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Understanding the regulation of Group B Streptococcal virulence factors

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Abstract

Bacterial infections remain a significant threat to the health of newborns and adults. Group B Streptococci (GBS) are Gram-positive bacteria that are common asymptomatic colonizers of healthy adults. However, this opportunistic organism can also subvert suboptimal host defenses to cause severe invasive disease and tissue damage. The increasing emergence of antibiotic-resistant GBS raises more concerns for sustained measures in treatment of the disease. A number of factors that are important for virulence of GBS have been identified. This review summarizes the functions of some well-characterized virulence factors, with an emphasis on how GBS regulates their expression. Regulatory and signaling molecules are attractive drug targets in the treatment of bacterial infections. Consequently, understanding signaling responses of GBS is essential for elucidation of pathogenesis of GBS infection and for the identification of novel therapeutic agents.

Keywords

bacteria; gene expression; Gram-positive; one-component system; pathogenesis; serine/threonine kinase; signaling; *Streptococcus agalactiae*; transcription; two-component system

Group B Streptococcal disease in newborns & adults

Invasive bacterial infections and the ensuing severe inflammatory response remains a significant cause of morbidity and mortality in human newborns and adults [1,2]. Group B Streptococci (GBS), or *Streptococcus agalactiae*, is the most common cause of life-threatening bacterial infection in human newborns. These bacteria are Gram-positive, β-hemolytic, chain-forming cocci that are normal residents of the vaginal flora in 25% of healthy women [3]. Transmission of GBS from colonized mothers to the newborn can occur *in utero* owing to ascending infection or during birth when the neonate aspirates contaminated amniotic/vaginal fluids. Affected newborns include preterm, low birth weight and full-term infants [4].

GBS disease in newborns is classified as early-onset disease (EOD) or late-onset disease (LOD), depending on the age of the infant at the time of disease manifestation. Maternal colonization is a prerequisite for EOD, and infection presents in infants within a few hours to days of life (≤7 days of age). EOD manifests as respiratory failure and pneumonia that rapidly progresses into bacteremia and septic shock syndrome [5]. In contrast, LOD is characterized by bloodstream infection, with a high risk of progression to meningitis. LOD can present in infants up to several months in age (7–90 days). While the acquisition of GBS for LOD is not

completely understood, vertical transmission, nosocomial acquisition and prematurity are recognized as risk factors [4,6–10].

Recent advances in awareness, diagnosis and treatment of GBS infections have significantly reduced neonatal mortality, particularly owing to EOD, from greater than 50% to 4–5% [11]. In the 1990s, guidelines for the prevention of GBS infections, which included surveillance programs and administration of antibiotics during labor and delivery (intrapartum antibiotic prophylaxis) were established [12,13]. Despite the success of intrapartum antibiotic prophylaxis in prevention of mother–infant transmission of GBS, rates of GBS-related stillbirths, prematurity and LOD have not decreased [14]. Serious morbidity in survivors of GBS infection ranges from 20–60% and includes neurological sequelae such as mental retardation, cerebral palsy and seizures [14,15].

GBS is also an emerging pathogen of adult humans. The elderly, immunocompromised and those with diabetes and malignancies are particularly at risk [16,17]. Clinical manifestations of adult GBS infection are varied and include skin, soft tissue and urinary tract infections, bacteremia, pneumonia, arthritis and endocarditis. The case fatality rate for GBS infection in elderly adults is estimated at 15% in the USA [16,17]. In addition, the recent emergence of GBS strains resistant to penicillin, clindamycin and erythromycin represents a significant concern in the treatment of infections [18–24]. Collectively, these observations reinforce the importance of GBS as a public health concern for human newborns and adults and the need for additional prevention and therapeutic strategies against GBS infection. A better understanding of the mechanisms of GBS pathogenesis is integral for the development of alternate therapeutic strategies.

Regulation of virulence factors for disease pathogenesis

Like many pathogenic bacteria, GBS encodes a myriad of virulence factors that are critical for its ability to cause disease. A few GBS virulence factors, such as the pore-forming toxins and the sialic acid-rich capsular polysaccharide (CPS) have been extensively studied for many years. As many as nine capsular serotypes: Ia, Ib and II–VIII have been characterized [25] and recently serotype IX was also described (see section on CPS) [26]. Genome analysis of the GBS strains representing the five major disease-causing capsular serotypes (Ia, Ib, II, III and V) indicate that the species can be defined by its pan genome [27]. This ‘pan’ or ‘whole’ genome includes a ‘core’ genome that represents genes present in all GBS strains and accounts for approximately 80% of any single GBS strain [27]. Furthermore, the pan genome also represents a dispensible genome comprised of genes that are uniquely present in only one or a few GBS strains [27]. The availability of the GBS genome sequence has accelerated our understanding of the pathogen’s strategy during disease progression. A number of additional virulence factors that are important for GBS adherence and invasion of host cells and evasion of host immunity have been described [28]. Despite these advances, one area that deserves greater attention is how the pathogen regulates expression of these virulence factors during infection. GBS normally resides as a commensal organism in maternal genital and lower gastrointestinal tracts but can transist into an invasive pathogen that infiltrates a diverse array of host niches, such as the intrauterine compartment, neonatal lung and multiple neonatal organs, including the brain. This indicates that GBS is efficiently able to adapt to changing host environments (see Figure 1 for the disease cycle of GBS as a neonatal pathogen). Appropriate expression of virulence factors in response to the host environment can provide a survival advantage to GBS. In pathogens such as *Salmonella*, inappropriate or constitutive expression of certain virulence factors compromises the pathogen’s ability to cause disease similar to the absence of these virulence factors [29,30]. Therefore, successful infection requires that pathogens appropriately express gene products in response to the host/external environment during infection. Understanding when and how GBS regulates the expression of virulence factors will be useful

in the development of alternate therapeutic strategies. This is critical because vaccines and anti-infectives targeted at a virulence or survival factor may not be as effective if their genes are not expressed at an optimal level in the environment/host niche where the vaccine or drug is administered. In addition, signal transduction systems (STS) that regulate the expression of virulence factors are also attractive drug targets for anti-infective therapies [31–33].

In all living organisms, regulation of gene expression in response to the external environment is accomplished by STS. These systems respond to an external signal and often regulate the function of DNA-binding transcription factors. The most common STS in bacteria are the two-component systems (TCS). TCS comprise a membrane-associated sensor histidine kinase (HK), which recognizes an external signal and phosphorylates its cognate response regulator (RR) at a conserved active site aspartate residue. This phosphorylation event usually alters the DNA binding affinity of the RR and changes gene expression to enable the organism to adapt to the environmental signal [34].

The genome sequence of GBS has revealed the presence of 17–20 TCS that can respond to changes in the external environment [27,35,36]. However, the roles of only four TCS, CovR/CovS, DltR/DltS, RgfC/RgfA and CiaR/CiaH are known [37–43]. GBS also encodes at least six stand-alone or one-component transcriptional regulators that regulate gene expression either in response to changes in the cytosol (e.g., small molecules, gases and so on) [44] or to the external environment through as yet unidentified sensors [45]. The functions of three of these six regulators, known as MtaR, RogB and RovS, have been examined (see sections later) [46–49]. Furthermore, all sequenced GBS strains encode signaling enzymes that are commonly present in eukaryotes. These are a serine/threonine kinase, Stk1 and its cognate phosphatase, Stp1, as well as a tyrosine kinase, CpsD and its cognate phosphatase, CpsB [50–53].

Given the vast number of signaling systems and transcriptional regulators encoded in the GBS genome (107 in total [27,35,36]) and the diverse array of host niches encountered by the pathogen during its disease cycle, GBS gene/virulence factor expression can be expected to be tightly regulated by its STS. In contrast to GBS, regulation of toxins and other virulence factors have been studied extensively in close relatives such as Group A Streptococcus and *Staphylococcus aureus* [54–57]. Below, the function and regulation of a few GBS virulence factors are summarized (see also Table 1 and Figure 2).

Pore-forming toxins

Pore-forming toxins are a critical component of pathogenesis in many disease causing bacteria. These toxins promote entry of the pathogen into host cells and facilitate their intracellular survival and systemic dissemination. The virulence of pathogens defective in expression of pore-forming toxins is severely attenuated [58,59]. GBS encodes at least two pore-forming toxins, known as β-hemolysin/cytolysin (β-H/C) and Christie Atkins Munch Peterson (CAMP) factor.

β-hemolysin/cytolysin

β-H/C, also known as CylE, is a surface-associated, pluripotent toxin that is crucial for various facets of GBS disease involving both the pathogen and the host. β-H/C promotes GBS invasion of host cell barriers such as the epithelial and endothelial cells of the lung and the blood–brain barrier (BBB) [60–64]. Furthermore, β-H/C impairs cardiac function, promotes liver failure and induces host inflammatory responses that contribute to neurological sequelae [64–67]. Hemolysin-deficient GBS mutants are attenuated for virulence in various animal models of GBS infection, including sepsis, pneumonia, meningitis and arthritis [64,66,68,69]. The structural gene for β-H/C was identified as *cylE* [70,71]. Recent studies have indicated that

other genes in the *cyl* operon such as *cylA, cylB, cylJ* and *cylK* are associated with the post-translational modification and secretion of β-H/C [72,73].

Studies on the importance of β-H/C in various facets of GBS disease have largely stemmed from properties of the β-H/C-deficient mutants and are supported by *in vitro* assays. However, biochemical and mechanistic insights into the pore-forming abilities of β-H/C and how β-H/C induces inflammatory responses in the host are still largely unknown. Furthermore, despite its crucial role in GBS disease, β-H/C has not been exploited as a target in the development of alternate therapeutic strategies. This is due to the fact that the toxin is not immunogenic and the protein is active only when associated with the bacterial surface or in the presence of high molecular weight stabilizers, thus imposing severe limitations on its purification and characterization for the development of therapeutic approaches (reviewed in [74]). These constraints may also account for the lack of information on its function.

Curiously, β-H/C (*cylE*) biosynthesis is associated with the production of an orange pigment [75,76] that promotes pathogen resistance to reactive oxygen species (ROS) [77]. Resistance to host-encoded reactive oxygen and nitrogen species is an important component of immune evasion by bacterial pathogens [78]. A combination of nuclear magnetic resonance and mass spectrometry identified the structure of the GBS orange pigment revealing it to be a rhamnopolyene with 12 unsaturated bonds [79]. The role of unsaturated bonds in the carotenoid family of pigments as a mechanism to quench ROS has been elucidated in other disease-causing bacteria including plant pathogens [80–82]. Deletion of the structural gene for β-H/C (*cylE*) abolishes pigment expression and hyper-hemolytic strains demonstrate increased pigmentation. Other genes in the *cyl* operon that are associated with fatty-acid biosynthesis are also thought to contribute to pigment biosynthesis. However, since allelic replacement of *cylE* completely abolishes pigment production, the nature of the link is not currently understood and requires further study. Whether the pleiotropic virulence phenotypes of β-H/C-deficient GBS seen *in vivo* can be solely attributed to the absence of hemolysin or may also be, in part, owing to the concomitant loss of pigment biosynthesis, remains to be clarified.

