0,01$ respectively). For this study a transcriptional fusion of the beta-galactosidase gene *lacZ* at the 5'end of the *cps* locus was constructed. The capsule gene expression was then measured by assaying beta-galactosidase activity.
In addition, another study has shown that the *hasABC* operon of *Streptococcus pyogenes* was significantly downregulated in a ΔccpA mutant (30). These bacteria are related pathogenic streptococci producing hyaluronic acid capsule. The *hasABC* operon is involved in the synthesis of this hyaloronic acid capsule. The hyaluronic acid capsule of *S. pyogenes* is different from the capsule of Streptococcus pneumoniae. However, the operon is phylogenetically related to the *cps3ABC* operon in *Streptococcus pneumoniae* and binding sites for CcpA were also found in *S. pneumoniae* (22, 31). This is further evidence that RegM and maybe another unknown protein similar to CcpA could regulate the expression of the *cps* locus.
As suggested by homology to CcpA, RegM is involved in the control of sugar pathways and could be a regulatory link between capsule production and the sugar pathways. This also means that glucose source and availability could have an effect on the capsule expression in *Streptococcus pneumoniae*. This will be further discussed in the paragraph *Sugar source and availability*.

SivS/R
One of the transcription regulators that has an effect on capsule expression in related pathogenic streptococci, is the two-component signal transduction system SivS/R in *Streptococcus iniae*. As stated before, two-component signal transduction systems play important roles in the expression of bacterial genes (including virulence related genes). A study has shown that SivS/R plays a role in the expression of the capsule (32). Real-time PCR analysis of *cpsA* revealed that the Δ*siv* mutant of *Streptococcus iniae* transcribed two-thirds less *cpsA* (the first gene of the capsule operon of S. iniae). The results indicate that sivS/R can regulate the capsule operon at the transcriptional level. The Δ*siv* mutant was also more susceptible to phagocytosis. This wasn't unexpected, because capsule inhibits phagocytosis and prevents opsonization by complement. SivS/R homologs are present in other streptococci although little is known about their significance. It is still unknown which external stimuli has an effect on this two-component signal transduction system.

Environmental factors

Streptococcus pneumoniae and related pathogenic Streptococci need to adapt in order to survive in a specific environmental niche. They sense the environment and respond to environmental factors/stimuli by up- or down-regulating virulence factors (including the capsule) that are essential for the survival in their environment. Two-component signal transduction systems play important roles in this adaptation process. The environmental factors, which (could) have an effect on two component signal transduction systems and the capsule expression of *S. pneumoniae*, are discussed below. These factors are Mg^{2+}, iron, oxygen, the human antimicrobial peptide LL-37, sugars and environmental factors in biofilms. These factors are also shown in *figure 2*.

Environmental factor Mg^{2+} and iron

As stated before, the expression of a number of virulence genes is controlled via the two-component sensor signal transduction system CovR/CovS. It is thought that the membrane-bound sensor CovS responds to environmental signal(s) by controlling the phosphorylation of the regulator CovR. When CovR gets phosphorylated, its affinity for binding to the promoter regions of Cov-regulated genes to repress transcription increases (33). One of the environmental signals that has an effect on this two-component sensor signal transduction system is Mg^{2+}.Gryllos et al (2003) showed that the addition of Mg^{2+} repressed the expression of group A Streptococcus hyaluronic capsule genes by up to 80% in a dose-dependent manner and was associated with a reduction in capsule polysaccharide (33). They also showed that the addition of Ca^{2+}, Mn^{2+}, or Zn^{2+} didn't have any effect on the expression. This means that the CovR/CovS system appears to be specific to Mg^{2+}.The authors suggest that when Mg^{2+} binds to the sensor CovS, CovS induces phosphorylation of the regulator CovR. This leads to the repression of Cov-regulated genes. Because there is a low concentration of Mg^{2+} in extracellular body fluids, the CovR/CovS system maintains in an inactive sate during the infection. This allows the bacteria to maximal express the essential virulence genes in a human host. As stated before, the orphan two-component signal transduction response regulator RitR in *Streptococcus pneumoniae* is very similar to CovR and binding sites for CovR were found in *S. pneumoniae.* This means that Mg^{2+} could also have an effect on the expression of *S. pneumoniae* polysaccharide capsule.