There is a growing body of evidence that GBS regulates the expression of β-H/C and its associated pigment using its STS **(**Figure 2). The TCS, comprising the DNA-binding RR, CovR and its cognate sensor HK, CovS, represses the transcription of *cyl* genes including *cylE* (β-H/C) and activates the expression of another pore-forming toxin called CAMP factor [37,38, 52]. CovR binds to the promoter of β-H/C (P_{cylX}) to repress its transcription [37,39]. CovS, phosphorylates CovR at a conserved aspartate residue, which enhances β-H/C repression [37–39,53]. In addition, it has been demonstrated that a serine/threonine kinase, Stk1, can also phosphorylate CovR at a threonine residue, which decreases aspartate phosphorylation and promoter binding [52,53]. Consequently, Stk1 relieves CovR repression of β-H/C and activation of CAMP factor [52,53]. This novel paradigm; where members of different signaling families interact to fine-tune gene expression, was also recently observed in another Streptococcal species, *Streptococcus pneumoniae* [83].

In GBS, it is suggested that the sensor kinases, Stk1 and CovS, are preferentially utilized by the pathogen to sense different host environments (e.g., colonising niches such as the vaginal mucosa versus invasive niches such as the bloodstream or the BBB) for appropriate expression of toxins and other factors during infection [53]. Such fine-tuned gene/virulence factor expression can increase the adaptability of GBS to host niches and is extremely beneficial for disease progression. Identification of the environmental/host signals recognized by CovS and Stk1 is essential for understanding how GBS regulates expression of toxins and other factors during infection.

The finding that a transcriptional regulator RovS, can also bind to, and activate the expression of *cylE* and other genes in the *cyl* operon [49] further demonstrates the complexity of β-H/C regulation **(**Figure 2). As RovS is a stand-alone regulator, it is likely that this regulator, similar to other members of its family, controls gene expression owing to changes in the cytosol (e.g., small molecules, gases and so on) [44]. Conversely, RovS may regulate gene expression in response to the external environment through an, as yet unidentified, sensor kinase. Since both CovR and RovS directly bind to the promoter of β-H/C, their regulation of β-H/C is likely to be triggered by their activating (or repressing) signal/s. It is noteworthy that *covR/S* does not regulate *rovS* (gbs1555) expression [37] and RovS does not appear to regulate transcription of either *covR/S* or its regulated genes, such as CAMP factor [49].

CAMP factor

CAMP factor is a secreted protein with pore-forming properties that has been suggested to be important for GBS pathogenesis [84–86]. Evidence that CAMP factor is important for virulence of GBS was provided by *in vivo* studies, which indicated that partially purified CAMP factor was lethal to rabbits, and co-administration of CAMP factor along with a sublethal dose of GBS can induce septicemia and death in mice [86,87]. In addition, CAMP factor was observed to oligomerize and form discrete pores on susceptible target membranes [88]. Observations that CAMP factor can bind to glycosylphosphotidylinositol-anchored proteins suggests that binding to its host-cell receptor may be necessary for cell lysis as observed with other bacterial toxins [89]. Surprisingly, Hensler *et al.* recently observed that a CAMP factor-deficient GBS strain is not attenuated for systemic virulence [90]. These data suggest that CAMP factor may be nonessential for GBS pathogenesis.

Given their pore-forming abilities, it is also likely that β-H/C may play a compensatory role for the absence of CAMP factor during systemic infection. If this hypothesis is correct, then CAMP factor may only be essential for GBS pathogenesis in host niches where β-H/C activity is diminished. For example, the normal human lung surfactant dipalmitoylphosphatidylcholine (DPPC) is antagonistic to β-H/C [77]. It is tempting to speculate that CAMP factor expression may be important to GBS in certain host niches like DPPC-containing environments (e.g., a normal human lung), whereas in others (e.g., a premature, surfactant deficient neonatal lung [77]), GBS may preferentially utilize β-H/C. Consistent with this hypothesis, GBS oppositely regulates β-H/C and CAMP factor using CovR/S and Stk1 **(**Figure 2).

The DNA-binding RR, CovR activates the expression of CAMP factor in GBS [37,38]. CovR deletion mutants in serotype Ia (strains 515 and A909), serotype V (strains 2603v/r and NCTC10/84) and serotype III (strain NEM316) are deficient for CAMP factor expression [37,38,52]. The CovR protein was also demonstrated to directly bind to the promoter of the gene encoding CAMP factor (*cfb*) suggesting that binding may be required for activation [91]. Aspartate phoshorylation of CovR by its sensor kinase CovS or low molecular weight phosphdonors (e.g., acetyl phosphate) enhances CovR function leading to enhanced activation of CAMP factor, whereas threonine phoshorylation of CovR by Stk1 decreases CovR-mediated activation of CAMP factor [53]. Thus GBS opposingly regulates the expression of its pore-forming toxins and modulates their expression using its sensor kinases, CovS and Stk1. Although CovR/S can exert a regulatory role on the expression of approximately 150 genes in GBS; 39 of these comprise the conserved regulon and include genes encoding the pore-forming β-H/C and CAMP factor **(**Table 1) [39].

Factors that promote immune evasion

In addition to the direct onslaught of pore-forming toxins that facilitate GBS survival in the host, it is imperative for GBS to subvert the host's innate immune defenses. To this end, GBS encodes a number of factors that either prevent its recognition by the host or provide resistance

to host defense mechanisms. A few factors that facilitate GBS immune evasion and our current understanding of their regulation are discussed.

Sialic acid-rich capsular polysaccharide

GBS are encapsulated by a sialic acid-rich CPS belonging to one of the ten capsular serotypes: Ia, Ib or II-IX. The CPS of GBS exemplifies a classical example of molecular mimicry. Since the CPS of GBS is decorated with sialic acid, a family of nine carbon sugars also commonly present on glycans of vertebrate cells, the host fails to recognize GBS as nonself. Consequently, CPS prevents complement factor C3 deposition and phagocytosis of GBS by the host's innate immune system [92,93]. The importance of CPS in adaptive immunity against GBS has also been described [94]. These studies showed that placental transfer of maternal antibodies can protect infants from invasive GBS infection and infants born to women with low CPS antibody titers may be at an increased risk for GBS infections [94]. Consistent with these findings, GBS strains devoid of CPS are avirulent in sepsis LD_{50} values of the CPS models of GBS infection; deficient strains were 10^3–10^5-fold greater than the isogenic wild-type strain [95–98].

Owing to its importance in GBS pathogenesis, CPS has been considered as a candidate for GBS vaccines. Thus, because of its low immunogenicity, conjugate vaccines of CPS complexed to highly immunogenic proteins were derived and these have significantly improved antibody responses and have also been successful in Phase I and II human trials [99–102]. A limiting factor in the widespread use of CPS-conjugate vaccines is the presence of at least nine structurally distinct and antigenically unique GBS capsular stereotypes. Successful prevention of GBS infections requires the administration of a polyvalent CPS vaccine against prominent disease-causing GBS serotypes (i.e., Ia, Ib, II, III and V) [103–105].

Recently, elegant studies have demonstrated that the CPS encoded by GBS can be modified by *O*-acetylation [106]. Since *O*-acetylation of bacterial polysaccharides can alter the immune/antibody response of the host to pathogens, such as *Neisseria meningitidis* [107] and *S. pneumoniae* 9V [108], the immune response of the host to the *O*-acetylated CPS of GBS requires further study. *O*-acetylation of CPS among GBS serotypes ranges from approximately 5–55% of the total CPS [106]. The enzyme responsible for *O*-acetylation of CPS is encoded by the gene *neuA*; a site-directed asparagine to alanine (N301A) mutation in *neuA* causes increased *O*-acetylation of CPS [109]. Since CPS preparations have employed a base treatment during extraction that destroyed the *O*-acetyl groups, it is likely that the CPS previously used in vaccine preparations were not *O*-acetylated. Consequently, the implications of *O*-acetylation of CPS on vaccine design, antibody responses and even pathogenesis of GBS are yet to be understood. Whether *O*-acetylation confers survival advantage to GBS in certain host environments also remains to be examined. The GBS strain with the N301A mutation in *neuA*, may prove useful to examine the virulence and survival benefits of CPS *O*-acetylation.

Despite its importance as an essential virulence factor, surprisingly little is known about how GBS regulates CPS during disease pathogenesis. Earlier studies have found that although important for systemic virulence, CPS interferes with GBS adherence and invasion of cultured epithelial and endothelial cells [110–112]. These observations indicate that GBS may regulate expression of CPS in response to the host/external environment. Differences in doubling time or changes in growth rate were suggested to affect the amount of CPS [113,114] and adherence and invasion of GBS to respiratory epithelial cells [115,116].

RogB, a transcriptional regulator of the RofA-like protein family regulates the transcription of *cps* genes **(**Figure 2) [48]. It is thought that RogB is a repressor or negative regulator of CPS because transcription of the first gene in the CPS operon, *cpsA*, increased in the absence of RogB in the GBS serotype III strain 6313. Whether RogB regulation of *cpsA* is accompanied

by changes in CPS levels and the relevance of this regulation during GBS infection requires further study. Furthermore, since the genetic locus encoding RogB is not conserved in all GBS strains/serotypes (e.g., the gene encoding RogB is not present in serotypeIa A909 or the serotype III strain COH1 [27]), its regulation of CPS is strain-specific.

Likewise, CovR/S also modestly regulates *cps* transcription in a strain-dependent manner. While CovR/S can activate *cps* gene expression in serotype III NEM316 by twofold **(**Figure 2) [37], this was not consistently seen in GBS serotype V 2603v/r and Ia strains 515 and A909 [39, Rajagopal L *et al.*, Unpub. Data], suggesting strain-specific regulation of CPS by CovR/S. Whether a universal regulator (i.e., present in all GBS strains) controls CPS expression in response to the external environment, such as during the transition from a commensal state, with presumably low CPS expression, to an invasive state with high CPS expression remains unknown. Identification of the environmental cues that trigger RogB and CovR/S regulation of CPS is necessary to advance our understanding of their role on CPS expression during invasive infection.

Superoxide dismutase

Pathogen resistance to host-encoded ROS is integral to host immune evasion. Apart from pigment (see section on β-H/C), GBS encodes a Mn^{2+} cofactored superoxide dismutase, SodA, for resistance to ROS and immune evasion. Superoxide dismutases convert singlet oxygen or superoxide anions (O^{2-}) to molecular oxygen (O_2) and H_2O_2, which are subsequently metabolized by catalases or peroxidases. Consequently, these enzymes enable pathogenic bacteria to resist oxidative stress during infection. A GBS *sodA* mutant was found to be significantly more sensitive to macrophages and showed impaired survival in the bloodstream and brain of mice [117]. Conversely, survival of the *sodA* mutant was not significantly impaired in the mouse liver or spleen. Taken together, these observations suggest that SodA confers survival benefits to GBS in specific host niches.

Regulation of *sodA* transcription in GBS is accomplished by RovS. A 2.2-fold decrease in *sodA* expression was observed in the *rovS* deletion mutant [49] suggesting that RovS positively regulates *sodA* transcription. Although RovS was shown to directly bind to the promoter region of *sodA* **(**Figure 2) [49], the role of this binding on *sodA* transcription and the signals that activate or repress the function of stand-alone regulators such as RovS are not known. Thus, RovS regulation of *sodA* during GBS infection is not completely understood and requires further study.

C5a peptidase

The GBS C5a peptidase (ScpB) is a serine protease that facilitates host immune evasion by the pathogen. ScpB, encoded by the gene *scpB*, can cleave and inactivate the human complement component C5a. Since C5a is important for the recruitment of neutrophils to the site of infection, its cleavage by ScpB impairs neutrophil recruitment [118]. Using knockout mice complemented with either murine or human C5a, it was concluded that the peptidase activity of ScpB specifically inactivates human C5a and not murine C5a [119]. ScpB can also promote binding of GBS to human epithelial cells and the extracellular matrix (ECM) protein fibronectin [120–122]. Studies have shown that the fibronectin adhesin activity of ScpB is independent of its peptidase activity and that ScpB binds to a site created by the juxtaposition of multiple fibronectin molecules [122,123]. The importance of ScpB in prevention of GBS infection stems from observations that immunization with either ScpB or ScpB–CPS conjugate vaccines promotes clearance of GBS from the mouse lung [124]. In addition, administration of ScpB encapsulated in microspheres was also observed to provide protection against GBS in animal models of infection [125].

Regulation of ScpB is accomplished by two independent TCS in GBS **(**Figure 2). During late exponential phase growth, an increase in *scpB* transcription, along with a concomitant increase in binding to the ECM protein fibrinogen, was observed in an *rgfC* mutant [42]. RgfC is a sensor HK and RgfA is the cognate DNA-binding RR. Whether RgfA directly binds to the promoter of *scpB* to repress its transcription is not known. It was also speculated that RgfB is a secreted peptide that may be important for quorum sensing (regulation in response to cell density) similar to the auto-inducing peptide of *Staphylococcus aureus* [42]. While these observations suggest growth phase-dependent repression of ScpB by RgfC/A and possibly RgfB, further investigations are necessary for a complete picture of ScpB regulation. As the first 355 codons of RgfC are missing in the serotype III strain NEM316 [35] and a point mutation in RgfA truncates its expression in the serotype Ia strain A909 [27], regulation of ScpB expression by RgfC/A is strain-specific.

The other TCS that regulates ScpB expression is CovR/S. Jiang *et al.* observed a 11–30-fold increase in *scpB* transcription in GBS serotype Ia (strain 515) and V (strain 2603v/r) *covR* mutants [38]. These data suggest that CovR is a repressor of ScpB, similar to its effect on β-H/C. However, ScpB is not part of the 39 gene-conserved CovR regulon [39] and studies have not demonstrated regulation of ScpB by CovR/S in GBS A909 [Rajagopal L *et al.*, Unpub. Data] suggesting that regulation of ScpB by CovR/S is also strain-specific. It is noteworthy that a NEM316 *covR/S* mutant (where CovR does not regulate ScpB) is hyper-adherent to host epithelial cells [37]. This indicates that although CovR/S regulation of ScpB may not be conserved, the regulation of factors that mediate adherence of GBS to host cells is (see section on adherence).

Serine proteinase

GBS can also cleave ECM components that facilitate its evasion of host immunity. A cell surface-associated serine protease known as CspA can cleave the human ECM protein fibrinogen [126]. Cleavage of fibrinogen to fibrin-like products thwarts the host immune system as it prevents recognition and phagocytic uptake of GBS. Consequently, GBS strains defective in CspA expression exhibit increased susceptibility to neutrophils and decreased virulence in a neonatal sepsis model of infection (a tenfold higher LD_{50}, [126]). Recent studies demonstrated that CpsA also readily cleaved chemokines such as growth-related oncogene-γ, neutrophil-activating peptide-2, and granulocyte chemotactic protein-2 and abolished their ability to attract and activate neutrophils [127]. Despite its importance to GBS virulence, not much is known regarding the regulation of CspA expression.

It is noteworthy that both serine proteases of GBS (ScpB and CspA) have a cell wall anchoring LPXTG motif [128,129]. These motifs are essential for cell wall targeting of these proteins by the major sortase enzyme, SrtA. Thus, it was anticipated that in the absence of SrtA, mislocalization of cell wall anchored proteins would lead to a dramatic decrease in virulence of GBS. Although the GBS *srtA* mutants displayed altered ScpB localization, fewer pili and decreased adherence to ECM proteins and host cells, they were not attenuated for systemic infection [129,130]. Since *srtA* mutants demonstrated a decreased colonization in animal models, these results emphasize the importance of cell-surface proteins in the colonization of GBS [130]. However, mechanisms that regulate *srtA* and *cspA* expression are still not completely elucidated.

Resistance to host antimicrobial peptides

Cationic antimicrobial peptides (AMPs) are components of the host innate immune system that play a critical role in combating bacterial infections [131]. AMPs are expressed by a number of host cells including epithelial cells, keratinocytes and leukocytes. An increase in expression of AMP occurs during injury and infection that provides resistance to invading organisms

[132]. Common classes of AMPs include defensins and cathelicindins. A number of mechanisms are employed by GBS to resist the action of these AMPs and a few are listed in below.

Changes in surface charge

The most common mechanism employed by pathogens, including GBS, to resist AMPs is to decrease the charge on their cell surface [133]. Since AMPs are positively charged and the bacterial cell surface is negatively charged, the initial interaction between them is electrostatic. To decrease the efficiency of this interaction, many Gram-positive pathogens incorporate substantial amounts of D-alanine into the lipotechoic acid (LTA) component of their cell wall. This decreases the net negative charge on the bacterial surface and thus, enables the pathogen to repel AMPs and prevent their access to the bacterial cell membrane. In GBS, incorporation of D-alanine into LTA is regulated by the *dltRSA-BCD* operon, which comprises two regulatory genes, *dltR* and -*S*, and four structural genes, *dltA–D* [40]. Deletion of the structural gene *dltA*, which encodes the D-alanine-D-alanyl carrier ligase, causes a severe attenuation of GBS in a neonatal rat virulence (100-fold higher LD_{50} sepsis model) and increases its susceptibility to defensins and phagocytes [41]. DltR is a DNA-binding RR that binds to promoter DNA to activate expression of the *dlt* operon and requires its cognate sensor HK DltS **(**Figure 2). The environmental signal/s perceived by the DltS sensor kinase is not known. Therefore, it is possible that during infection or during contact with host cells, GBS may modulate expression of the *dlt* genes to alter their surface charge and repel host AMPs. Support for this hypothesis is provided by recent observations that human AMP, such as LL37, can stimulate signaling by the CovR/S system in Group A Streptococcus [134,135].

Penicillin-binding proteins

Bacterial penicillin-binding proteins (PBPs) are typically involved in peptidogylcan biosynthesis and are the site of action for the β-lactam family of antibiotics such as penicillin [136–138]. Surprisingly, GBS mutants lacking the gene *ponA*, which encodes PBP1a were similar to the wild-type strain for sensitivity to penicillin [139]. A signature-tagged mutagenesis screen revealed that the *ponA* mutant was attenuated for virulence (100-fold higher LD_{50}) in a neonatal sepsis model of infection [140]. Subsequent studies showed that the *ponA* mutant was cleared quicker from the lungs of neonatal rats and was also more sensitive to killing by neutrophils and alveolar macrophages [139,141]. These phenotypes are likely owing to the fact that the GBS *ponA* mutant is more susceptible than the wild-type to the action of different classes of host AMPs [142]. Given that the *ponA* mutant did not demonstrate an altered surface charge or remarkable changes in cell-wall peptidoglycan, how PBP1a enables GBS to resist AMPs and innate airway defenses in the neonatal lung remains to be resolved [141,142]. Regulatory systems that affect *ponA* transcription have not been identified.

Likewise, the regulation and function of other PBPs encoded by GBS are also not completely understood. However, GBS strains resistant to penicillin were found to contain spontaneous mutations that conferred amino-acid substitutions (e.g., Q577E or V405A) to the PBP2X protein [22–24]. These results confirm that PBP2X is a site of action for the β-lactam family of antibiotics. The effect of these amino-acid substitutions on growth and/or virulence of GBS is not known.

Pili

Recent studies demonstrate that GBS encodes small cell-surface appendages known as pili [128,143]. Pili mediate GBS resistance to AMPs and also facilitate adherence and attachment of the pathogen to host cells [128,144–146]. Two genetic loci are responsible for the pilus-like structures in GBS and are known as pilus islands-1 and -2 (PI-1 and PI-2, respectively). PI-2 exists in two variant forms known as PI-2a and PI-2b [147]. Sequenced GBS strains were found

to encode the genes comprised in at least one of these loci. Homologs of PilB constitute the major structural component of the GBS pili; two accessory proteins, which are homologs of PilA and PilC, are also associated with the GBS pili [128,145].

GBS strains lacking PilB demonstrate decreased virulence and increased susceptibility to phagocytes and AMPs [145,146]. However, the mechanism by which pili enable GBS to resist AMPs is not understood. GBS adherence to epithelial cells was reduced in mutants lacking PilA and PilC [128], indicating a pleiotropic role of pili in disease pathogenesis. Since *Lactococcus lactis* strains expressing these pilus proteins can stimulate the production of protective antibodies against GBS [148], exploiting pili for a vaccine design and development has gained significant attention [103,149,150].

RogB can positively regulate pilus gene expression in GBS. The gene encoding RogB is located approximately 284 bp from PI-2a and the transcription of genes in this locus is decreased in the absence of RogB in the GBS strain NEM316 **(**Figure 2) [128]. Since RogB or its homologs are not physically associated with PI-1 or PI-2b, it is likely that these loci are not regulated by RogB [128,129]. As RogB is not conserved in GBS strains and is not likely to control all PIs, our understanding of the mechanisms that enables GBS to regulate expression of its pili is still incomplete. However, RogB was implied to regulate other factors that promote GBS adherence to host cells (see below).

Adherence & invasion of host-cell surfaces

Adherence of GBS of host-cell surfaces is important for mucosal colonization and subsequent invasion. GBS avidly adheres and invades a number of host cells, including vaginal epithelial cells, lung epithelial and endothelial cells and the critical BBB comprised of a single layer of micro-vascular endothelial cells. These interactions often involve the initial binding of GBS to ECM proteins such as fibrinogen, fibronectin and laminin, which facilitate subsequent interactions with host-cell surface integrins and entry into the host cell [28]. A few factors that enable GBS to bind ECM proteins and invasion of host cells are discussed below.

Fibrinogen-binding proteins

GBS binding to ECM fibrinogen is mediated by two fibrinogen-binding proteins known as FbsA and -B [151,152]. FbsA is a surface-associated protein encoded by a few GBS strains/serotypes (e.g., serotype Ia, strain A909; serotype III, strains NEM316, 6313 [27]) whereas FbsB is found in all GBS strains sequenced thus far [27]. Purified FbsA and FbsB proteins can bind to both soluble and immobilized fibrinogen [151,152]. GBS mutants lacking FbsA demonstrate decreased binding to human fibrinogen and are more sensitive to phagocytes compared with their isogenic wild-type [151]. In contrast to FbsA, a GBS FbsB mutant is not attenuated for fibrinogen binding but invasion into lung epithelial cells is severly impaired [153]. These results suggest that FbsA mediates adherence and FbsB promotes entry of GBS into host cells.