Iron also has an effect on the capsule expression of *S. pneumoniae.* Gupta et al. (2009) have shown by RT-PCR that the transcription of *cps4A* in *S. pneumoniae* TIGR4 (a capsular type 4 strain) were highly upregulated in the presence of the iron sources ferritin hemin and hemoglobin compared to the expression of *cps4A* in *S. pneumoniae* in chemically defined medium (CDM) (11, 12 and 23 fold, respectively) (34). However the standard deviations of haemoglobin were high. This means that the effect of haemoglobin could be much lower. High iron levels are present in the bloodstream where *S. pneumoniae* needs maximum CPS biosynthesis to protect itself against opsonophagocytosis (22).

Antimicrobial peptides

Antimicrobial peptides (AP) are small, usually cationic, peptides that have antimicrobial activity against bacteria, viruses and/or fungi and are produced by the human host to protect itself against an infection with bacteria (35). They exert their antimicrobial effect by damaging microbial cell membranes. However, some bacteria, among which *S. pneumoniae*, have also developed "weapons" to resist antimicrobial peptides. One of the strategies for resistance to antimicrobial peptides is de production of a polysaccharide capsule. Anionic capsular polysaccharides block the bactericidal activity of cationic APs by binding them, thereby reducing the amount of antimicrobial peptides reaching the bacterial surface (36). In group A streptococi, small amounts of antimicrobial peptides might serve a signalling function via the sensor CovS (see also *Environmental factor Mg^{2+}*), because it shown that sub-inhibitory concentrations of the human antimicrobial cathelicidin peptide LL-37, but not other APs, significantly ($p<0.009$ at 100 nM AP) stimulate expression of CovR/CovS-regulated genes including the hasABC operon (37). This leads to an increase in resistance to opsonophagocystis. Paradoxically, the production of AP LL-37 as a protection mechanism led to increased Group A streptococci pathogenicity during the human infection.

As mentioned before, the *hasABC* operon is phylogenetically related to the *cps3ABC* operon in *Streptococcus pneumoniae.* And because it is phylogenetically related to the *cps3ABC* operon, LL-37 could have an effect on the biosynthesis of the *Streptococcus pneumoniae* serotype 3 capsule. In addition, CovR looks also similar to RitR and binding sites for CovR were found in *S. pneumoniae* (see also *The orphan two-component signal transduction*

response regulator RitR). This means that LL-37 may also have an influence on the orphan two-component signal transduction response regulator RitR.

Oxygen

Streptococcus pneumonia displays phase variation between opaque and transparent. Phase variation is the molecular mechanism that leads to the switching of the gene expression state (7). Due to higher amounts of capsular polysaccharide, opaque variants are more resistant to opsonophagocytosis (8). In contrast, the transparent variants of *S. pneumoniae* produce smaller amounts of capsular polysaccharides and are more prone to opsonophagocytosis. The reduction in capsular polysaccharides allows enhanced adherence to human epithelial cells and leads also to enhanced colonization of the nasopharynx.

The environmental factor oxygen has different effects on the opaque and transparent variants. There is evidence that oxygen may be an important factor in the ability to regulate capsule synthesis. A study has shown that in opaque variants, reduced oxygen leads to enhanced production of capsular polysaccharide, whereas in transparent variants, capsule synthesis stays low in both anaerobic and aerobic conditions (38). This study has also shown that the inhibitory effect of oxygen on CPS expression in opaque variants was associated with a decrease in tyrosine phosphorylation of CpsD. CpsD is an autophosphorylating protein-tyrosine kinase and a regulator of capsular polysaccharide synthesis. Tyrosine phosphorylation modulates the expression and activity of CpsD.

In patients with an invasive infection, *S. pneumoniae* isolated from the nasopharynx had a transparent phenotype without phosphotyrosine on CpsD and *S. pneumoniae* isolated from blood had an opaque phenotype that phosphorylates CpsD in response to oxygen. This means that differences in oxygen concentrations may possibly allow the bacteria to adapt in these different environments (38).

Sugar source and availability

As we know, there are different concentrations of sugars in the nasopharynx, in blood, etc. and this might have an effect on the capsule expression of *S. pneumoniae*. Glucose concentrations are usually very low in healthy nasopharyngeal secretions and in this environment reduced amounts of capsular polysaccharide are needed for enhanced attachment to epithelial cells. During invasion, *S. pneumoniae* encounter much higher glucose concentrations in the blood stream where it needs maximum CPS biosynthesis to protect itself against opsonophagocytosis (22).