FbsA expression is regulated by at least three different STS in GBS serotype III. Regulation of FbsA expression is accomplished by RogB, RovS and CovR/S **(**Figure 2). RogB, the stand-alone transcriptional regulator that is also unique to certain GBS strains, positively regulates *fbsA* transcription in a growth phase-dependent manner [48]. GBS mutants lacking RogB demonstrate decreased *fbsA* transcription and fibrinogen binding. Curiously, the virulence properties of a RogB mutant have not been examined in various animal models of GBS infection. Given that RogB is predicted to be a positive regulator of adherence factors such as FbsA and Pili and a negative regulator of CPS (see previous sections), examination of the global role of RogB in GBS gene expression will expand our understanding on the role of this regulator in GBS pathogenesis.

Apart from RogB, RovS was shown to bind directly to the promoter of *fbsA* and negatively regulate its transcription [49]. Consistent with these findings, the *rovS* mutant displays a moderate increase in both fibrinogen binding and adherence to lung epithelial cells. In addition, expression of the previously mentioned positive regulator of *fbsA* (RogB) is also decreased in the *rovS* mutant; however, RovS did not directly bind to the promoter region of *rogB* [49]. Whether RogB regulation of *fbsA* is independent of RovS is not known. Delineation of regulation by RogB and RovS is essential for a complete understanding of these regulatory proteins on FbsA expression, adherence of GBS to host cells and GBS virulence.

FbsA is also negatively regulated by the CovR/S system. Transcription of *fbsA* increased 4.6-fold in the absence of the CovR/S TCS in the GBS serotype III strain NEM316, which encodes both RogB and RovS. Whether CovR binds directly to the promoter of *fbsA* to control its expression is not known. As expression of RovS (gbs1555) and RogB (gbs1479) is not altered in a NEM316 Δ*covSR* mutant [37], it is likely that CovR/S and RovS/RogB-mediated control of *fbsA* are independent of each other but may be mediated by their activating (or repressing) signals.

Laminin-binding protein

Adherence of GBS to host-cell laminin is accomplished by the laminin-binding protein Lmb. GBS mutants lacking *lmb* show decreased adherence to human laminin and to human brain microvascular endothelial cells [154,155]. Although Lmb is thought to promote GBS colonization and translocation into the bloodstream, the mechanisms that regulate its expression are not known. While the genes *lmb* and *scpB* are located next to each other on the same DNA contig, the presence of approximately 200 bp intergenic region between these genes in serotypes Ia and III suggests that they are not similarly regulated in these strains. Thus, regulation of *lmb* expression in GBS is not completely understood and requires further study.

Serine-rich repeat proteins

Serine-rich repeat (Srr) proteins of the streptococcal and staphylococcal protein family were recently identified in GBS. Members of this family are comprised of highly glycosylated, surface localized, serine-rich proteins that have adhesive functions and are encoded in operons with dedicated secretion systems [156,157]. The Srr proteins of GBS contain a signal peptide of approximately 50 amino acids, regions of Srrs and a LPXTG cell-wall anchoring motif [157,158]. GBS serotype Ia, Ib, Ic, II, V and certain serotype III strains (e.g., NEM316) share a common Srr protein called Srr-1 [158]. Other GBS serotype III strains (e.g., J48) encode the Srr protein Srr-2 [158]. The amino acids that alternate with serine in the repeat region of Srr-2 are valine, isoleucine, threonine, asparagine and glutamic acid. In contrast alanine, threonine and methionine alternate with serine in Srr-1 [158]. Srr-2 has limited homology to Srr-1 (<20%) and GBS strains encoding Srr-2 are more virulent in neonatal sepsis model of infections (1000-fold higher LD_{90} values) compared with Srr-1 strains [158].

Recent studies have shown that the GBS Srr-1 protein binds to a 64 kDa protein in human saliva identified as human keratin [159]. The amino acids located at positions 485–682 that precede the large Srr region in Srr-1 was identified as the region important for keratin binding [159]. Furthermore, these studies also showed that the 255 C-terminal amino acids of human keratin are required for Srr-1 binding. A comparison of adherence and invasion of human epithelial HEp2 cells revealed that Srr-1 promotes GBS adherence to these cells. The *srr-1* deletion mutant displayed a 75% decrease in adherence but showed no decrease in invasion of HEp2 cells [159]. Taken together, these results indicate the importance of Srr proteins to adherence and virulence of GBS. Although a putative RofA-like transcription factor is encoded within the Srr-1 operon, not much is known about its role in *srr-1* transcription.

GBS immunogenic bacterial adhesion

Adherence of GBS to host cells is also facilitated by the newly discovered cell-surface GBS immunogenic bacterial adhesin (BibA) [160]. A total of four different forms of BibA are encoded by various GBS strains/serotypes and include cell-wall associated and secreted forms. The contribution of each of these variant forms to GBS pathogenesis requires further study. However, in serotype V, the cell-surface associated form of BibA promotes adherence of GBS to human cervical and lung epithelial cells, binds to a regulator of the classical complement pathway known as C4-binding protein and is important for GBS resistance to phagocytosis and virulence [160]. Although changes in *bibA* transcription were observed at higher temperatures (see section on adaptive responses) [161], signaling systems that regulate *bibA* have not been identified.

αC protein

The surface-anchored αC protein (ACP) is important for GBS virulence and mediates GBS invasion of human cervical epithelial cells. ACP is commonly expressed on the surface of GBS serotypes Ia, Ib and II and are uncommon in serotype III GBS strains [162,163]. ACP promotes GBS internalization into host cells through its interaction with the host-cell glycosaminoglycan [164]. Consequently, mutations that alter glycosaminoglycan binding render GBS deficient in cervical epithelial cell invasion [165]. Furthermore, ACP can also promote GBS invasion of these host cells by binding to $\alpha_1\beta_1$-integrins on the epithelial cell surface [166]. The mechanisms involved in the regulation of ACP expression are not understood.

Invasion-associated gene

The invasion-associated gene, *iagA*, was identified as an important virulence factor for GBS meningitis. IagA was identified in a screen for GBS mutants that showed loss of BBB penetration [167]. Mice infected with the *iagA* deletion mutant developed bacteremia at similar efficiencies as the wild-type GBS strain [167]. However, the *iagA* mutant demonstrated decreased BBB penetration and meningitis infection when compared with the isogenic wild-type [167]. IagA is a glycolipid known as diglucosyldiacylglycerol, which facilitates membrane anchoring of LTA. In an *iagA* mutant, LTA is shed into the growth media, which interestingly can inhibit BBB invasion even by wild-type GBS [167]. Factors that regulate expression of IagA and whether contact with BBB can alter *iagA* expression is not known.

Other functions that contribute to GBS disease pathogenesis

Hyaluronate lyase

A secreted protease known as hyaluronate lyase (HlyB), encoded by the gene *hlyB* is important for GBS pathogenesis. [168,169]. Hyalurons are major components of human tissue and higher concentrations are found in the placenta, amniotic fluid and in the lung [170–172]. Since hyaluronate lyase can cleave hyaluron [172–174], this protein is thought to facilitate the spread of GBS during infection. Consistent with this hypothesis, high concentrations of hyaluronate lyase were reported in neonates with bloodstream infections compared with neonates that were asymptomatically colonized with GBS [175]. Furthermore, GBS infection in a piglet pneumonia model correlated with a concomitant decrease in pulmonary hyaluron, suggesting an increase in HylB activity [176]. Whether transcription of *hylB* is altered during GBS infection is not known and the STS that regulate *hylB* transcription have not been identified.

Methionine uptake

The GBS methionine transport regulator (MtaR) belongs to the LysR family of stand-alone transcriptional regulators [46]. Although divergently transcribed from the CPS operon, MtaR does not regulate expression of the *cps* genes. Likewise, a *mtaR* mutant did not demonstrate

altered CPS levels [46]. However, an MtaR mutant displayed severe attenuation (1000-fold higher LD_{50}) in the neonatal rat sepsis model of GBS virulence and was impaired for methionine transport/uptake. The genes regulated by MtaR, which facilitate methionine transport were recently identified; microarray analysis revealed a 2.5–5-fold decrease in the expression of a putative methionine transporter (*metNPQ*). Furthermore, the *mtaR* deletion mutant also demonstrated a moderate decrease in expression of genes involved in arginine biosynthesis and the serine protease CspA, and a slight increase in expression of FbsB [47]. It is likely that both decreased nutrient acquisition and suboptimal expression of CpsA contributes to the attenuated virulence of the *mtaR* mutant [46,47].

Adaptive responses of GBS

Given the wide range of host niches encountered by GBS during its disease cycle, two studies have attempted to address changes in gene expression in response to the external environment. A summary of these results is discussed.

GBS adaptation to changes in doubling time & dissolved oxygen

Johri *et al*. examined changes in transcription and surface protein expression when GBS was subjected to *in vitro* growth conditions that mimic low-invasive and high-invasive states [177]. GBS serotype V 2603v/r was grown in a chemostat at a doubling time of 1.8 h in the presence of 12% DO_2 (dissolved oxygen), which represents a high-invasive state. Two low-invasive conditions (similar doubling time without dissolved oxygen [1.8 h, 0% DO_2] and increased doubling time with DO_2 [11h, 12% DO $_2$]) were also included in their analysis [177]. GBS invasion of human cervical epithelial cells supported the hypothesis that these growth conditions mimicked low and high invasive states, respectively [177]. Protein analysis using liquid chromatography-tandem mass spectrometry revealed that during high-invasive conditions, surface expression of lipoproteins, proteins involved in iron and amino-acid transport, cell division, cell surface proteases, PBPs, DltD, CpsC, and regulators such as HKs and Stk1 were increased. These results support the hypothesis that these factors may play an important role during GBS invasion. For a number of these proteins, increases in their surface expression is correlated with a concomitant increase in their transcription [177]. Notably, an increase in expression of the pluripotent β-H/C was not observed under high invasive conditions. Whether increased expression or secretion of CAMP factor was observed under these conditions is not known. It would be of interest to determine if increased expression of the same genes/proteins is also seen in other GBS strains/serotypes under these growth conditions. Since these studies were performed using GBS grown in a chemostat, the importance of these findings during GBS infection and pathogenesis warrants further investigation.

GBS adaptation to temperature & human blood

Recently, Mereghetti *et al*. examined global changes in the GBS transcriptome during growth at higher temperatures, which mimic febrile conditions in the host [161]. At a high temperature (40°C), the authors observed increased transcription of *cylE*, encoding β-H/C, even when GBS were grown in laboratory media. This increase in β-H/C gene expression was growth phase-dependent, ranged between 2–4.8-fold, and also correlated to greater hemolytic activity. As transcription of the regulators of β-H/C, such as CovR/CovS and Stk1, was not significantly affected under these conditions [161], these results suggest that β-H/C transcription is most likely altered by signaling mediated by the sensor kinases Stk1 and/or CovS.

Increased expression of genes encoding cell surface proteins and those involved in iron acquisition and nucleotide biosynthesis were also observed at 40°C [161]. These results suggest that during infection, GBS may scavenge iron from the environment using its siderophores

[178] and synthesize purines and pyridimines using its *de novo* pathway. Previous studies have demonstrated that while GBS are auxotrophic for the biosynthesis of certain amino acids, they can synthesize purines and pyrimidines *denovo* [46,51]. While iron siderophore (*fhu*) mutants did not demonstrate attenuated virulence in a neonatal sepsis model of infection [178], enhanced expression of siderophore genes under conditions that mimic fever suggests that the *fhu* mutants would demonstrate decreased survival in a febrile model of GBS infection [161].

In a subsequent study, Mereghetti *et al.* examined changes in the GBS transcriptome during its adaptation to human blood [179]. As expected, rapid changes in gene expression (i.e., within 30 min) were observed when GBS were shifted from the laboratory growth media to human blood. A number of genes encoding proteins involved in cell division and cell-wall metabolism, including the CPS synthesis genes, were downregulated during GBS adaptation to human blood. Conversely, a substantial increase in the expression of genes encoding proteins related to stress adaptation, such as a universal stress protein, SodA, chaperones and proteases, was observed. Transcription of GBS factors, which play a role in adherence and cleavage of host ECM proteins, such as FbsA and BipA, were also significantly upregulated in human blood, with changes ranging from 1.9–6.9-fold. In addition, increased expression of up to ten transcriptional regulators was observed in human blood providing evidence for transcriptional regulation during GBS adaptation to different host environments [179].

The authors also examined the effect of temperature on GBS gene expression in human blood [179]. Interestingly, after the initial adaptation to human blood, the GBS transcriptome appeared fairly stable between 37–40°C. However, expression of genes encoding the pore-forming β-H/C and CAMP factor was reported to be slightly upregulated at 40°C in human blood compared with 37°C and ranged from 0.9–1.7-fold. Given this slight change in transcription and that human blood is an *exvivo* model of infection [179], further studies are required for a complete understanding of the regulation of toxins and other factors during GBS infection.

Conclusion & future perspective

The persistence of GBS infections in human newborns and in high-risk adult populations emphasizes the need for additional prevention and therapeutic measures. Although intrapartum antibiotic therapy has successfully decreased the incidence of EOD, these measures have not decreased or prevented LOD, GBS-related stillbirth and prematurity. Furthermore, these measures do not address prevention or treatment of GBS infection in human adults. The recent emergence of antibiotic-resistant GBS strains, particularly those resistant to penicillin, imposes a significant threat to the successful treatment of infections, as is now the case with the related Gram-positive pathogen *S. aureus*. Current strategies have focused on the development of effective vaccines for prevention of GBS infection [150]. However, it is important to remember that vaccines are not suitable for the treatment of infections. Consequently, identification of alternate drug targets is essential for future therapeutic measures against GBS disease. Elucidation of the molecular mechanisms that underlie GBS disease pathogenesis is pivotal for the identification of drug targets and also in the development of novel vaccines. The availability of the GBS genome has increased our understanding of factors that contribute to various facets of GBS infections. Molecular insights into the mechanism of action of some of these factors in GBS adherence and invasion of host cells and in evasion of host immunity have been elucidated **(**Table 1). Factors, such as the CPS, have been examined for their efficacy in the prevention of GBS infection and are in clinical trials. In addition, numerous other surface-associated virulence factors, including C5a peptidase and pili, have been tested for their ability to mount an immune response against GBS infection. These investigations are ongoing and will identify novel candidate vaccine targets.

Despite these advances, our understanding of the molecular events that dictate the transition of an otherwise commensal organism to an invasive pathogen is still at its infancy. In order to comprehend GBS pathogenesis, significant progress will have to be made to understand how this pathogen adapts to the various host environments encountered during its disease cycle. The nominal advances made in this area have already revealed the adaptable nature of GBS and novel paradigms that enable the pathogen to fine-tune gene expression for disease progression. In contrast to GBS, signaling responses for environmental adaptation have been extensively studied in related Gram-positive pathogens such as Group A Streptococcus and *S. aureus*. Extracellular signals perceived by some of the STS in these pathogens have also been identified and include changes in magnesium concentration, the presence of quorum sensing molecules and AMPs [56,134,180–182]. Furthermore, inhibitors targeted at STS to decrease bacterial resistance to antibiotics have also been explored [31,32]. The function of the GBS STS, such as the genes they regulate and the signals they perceive to enable successful environmental adaptation for infection and disease progression, are not completely understood. Given that antibiotic resistance is on the rise, advances in GBS environmental adaptation will be in the forefront as this knowledge is critical to effectively treat GBS infections. Many of these discoveries may also challenge existing paradigms to reveal the versatile nature of the pathogen. A deeper understanding of how GBS regulates expression of its virulence/survival factors, particularly for those being considered as vaccine or drug targets, is imperative for the success of alternate therapeutic strategies to combat this opportunistic pathogen that affects our most vulnerable populations.

Executive summary

Group B Streptococcus disease in human newborns & adults

- Group B Streptococcus (GBS) disease in human newborns manifests as early-onset disease or late-onset disease.
- Intrapartum antibiotic therapy has successfully reduced incidences of early-onset disease but has had no effect on incidences of late-onset disease, GBS-related stillbirth or prematurity.
- GBS is also a pathogen of adults. The elderly, immunocompromised and those with underlying conditions such as diabetes or malignanicies are particularly at risk.
- The emergence of GBS strains resistant to penicillin, clindamycin and erythromycin reinforces the need for the development of alternate prevention and therapeutic measures.

Regulation of virulence factors

- Although the GBS genome indicates the presence of multiple signal transduction systems, the roles of only a few are known.
- Transcription of the pore-forming toxins, sialic acid-rich capsular polysaccharide, fibrinogen-binding proteins, C5a peptidase, serine protease and other virulence factors are regulated by GBS signal transduction systems.
- GBS alters its gene expression to respond to changes in the external environment such as oxygen concentration, temperature and presence of human blood.

Regulation during infection

- GBS encodes a number of factors for adherence, invasion and dissemination within the host.

- A number of factors also promote immune evasion and nutrient acquisition during GBS infection.
- How GBS responds to its external environment to appropriately express this large repertoire of factors for colonization or invasion of the host are not understood.

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Bibliography

Papers of special note have been highlighted as:

- of interest

- of considerable interest

1. Berner R. Significance, management and prevention of *Streptococcus agalactiae* infection during the perinatal period. Expert Rev Anti Infect Ther 2004;2:427–437. [PubMed: 15482207]
2. van der Poll T, Opal SM. Host–pathogen interactions in sepsis. Lancet Infect Dis 2008;8:32–43. [PubMed: 18063412]
3. Campbell JR, Hillier SL, Krohn MA, Ferrieri P, Zaleznik DF, Baker CJ. Group B Streptococcal colonization and serotype-specific immunity in pregnant women at delivery. Obstet Gynecol 2000;96:498–503. [PubMed: 11004347]
4. Mullaney DM. Group B Streptococcal infections in newborns. J Obstet Gynecol Neonatal Nurs 2001;30:649–658.
5. Cowgill K, Taylor TH Jr, Schuchat A, Schrag S. Report from the CDC. Awareness of perinatal Group B Streptococcal infection among women of childbearing age in the United States, 1999 and 2002. J Womens Health (Larchmt) 2003;12:527–532. [PubMed: 13678508]
6. Lin FY, Weisman LE, Troendle J, Adams K. Prematurity is the major risk factor for late-onset Group B Streptococcus disease. J Infect Dis 2003;188:267–271. [PubMed: 12854082]
7. Schrag SJ, Zywicki S, Farley MM, et al. Group B Streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med 2000;342:15–20. [PubMed: 10620644]
8. Boyer KM, Vogel LC, Gotoff SP, Gadzala CA, Stringer J, Maxted WR. Nosocomial transmission of bacteriophage type 7/11/12 Group B Streptococci in a special care nursery. AMA Am J Dis Child 1980;134:964–966.
9. Tumbaga PF, Philip AGS. Perinatal Group B Streptococcal infections: past, present and future. NeoReviews 2003;4:65–72.
10. Pettersson K. Perinatal infection with Group B Streptococci. Semin Fetal Neonatal Med 2007;12:193–197. [PubMed: 17347062]
11. Dermer P, Lee C, Eggert J, Few B. A history of neonatal Group B Streptococcus with its related morbidity and mortality rates in the United States. J Pediatr Nurs 2004;19:357–363. [PubMed: 15614260]

12. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. Guidelines for prevention of Group B Streptococcal (GBS) infection by chemoprophylaxis. Pediatrics 1992;90:775–778. [PubMed: 1408555]

13. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. Revised guidelines for prevention of early-onset Group B Streptococcal (GBS) infection. Pediatrics 1997;99:489–496. [PubMed: 9041310]

14. Gibbs RS, Schrag S, Schuchat A. Perinatal infections due to Group B Streptococci. Obstet Gynecol 2004;104:1062–1076. [PubMed: 15516403]

15. Lukacs SL, Schoendorf KC, Schuchat A. Trends in sepsis-related neonatal mortality in the United States, 1985–1998. Pediatr Infect Dis J 2004;23:599–603. [PubMed: 15247595]

16. Edwards MS, Baker CJ. Group B Streptococcal infections in elderly adults. Clin Infect Dis 2005;41:839–847. [PubMed: 16107984]

17. Farley MM. Group B Streptococcal disease in nonpregnant adults. Clin Infect Dis 2001;33:556–561. [PubMed: 11462195]

18. Andrews JI, Diekema DJ, Hunter SK, et al. Group B Streptococci causing neonatal bloodstream infection: antimicrobial susceptibility and serotyping results from SENTRY centers in the western hemisphere. Am J Obstet Gynecol 2000;183:859–862. [PubMed: 11035326]

19. Bland ML, Vermillion ST, Soper DE, Austin M. Antibiotic resistance patterns of Group B Streptococci in late third-trimester rectovaginal cultures. Am J Obstet Gynecol 2001;184:1125–1126. [PubMed: 11349176]

20. Simoes JA, Aroutcheva AA, Heimler I, Faro S. Antibiotic resistance patterns of Group B Streptococcal clinical isolates. Infect Dis Obstet Gynecol 2004;12:1–8. [PubMed: 15460188]

21. Heelan JS, Hasenbein ME, McAdam AJ. Resistance of Group B Streptococcus to selected antibiotics, including erythromycin and clindamycin. J Clin Microbiol 2004;42:1263–1264. [PubMed: 15004089]

22▪. Dahesh S, Hensler ME, van Sorge NM, et al. Point mutation in the Group B Streptococcal *pbp2x* gene conferring decreased susceptibility to β-lactam antibiotics. Antimicrob Agents Chemother 2008;52:2915–2918. [PubMed: 18541727]Along with [23] and [24], reports on the mechanisms of spontaneous resistance to penicillin in Group B Streptococci (GBS)

23▪. Kimura K, Suzuki S, Wachino J, et al. First molecular characterization of Group B Streptococci with reduced penicillin susceptibility. Antimicrob Agents Chemother 2008;52:2890–2897. [PubMed: 18490507]Along with [22] and [24], reports on the mechanisms of spontaneous resistance to penicillin in GBS