UDP-glucose and UDP-glucuronic substrates are required for the synthesis of the type 3 capsule. Ventura et al. (2006) have shown that when one of these substrates is missing capsular polysaccharide chain lengths are reduced and chain ejection increases (39). It has been suggested that at least in S. *pneumoniae* serotype 3, capsular polysaccharide chain length can be adjusted by sugar availability in the environment.

Additionally, Philippe Giammarinaro and James C. Paton (2002) have shown that the expression of the capsule of *S. pneumoniae* is down-regulated in semi-synthetic medium supplemented with lactose and this means that capsule synthesis is dependent on the carbon source (9).

Environmental conditions in Biofilms

S*treptococcus pneumonia* can undergo spontaneous phase variation between transparent and an opaque colony phenotypes. But there are also non-phase-variable colony variants. Magee Allegrucci and Karin Sauer have demonstrated that S. pneumoniae serotype 19 generated acapsular and mucoid non-phase-variable colony variants when grown as biofilms and harbored single nucleotide polymorphisms (SNP) in *cps19F* (8). Biofilms have several

advantages to bacteria, including physical protection and antimicrobial resistance. Biofilms of *S.pneumoniae* that are defective in the production of hydrogen peroxide, the oxidizing chemical, showed a significant decrease in variant formation. Furthermore, the elevated hydrogen peroxide concentrations present under biofilm growth conditions co-occur with increased rates of mutation.
These findings provide evidence that the formation of non-phase-variable biofilm-derived colony variants isn't caused by selection for the variants which are better equipped for biofilm growth and adherence, but is caused by increased rates of mutation induced by oxidative stress conditions (8).The authors of the study also suggest that development of non-phase-variable colony variants is caused by hydrogen peroxide and environmental conditions specific to biofilms.