24▪. Nagano N, Nagano Y, Kimura K, Tamai K, Yanagisawa H, Arakawa Y. Genetic heterogeneity in *pbp* genes among clinically isolated Group B Streptococci with reduced penicillin susceptibility. Antimicrob Agents Chemother 2008;52(12):4258–4267. [PubMed: 18809936]Along with [22] and [23], reports on the mechanisms of spontaneous resistance to penicillin in GBS

25. Cieslewicz MJ, Chaffin D, Glusman G, et al. Structural and genetic diversity of Group B Streptococcus capsular polysaccharides. Infect Immun 2005;73:3096–3103. [PubMed: 15845517]

26. Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a proposed new *Streptococcus agalactiae* serotype. J Clin Microbiol 2007;45:2929–2936. [PubMed: 17634306]

27. Tettelin H, Masignani V, Cieslewicz MJ, et al. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial ‘pan-genome’. Proc Natl Acad Sci USA 2005;102:13950–13955. [PubMed: 16172379]

28▪. Maisey HC, Doran KS, Nizet V. Recent advances in understanding the molecular basis of Group B Streptococcus virulence. Expert Rev Mol Med 2008;10:e27. [PubMed: 18803886]Comprehensive review on GBS pathogenesis with an emphasis on its interactions with the host

29. Miller SI, Mekalanos JJ. Constitutive expression of the *phoP* regulon attenuates *Salmonella* virulence and survival within macrophages. J Bacteriol 1990;172:2485–2490. [PubMed: 2185222]

30. Mouslim C, Delgado M, Groisman EA. Activation of the RcsC/YojN/RcsB phosphorelay system attenuates *Salmonella* virulence. Mol Microbiol 2004;54:386–395. [PubMed: 15469511]

31. Stephenson K, Hoch JA. Virulence- and antibiotic resistance-associated two-component signal transduction systems of Gram-positive pathogenic bacteria as targets for antimicrobial therapy. Pharmacol Ther 2002;93:293–305. [PubMed: 12191621]

32. Johnson CD, Liu LX. Novel antimicrobial targets from combined pathogen and host genetics. Proc Natl Acad Sci USA 2000;97:958–959. [PubMed: 10655466]

33. Cohen P. Protein kinases – the major drug targets of the twenty-first century? Nat Rev Drug Discov 2002;1:309–315. [PubMed: 12120282]

34. Hoch, J.; Silhavy, TJ. Two-Component Signal Transduction. ASM Press; VA, USA: 1995.

35. Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. Mol Microbiol 2002;45:1499–1513. [PubMed: 12354221]

36. Tettelin H, Masignani V, Cieslewicz MJ, et al. Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V *Streptococcus agalactiae*. Proc Natl Acad Sci USA 2002;99:12391–12396. [PubMed: 12200547]

37▪▪. Lamy MC, Zouine M, J Fert, et al. CovS/CovR of Group B Streptococcus: a two-component global regulatory system involved in virulence. Mol Microbiol 2004;54:1250–1268. [PubMed: 15554966] One of two independent studies on the CovR/S system in GBS

38▪▪. Jiang SM, Cieslewicz MJ, Kasper DL, Wessels MR. Regulation of virulence by a two-component system in Group B Streptococcus. J Bacteriol 2005;187:1105–1113. [PubMed: 15659687]One of two independent studies on the CovR/S system in GBS

39. Jiang SM, Ishmael N, Hotopp JD, et al. Variation in the Group B Streptococcus CsrRS regulon and effects on pathogenicity. J Bacteriol 2008;190:1956–1965. [PubMed: 18203834]

40. Poyart C, Lamy MC, Boumaila C, Fiedler F, Trieu-Cuot P. Regulation of D-alanyl-lipotechoic acid biosynthesis in *Streptococcus agalactiae* involves a novel two-component regulatory system. J Bacteriol 2001;183:6324–6334. [PubMed: 11591677]

41▪▪. Poyart C, Pellegrini E, Marceau M, et al. Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid is due to an increased susceptibility to defensins and phagocytic cells. Mol Microbiol 2003;49:1615–1625. [PubMed: 12950925]Interesting article on regulation of GBS resistance to antimicrobial peptides by the two-component systems DltR/S

42▪. Spellerberg B, Rozdzinski E, Martin S, Weber-Heynemann J, Lutticken R. *rgf* encodes a novel two-component signal transduction system of *Streptococcus agalactiae*. Infect Immun 2002;70:2434–2440. [PubMed: 11953380]Describes regulation of C5a peptidase by RgfB/C

43▪. Quach D, van Sorge NM, Kristian SA, Bryan JD, Shelver DW, Doran KS. The CiaR response regulator in Group B Streptococcus promotes intracellular survival and resistance to innate immune defenses. J Bacteriol. 2008(Epub ahead of print). Describes the role of CiaR/H in GBS virulence

44. Ulrich LE, Koonin EV, Zhulin IB. One-component systems dominate signal transduction in prokaryotes. Trends Microbiol 2005;13:52–56. [PubMed: 15680762]

45. Kreikemeyer B, McIver KS, Podbielski A. Virulence factor regulation and regulatory networks in *Streptococcus pyogenes* and their impact on pathogen–host interactions. Trends Microbiol 2003;11:224–232. [PubMed: 12781526]

46. Shelver D, Rajagopal L, Harris TO, Rubens CE. MtaR, a regulator of methionine transport, is critical for survival of Group B Streptococcus *in vivo*. J Bacteriol 2003;185:6592–6599. [PubMed: 14594832]

47▪. Bryan JD, Liles R, Cvek U, Trutschl M, Shelver D. Global transcriptional profiling reveals *Streptococcus agalactiae* genes controlled by the MtaR transcription factor. BMC Genomics 2008;9:607. [PubMed: 19087320]Reports genes regulated by MtaR

48▪. Gutekunst H, Eikmanns BJ, Reinscheid DJ. Analysis of RogB-controlled virulence mechanisms and gene repression in *Streptococcus agalactiae*. Infect Immun 2003;71:5056–5064. [PubMed: 12933848]Describes regulation of CpsA and FbsA by RogB

49▪. Samen UM, Eikmanns BJ, Reinscheid DJ. The transcriptional regulator RovS controls the attachment of *Streptococcus agalactiae* to human epithelial cells and the expression of virulence genes. Infect Immun 2006;74:5625–5635. [PubMed: 16988238]Describes regulation of CylE, SodA and FbsA by RovS

50. Rajagopal L, Clancy A, Rubens CE. A eukaryotic type serine/threonine kinase and phosphatase in *Streptococcus agalactiae* reversibly phosphorylate an inorganic pyrophosphatase and affect growth, cell segregation, and virulence. J Biol Chem 2003;278:14429–14441. [PubMed: 12562757]

51. Rajagopal L, Vo A, Silvestroni A, Rubens CE. Regulation of purine biosynthesis by a eukaryotic-type kinase in *Streptococcus agalactiae*. Mol Microbiol 2005;56:1329–1346. [PubMed: 15882424]

52. Rajagopal L, Vo A, Silvestroni A, Rubens CE. Regulation of cytotoxin expression by converging eukaryotic-type and two-component signaling mechanisms in *Streptococcus agalactiae*. Mol Microbiol 2006;62:941–957. [PubMed: 17005013]

53▪. Lin WJ, Walthers D, Connelly JE, et al. Threonine phosphorylation prevents promoter DNA binding of the Group B Streptococcus response regulator CovR. Mol Microbiol. 2009(Epub ahead of print). Elucidates the link between two component (CovR) and serine/threonine kinase (Stk1) signaling systems in GBS

54. Churchward G. The two faces of Janus: virulence gene regulation by CovR/S in Group A Streptococci. Mol Microbiol 2007;64:34–41. [PubMed: 17376070]

55. Hondorp ER, McIver KS. The Mga virulence regulon: infection where the grass is greener. Mol Microbiol 2007;66:1056–1065. [PubMed: 18001346]

56. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 2003;48:1429–1449. [PubMed: 12791129]

57. Novick RP. Medicine Combating impervious bugs. Science 2008;319:910–911. [PubMed: 18276877]

58. Aroian R, van der Goot FG. Pore-forming toxins and cellular non-immune defenses (CNIDs). Curr Opin Microbiol 2007;10:57–61. [PubMed: 17234446]

59. Gonzalez MR, Bischofberger M, Pernot L, van der Goot FG, Freche B. Bacterial pore-forming toxins: the (w)hole story? Cell Mol Life Sci 2008;65:493–507. [PubMed: 17989920]

60. Nizet V, Gibson RL, Chi EY, Framson PE, Hulse M, Rubens CE. Group B Streptococcal β-hemolysin expression is associated with injury of lung epithelial cells. Infect Immun 1996;64:3818–3826. [PubMed: 8751934]

61. Nizet V, Gibson RL, Rubens CE. The role of Group B Streptococci β-hemolysin expression in newborn lung injury. Adv Exp Med Biol 1997;418:627–630. [PubMed: 9331730]

62. Gibson RL, Nizet V, Rubens CE. Group B Streptococcal β-hemolysin promotes injury of lung microvascular endothelial cells. Pediatr Res 1999;45:626–634. [PubMed: 10231855]

63. Doran KS, Chang JC, Benoit VM, Eckmann L, Nizet V. Group B Streptococcal β-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleukin-8. J Infect Dis 2002;185:196–203. [PubMed: 11807693]

64. Doran KS, Liu GY, Nizet V. Group B Streptococcal β-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. J Clin Invest 2003;112:736–744. [PubMed: 12952922]

65. Hensler ME, Miyamoto S, Nizet V. Group B Streptococcal β-hemolysin/cytolysin directly impairs cardiomyocyte viability and function. PLoS ONE 2008;3:e2446. [PubMed: 18560574]

66. Ring A, Braun JS, Pohl J, Nizet V, Stremmel W, Shenep JL. Group B Streptococcal β-hemolysin induces mortality and liver injury in experimental sepsis. J Infect Dis 2002;185:1745–1753. [PubMed: 12085320]

67. Ring A, Braun JS, Nizet V, Stremmel W, Shenep JL. Group B Streptococcal β-hemolysin induces nitric oxide production in murine macrophages. J Infect Dis 2000;182:150–157. [PubMed: 10882592]

68. Hensler ME, Liu GY, Sobczak S, Benirschke K, Nizet V, Heldt GP. Virulence role of Group B Streptococcus β-hemolysin/cytolysin in a neonatal rabbit model of early-onset pulmonary infection. J Infect Dis 2005;191:1287–1291. [PubMed: 15776375]

69. Puliti M, Nizet V, von Hunolstein C, et al. Severity of Group B Streptococcal arthritis is correlated with β-hemolysin expression. J Infect Dis 2000;182:824–832. [PubMed: 10950777]

70. Pritzlaff CA, Chang JC, Kuo SP, Tamura GS, Rubens CE, Nizet V. Genetic basis for the β-haemolytic/cytolytic activity of Group B Streptococcus. Mol Microbiol 2001;39:236–247. [PubMed: 11136446]

71. Spellerberg B, Pohl B, Haase G, Martin S, Weber-Heynemann J, Lutticken R. Identification of genetic determinants for the hemolytic activity of *Streptococcus agalactiae* by ISS1 transposition. J Bacteriol 1999;181:3212–3219. [PubMed: 10322024]

72. Forquin MP, Tazi A, Rosa-Fraile M, Poyart C, Trieu-Cuot P, Dramsi S. The putative glycosyltransferase-encoding gene *cylJ* and the Group B Streptococcus (GBS)-specific gene *cylK* modulate hemolysin production and virulence of GBS. Infect Immun 2007;75:2063–2066. [PubMed: 17283108]

73. Gottschalk B, Broker G, Kuhn M, Aymanns S, Gleich-Theurer U, Spellerberg B. Transport of multidrug resistance substrates by the *Streptococcus agalactiae* hemolysin transporter. J Bacteriol 2006;188:5984–5992. [PubMed: 16885467]
74. Nizet V. Streptococcal β-hemolysins: genetics and role in disease pathogenesis. Trends Microbiol 2002;10:575–580. [PubMed: 12564994]
75. Spellerberg B, Martin S, Brandt C, Lutticken R. The *cyl* genes of *Streptococcus agalactiae* are involved in the production of pigment. FEMS Microbiol Lett 2000;188:125–128. [PubMed: 10913694]
76. Tapsall JW. Pigment production by Lancefield-Group-B Streptococci (*Streptococcus agalactiae*). J Med Microbiol 1986;21:75–81. [PubMed: 3512836]
77. Liu GY, Doran KS, Lawrence T, et al. Sword and shield: linked Group B Streptococcal β-hemolysin/cytolysin and carotenoid pigment function to subvert host phagocyte defense. Proc Natl Acad Sci USA 2004;101:14491–14496. [PubMed: 15381763]
78. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol 2004;2:820–832. [PubMed: 15378046]
79. Rosa-Fraile M, Rodriguez-Granger J, Haidour-Benamin A, Cuerva JM, Sampedro A. Granadaene: proposed structure of the GroupB Streptococcus polyenic pigment. Appl Environ Microbiol 2006;72:6367–6370. [PubMed: 16957264]
80. Krinsky NI, Yeum KJ. Carotenoid-radical interactions. Biochem Biophys Res Commun 2003;305:754–760. [PubMed: 12763057]
81. Mathews MM, Sistrom WR. Function of carotenoid pigments in non-photosynthetic bacteria. Nature 1959;184(Suppl 24):1892–1893. [PubMed: 14422277]
82. Rajagopal L, Sundari CS, Balasubramanian D, Sonti RV. The bacterial pigment xanthomonadin offers protection against photodamage. FEBS Lett 1997;415:125–128. [PubMed: 9350981]
83▪. Ulijasz AT, Falk SP, Weisblum B. Phosphorylation of the RitR DNA-binding domain by a Ser–Thr phosphokinase: implications for global gene regulation in the streptococci. Mol Microbiol 2008;71(2):382–390. [PubMed: 19040630]Recent evidence for the link between response regulators and ser/thr kinases in other streptococci
84. Lang S, Palmer M. Characterization of *Streptococcus agalactiae* CAMP factor as a pore-forming toxin. J Biol Chem 2003;278:38167–38173. [PubMed: 12835325]
85. Podbielski A, Blankenstein O, Lutticken R. Molecular characterization of the *cfb* gene encoding Group B Streptococcal CAMP-factor. Med Microbiol Immunol (Berl) 1994;183:239–256. [PubMed: 7715536]
86. Jurgens D, Sterzik B, Fehrenbach FJ. Unspecific binding of Group B Streptococcal cocytolysin (CAMP factor) to immunoglobulins and its possible role in pathogenicity. J Exp Med 1987;165:720–732. [PubMed: 3546580]
87. Skalka B, Smola J. Lethal effect of CAMP-factor and UBERIS-factor – a new finding about diffusible exosubstances of *Streptococcus agalactiae* and *Streptococcus uberis*. Zentralbl Bakteriol A 1981;249:190–194. [PubMed: 7023125]
88. Lang S, Palmer M. Characterization of *Streptococcus agalactiae* CAMP factor as a pore-forming toxin. J Biol Chem 2003;278:38167–38173. [PubMed: 12835325]
89. Lang S, Xue J, Guo Z, Palmer M. *Streptococcus agalactiae* CAMP factor binds to GPI-anchored proteins. Med Microbiol Immunol 2007;196:1–10. [PubMed: 16773378]
90▪. Hensler ME, Quach D, Hsieh CJ, Doran KS, Nizet V. CAMP factor is not essential for systemic virulence of Group B Streptococcus. Microb Pathog 2008;44:84–88. [PubMed: 17870297] Examined virulence properties of a Christie Atkins Munch Peterson factor deficient GBS strain
91. Jiang SM, Ishmael N, Hotopp JD, et al. Variation in the Group B Streptococcus CsrRS regulon and effects on pathogenicity. J Bacteriol 2008;190:1956–1965. [PubMed: 18203834]
92. Marques MB, Kasper DL, Pangburn MK, Wessels MR. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III Group B Streptococci. Infect Immun 1992;60:3986–3993. [PubMed: 1398910]
93. Campbell JR, Baker CJ, Edwards MS. Deposition and degradation of C3 on type III Group B Streptococci. Infect Immun 1991;59:1978–1983. [PubMed: 2037359]

94. Baker CJ, Kasper DL. Correlation of maternal antibody deficiency with susceptibility to neonatal Group B Streptococcal infection. N Engl J Med 1976;294:753–756. [PubMed: 768760]

95. Rubens CE, Wessels MR, Heggen LM, Kasper DL. Transposon mutagenesis of type III Group B Streptococcus: correlation of capsule expression with virulence. Proc Natl Acad Sci USA 1987;84:7208–7212. [PubMed: 2823254]

96. Wessels MR, Rubens CE, Benedi VJ, Kasper DL. Definition of a bacterial virulence factor: sialylation of the Group B Streptococcal capsule. Proc Natl Acad Sci USA 1989;86:8983–8987. [PubMed: 2554337]

97. Martin TR, Ruzinski JT, Rubens CE, Chi EY, Wilson CB. The effect of type-specific polysaccharide capsule on the clearance of Group B Streptococci from the lungs of infant and adult rats. J Infect Dis 1992;165:306–314. [PubMed: 1730897]

98. Wessels MR, Haft RF, Heggen LM, Rubens CE. Identification of a genetic locus essential for capsule sialylation in type III Group B Streptococci. Infect Immun 1992;60:392–400. [PubMed: 1309720]

99. Marques MB, Kasper DL, Shroff A, Michon F, Jennings HJ, Wessels MR. Functional activity of antibodies to the group B polysaccharide of Group B Streptococci elicited by a polysaccharide-protein conjugate vaccine. Infect Immun 1994;62:1593–1599. [PubMed: 8168919]

100. Paoletti LC, Kasper DL. Glycoconjugate vaccines to prevent Group B Streptococcal infections. Expert Opin Biol Ther 2003;3:975–984. [PubMed: 12943456]

101. Paoletti LC, Kasper DL, Michon F, et al. An oligosaccharide-tetanus toxoid conjugate vaccine against type III Group B Streptococcus. J Biol Chem 1990;265:18278–18283. [PubMed: 2120228]

102. Paoletti LC, Kasper DL. Conjugate vaccines against Group B Streptococcus types IV and VII. J Infect Dis 2002;186:123–126. [PubMed: 12089673]

103. Johri AK, Paoletti LC, Glaser P, et al. Group B Streptococcus: global incidence and vaccine development. Nat Rev Microbiol 2006;4:932–942. [PubMed: 17088932]

104. Edwards MS. Group B Streptococcal conjugate vaccine: a timely concept for which the time has come. Hum Vaccin 2008;4:444–448. [PubMed: 18728401]

105. Paoletti LC, Guttormsen HK, Christian MS, Hoberman AM, McInnes P. Neither antibody to a Group B Streptococcal conjugate vaccine nor the vaccine itself is teratogenic in rabbits. Hum Vaccin 2008;4:435–443. [PubMed: 18443428]

106▪. Lewis AL, Nizet V, Varki A. Discovery and characterization of sialic acid *O*-acetylation in Group B Streptococcus. Proc Natl Acad Sci USA 2004;101:11123–11128. [PubMed: 15263085]First report that describes *O*-acetylation of the GBS capsular polysaccharide

107. Berry DS, Lynn F, Lee CH, Frasch CE, Bash MC. Effect of *O*-acetylation of *Neisseria meningitidis* serogroup A capsular polysaccharide on development of functional immune responses. Infect Immun 2002;70:3707–3713. [PubMed: 12065513]

108. McNeely TB, Staub JM, Rusk CM, Blum MJ, Donnelly JJ. Antibody responses to capsular polysaccharide backbone and *O*-acetate side groups of *Streptococcus pneumoniae* type 9V in humans and rhesus macaques. Infect Immun 1998;66:3705–3710. [PubMed: 9673252]

109. Lewis AL, Cao H, Patel SK, et al. NeuA sialic acid *O*-acetylesterase activity modulates *O*-acetylation of capsular polysaccharide in Group B Streptococcus. J Biol Chem 2007;282:27562–27571. [PubMed: 17646166]

110. Hulse ML, Smith S, Chi EY, Pham A, Rubens CE. Effect of type III Group B Streptococcal capsular polysaccharide on invasion of respiratory epithelial cells. Infect Immun 1993;61:4835–4841. [PubMed: 8406885]

111. Tamura GS, Kuypers JM, Smith S, Raff H, Rubens CE. Adherence of Group B Streptococci to cultured epithelial cells: roles of environmental factors and bacterial surface components. Infect Immun 1994;62:2450–2458. [PubMed: 8188370]

112. Nizet V, Kim KS, Stins M, et al. Invasion of brain microvascular endothelial cells by Group B Streptococci. Infect Immun 1997;65:5074–5081. [PubMed: 9393798]

113. Paoletti LC, Ross RA, Johnson KD. Cell growth rate regulates expression of Group B Streptococcus type III capsular polysaccharide. Infect Immun 1996;64:1220–1226. [PubMed: 8606082]

114. Ross RA, Madoff LC, Paoletti LC. Regulation of cell component production by growth rate in the Group B Streptococcus. J Bacteriol 1999;181:5389–5394. [PubMed: 10464211]