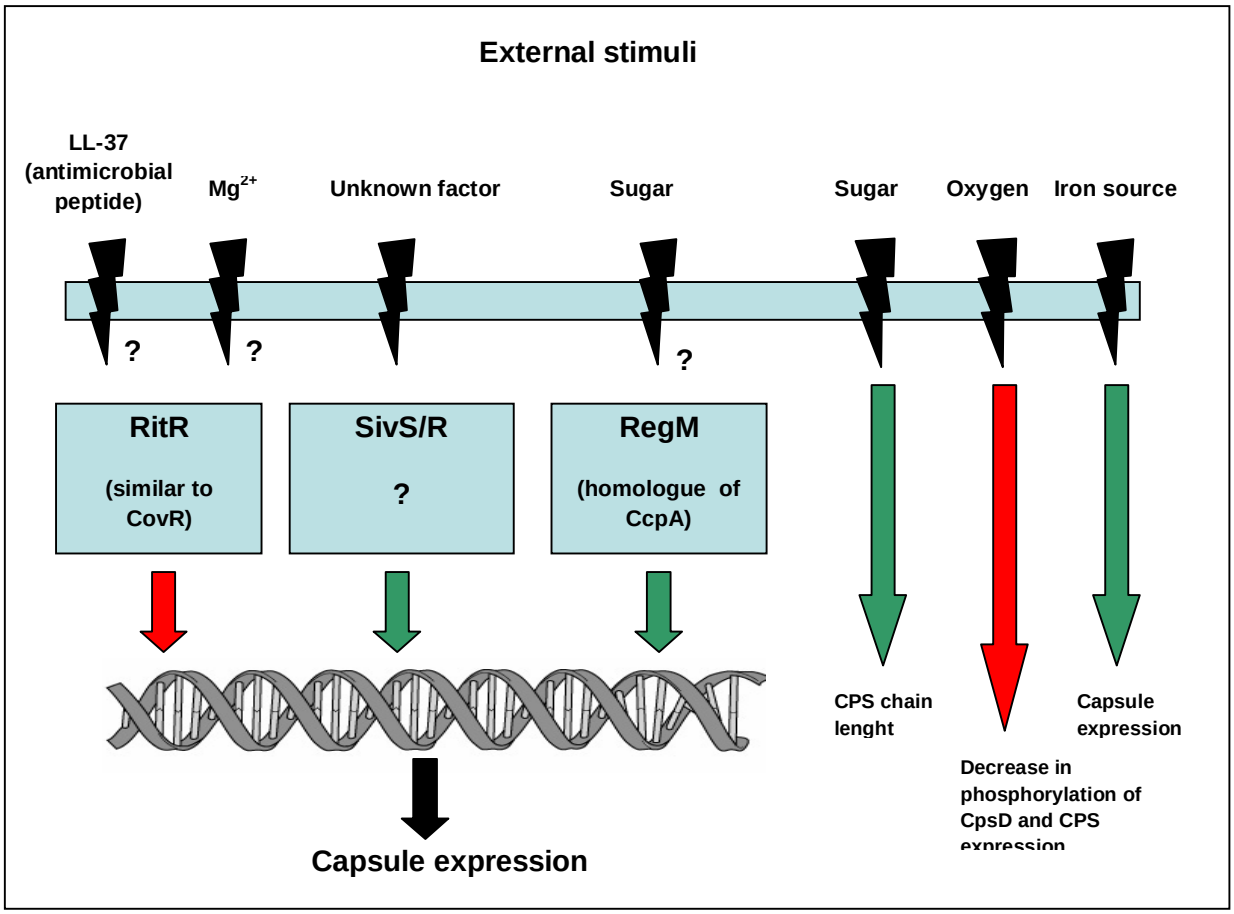

Figure 2. Schematic view of the environmental factors and transcriptional regulators that (could (**?**)) play a role in the biosynthesis of the capsule in *S. pneumoniae*. The factors/regulators with a green arrow have a stimulating effect on capsule biosynthesis and the factors/regulators with a red arrow have an inhibiting effect on capsule biosynthesis.

Conclusion/Discussion

In conclusion, two-component signal transduction systems and other transcription regulators play very important roles in the regulation of capsule synthesis in *Streptococcus pneumoniae* and related pathogenic streptococci. Most of the environmental factors and transcriptional regulators described in this paper had a significant and strong effect on the expression of capsule genes and capsule. This means that these factors and regulators play important roles in the virulence of *S. pneumoniae*.
The two-component sensor signal transduction system CovS/CovR in related pathogenic streptococci plays a very important role in the regulation of genes (including capsule genes).

The environmental factors Mg^{2+} and human antimicrobial peptide LL-37 have an effect on this system. These environmental factors could also have an effect on the capsule biosynthesis in *Streptococcus pneumoniae,* because of the similarities between its RitR and CovR, and the binding sites for CovR. The orphan two-component signal transduction RitR, which is involved in iron uptake regulation, is a response regulator in *S. pneumoniae* that downregulates the expression of the gene *ugd*, which encodes for UDP glucose dehydrogenase. UDP glucose dehydrogenase is very important for the synthesis of the capsule. The environmental factors Mg^{2+} and LL-37 could lead to a downregulation of the *ugd* gene in *S. pneumoniae*. It seems unlikely that iron has an effect on RitR, because high iron concentrations would lead to a reduced expression of the capsule. This is contradictory, because in the blood stream *S. pneumoniae* needs maximum CPS biosynthesis.
Another two-component sensor signal transduction system in related pathogenic streptococci that plays a role in the synthesis of the capsule is SivS/R in *Streptococcus iniae*. SivS/R homologs are present in other streptococci although little is known about their significance and further studies are needed to determine how this regulation contributes to disease pathogenesis. It is unknown which external stimuli has an effect on this system,
But there is also a transcription factor that plays an important role in capsule synthesis in *Streptococcus pneumoniae*. This is the transcription factor RegM. As suggested by homology to CcpA, RegM is involved in the control of sugar pathways and could be a regulatory link between capsule production and the sugar pathways. Furthermore binding sites for CcpA were found in *S. pneumoniae.* This means that glucose source and availability could have an effect on the capsule expression in *S. pneumoniae*.
Besides the environmental factors Mg^{2+}, iron source, human antimicrobial peptide LL-37 and sugar source and availability, environmental factors such as oxygen and environmental factors in biofilms also play important roles. Reduced oxygen leads to enhanced production of capsular polysaccharide in opaque variants of *Streptococcus pneumoniae,* and hydrogen peroxide and environmental conditions specific to biofilms cause formation of non-phase-variable colony variants.
It is clear that there are a lot of factors and systems involved in the capsule synthesis in *S. pneumoniae* and related pathogenic streptococci. The underlying mechanisms are very complex and there is still little known about these mechanisms. These new findings provide new insights into the mechanisms and environmental factors involved in capsule biosynthesis. New insights in this field can lead to better prevention and treatment strategies. Further studies are still needed to find out if the environmental factors such as Mg^{2+} and LL-37 also have an effect on the capsule biosynthesis in *Streptococcus pneumoniae* and to find out if the transcription regulators, that are very similar to the transcription regulators playing important roles in related pathogenic streptococci, also play important roles in *Streptococcus pneumoniae*.

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