115. Johri AK, Patwardhan V, Paoletti LC. Growth rate and oxygen regulate the interactions of Group B Streptococcus with polarized respiratory epithelial cells. Can J Microbiol 2005;51:283–286. [PubMed: 15980889]
116. Malin G, Paoletti LC. Use of a dynamic *in vitro* attachment and invasion system (DIVAS) to determine influence of growth rate on invasion of respiratory epithelial cells by Group B Streptococcus. Proc Natl Acad Sci USA 2001;98:13335–13340. [PubMed: 11687649]
117. Poyart C, Pellegrini E, Gaillot O, Boumaila C, Baptista M, Trieu-Cuot P. Contribution of Mn-cofactored superoxide dismutase (SodA) to the virulence of *Streptococcus agalactiae*. Infect Immun 2001;69:5098–5106. [PubMed: 11447191]
118. Takahashi S, Nagano Y, Nagano N, Hayashi O, Taguchi F, Okuwaki Y. Role of C5a-ase in Group B Streptococcal resistance to opsonophagocytic killing. Infect Immun 1995;63:4764–4769. [PubMed: 7591133]
119. Bohnsack JF, Widjaja K, Ghazizadeh S, et al. A role for C5 and C5a-ase in the acute neutrophil response to Group B Streptococcal infections. J Infect Dis 1997;175:847–855. [PubMed: 9086140]
120. Cheng Q, Stafslien D, Purushothaman SS, Cleary P. The Group B Streptococcal C5a peptidase is both a specific protease and an invasin. Infect Immun 2002;70:2408–2413. [PubMed: 11953377]
121. Beckmann C, Waggoner JD, Harris TO, Tamura GS, Rubens CE. Identification of novel adhesins from Group B Streptococci by use of phage display reveals that C5a peptidase mediates fibronectin binding. Infect Immun 2002;70:2869–2876. [PubMed: 12010974]
122. Tamura GS, Hull JR, Oberg MD, Castner DG. High-affinity interaction between fibronectin and the Group B Streptococcal C5a peptidase is unaffected by a naturally occurring four-amino-acid deletion that eliminates peptidase activity. Infect Immun 2006;74:5739–5746. [PubMed: 16988251]
123. Hull JR, Tamura GS, Castner DG. Interactions of the streptococcal C5a peptidase with human fibronectin. Acta Biomater 2008;4:504–513. [PubMed: 18313373]
124. Cheng Q, Debol S, Lam H, et al. Immunization with C5a peptidase or peptidase-type III polysaccharide conjugate vaccines enhances clearance of Group B Streptococci from lungs of infected mice. Infect Immun 2002;70:6409–6415. [PubMed: 12379721]
125. Santillan DA, Andracki ME, Hunter SK. Protective immunization in mice against Group B Streptococci using encapsulated C5a peptidase. Am J Obstet Gynecol 2008;198:114 e1–e6. [PubMed: 17905172]
126. Harris TO, Shelver DW, Bohnsack JF, Rubens CE. A novel streptococcal surface protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen. J Clin Invest 2003;111:61–70. [PubMed: 12511589]
127. Bryan JD, Shelver DW. *Streptococcus agalactiae* CspA is a serine protease that inactivates chemokines. J Bacteriol. 2008(Epub ahead of print)
128. Dramsi S, Caliot E, Bonne I, et al. Assembly and role of pili in Group B Streptococci. Mol Microbiol 2006;60:1401–1413. [PubMed: 16796677]
129. Nobbs AH, Rosini R, Rinaudo CD, Maione D, Grandi G, Telford JL. Sortase A utilizes an ancillary protein anchor for efficient cell wall anchoring of pili in *Streptococcus agalactiae*. Infect Immun 2008;76:3550–3560. [PubMed: 18541657]
130. Lalioui L, Pellegrini E, Dramsi S, et al. The SrtA sortase of *Streptococcus agalactiae* is required for cell wall anchoring of proteins containing the LPXTG motif, for adhesion to epithelial cells, and for colonization of the mouse intestine. Infect Immun 2005;73:3342–3350. [PubMed: 15908360]
131. Hancock RE, Diamond G. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol 2000;8:402–410. [PubMed: 10989307]
132. Nizet V, Gallo RL. Surviving innate immunity. Trends Microbiol 2002;10:358–359. [PubMed: 12160630]
133. Peschel A. How do bacteria resist human antimicrobial peptides? Trends Microbiol 2002;10:179–186. [PubMed: 11912025]
134. Gryllos I, Tran-Winkler HJ, Cheng MF, et al. Induction of Group A Streptococcus virulence by a human antimicrobial peptide. Proc Natl Acad Sci USA 2008;105:16755–16760. [PubMed: 18936485]

135. Froehlich BJ, Bates C, Scott JR. *Streptococcus pyogenes* CovR/S mediates growth in iron starvation and in the presence of the human cationic antimicrobial peptide LL-37. J Bacteriol 2009;191(2): 673–677. [PubMed: 18996992]

136. Spratt BG, Cromie KD. Penicillin-binding proteins of gram-negative bacteria. Rev Infect Dis 1988;10:699–711. [PubMed: 3055170]

137. Georgopapadakou NH, Liu FY. Penicillin-binding proteins in bacteria. Antimicrob Agents Chemother 1980;18:148–157. [PubMed: 7416741]

138. Zapun A, Contreras-Martel C, Vernet T. Penicillin-binding proteins and β-lactam resistance. FEMS Microbiol Rev 2008;32:361–385. [PubMed: 18248419]

139. Jones AL, Needham RH, Clancy A, Knoll KM, Rubens CE. Penicillin-binding proteins in *Streptococcus agalactiae*: a novel mechanism for evasion of immune clearance. Mol Microbiol 2003;47:247–256. [PubMed: 12492868]

140. Jones AL, Knoll KM, Rubens CE. Identification of *Streptococcus agalactiae* virulence genes in the neonatal rat sepsis model using signature-tagged mutagenesis. Mol Microbiol 2000;37:1444–1455. [PubMed: 10998175]

141. Jones AL, Mertz RH, Carl DJ, Rubens CE. A streptococcal penicillin-binding protein is critical for resisting innate airway defenses in the neonatal lung. J Immunol 2007;179:3196–3202. [PubMed: 17709535]

142. Hamilton A, Popham DL, Carl DJ, Lauth X, Nizet V, Jones AL. Penicillin-binding protein 1a promotes resistance of Group B Streptococcus to antimicrobial peptides. Infect Immun 2006;74:6179–6187. [PubMed: 17057092]

143. Lauer P, Rinaudo CD, Soriani M, et al. Genome analysis reveals pili in Group B Streptococcus. Science 2005;309:105. [PubMed: 15994549]

144. Pezzicoli A, Santi I, Lauer P, et al. Pilus backbone contributes to Group B Streptococcus paracellular translocation through epithelial cells. J Infect Dis 2008;198:890–898. [PubMed: 18694342]

145. Maisey HC, Hensler M, Nizet V, Doran KS. Group B Streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells. J Bacteriol 2007;189:1464–1467. [PubMed: 17041051]

146. Maisey HC, Quach D, Hensler ME, et al. A Group B Streptococcal pilus protein promotes phagocyte resistance and systemic virulence. FASEB J 2008;22:1715–1724. [PubMed: 18198218]

147. Rosini R, Rinaudo CD, Soriani M, et al. Identification of novel genomic islands coding for antigenic pilus-like structures in *Streptococcus agalactiae*. Mol Microbiol 2006;61:126–141. [PubMed: 16824100]

148. Buccato S, Maione D, Rinaudo CD, et al. Use of *Lactococcus lactis* expressing pili from Group B Streptococcus as a broad-coverage vaccine against streptococcal disease. J Infect Dis 2006;194:331–340. [PubMed: 16826481]

149. Telford JL, Barocchi MA, Margarit I, Rappuoli R, Grandi G. Pili in Gram-positive pathogens. Nat Rev Microbiol 2006;4:509–519. [PubMed: 16778837]

150▪. Maione D, Margarit I, Rinaudo CD, et al. Identification of a universal Group B Streptococcus vaccine by multiple genome screen. Science 2005;309:148–150. [PubMed: 15994562]Explores cell surface proteins as a universal vaccine for GBS

151. Schubert A, Zakikhany K, Schreiner M, et al. A fibrinogen receptor from Group B Streptococcus interacts with fibrinogen by repetitive units with novel ligand binding sites. Mol Microbiol 2002;46:557–569. [PubMed: 12406229]

152. Jacobsson K. A novel family of fibrinogen-binding proteins in *Streptococcus agalactiae*. Vet Microbiol 2003;96:103–113. [PubMed: 14516712]

153. Gutekunst H, Eikmanns BJ, Reinscheid DJ. The novel fibrinogen-binding protein FbsB promotes *Streptococcus agalactiae* invasion into epithelial cells. Infect Immun 2004;72:3495–3504. [PubMed: 15155657]

154. Spellerberg B, Rozdzinski E, Martin S, et al. Lmb, a protein with similarities to the LraI adhesin family, mediates attachment of *Streptococcus agalactiae* to human laminin. Infect Immun 1999;67:871–878. [PubMed: 9916102]

155. Tenenbaum T, Spellerberg B, Adam R, Vogel M, Kim KS, Schroten H. *Streptococcus agalactiae* invasion of human brain microvascular endothelial cells is promoted by the laminin-binding protein Lmb. Microbes Infect 2007;9:714–720. [PubMed: 17400016]

156. Siboo IR, Chambers HF, Sullam PM. Role of SraP, a serine-rich surface protein of *Staphylococcus aureus*, in binding to human platelets. Infect Immun 2005;73:2273–2280. [PubMed: 15784571]

157. Takamatsu D, Bensing BA, Cheng H, et al. Binding of the *Streptococcus gordonii* surface glycoproteins GspB and Hsa to specific carbohydrate structures on platelet membrane glycoprotein Ibα. Mol Microbiol 2005;58:380–392. [PubMed: 16194227]

158. Seifert KN, Adderson EE, Whiting AA, Bohnsack JF, Crowley PJ, Brady LJ. A unique serine-rich repeat protein (Srr-2) and novel surface antigen (ε) associated with a virulent lineage of serotype III *Streptococcus agalactiae*. Microbiology 2006;152:1029–1040. [PubMed: 16549667]

159. Samen U, Eikmanns BJ, Reinscheid DJ, Borges F. The surface protein Srr-1 of *Streptococcus agalactiae* binds human keratin 4 and promotes adherence to epithelial HEp-2 cells. Infect Immun 2007;75:5405–5414. [PubMed: 17709412]

160. Santi I, Scarselli M, Mariani M, et al. BibA: a novel immunogenic bacterial adhesin contributing to Group B Streptococcus survival in human blood. Mol Microbiol 2007;63:754–767. [PubMed: 17212592]

161▪. Mereghetti L, Sitkiewicz I, Green NM, Musser JM. Remodeling of the *Streptococcus agalactiae* transcriptome in response to growth temperature. PLoS ONE 2008;3:e2785. [PubMed: 18665215] Adaptation of GBS to conditions that mimic fever

162. Michel JL, Madoff LC, Kling DE, Kasper DL, Ausubel FM. Cloned α and β C-protein antigens of Group B Streptococci elicit protective immunity. Infect Immun 1991;59:2023–2028. [PubMed: 1674738]

163. Johnson DR, Ferrieri P. Group B Streptococcal Ibc protein antigen: distribution of two determinants in wild-type strains of common serotypes. J Clin Microbiol 1984;19:506–510. [PubMed: 6201506]

164. Baron MJ, Bolduc GR, Goldberg MB, Auperin TC, Madoff LC. αC protein of Group B Streptococcus binds host cell surface glycosaminoglycan and enters cells by an actin-dependent mechanism. J Biol Chem 2004;279:24714–24723. [PubMed: 15044471]

165. Baron MJ, Filman DJ, Prophete GA, Hogle JM, Madoff LC. Identification of a glycosaminoglycan binding region of the αC protein that mediates entry of Group B Streptococci into host cells. J Biol Chem 2007;282:10526–10536. [PubMed: 17259175]

166. Bolduc GR, Madoff LC. The Group B Streptococcal α C protein binds α1β1-integrin through a novel KTD motif that promotes internalization of GBS within human epithelial cells. Microbiology 2007;153:4039–4049. [PubMed: 18048918]

167. Doran KS, Engelson EJ, Khosravi A, et al. Blood–brain barrier invasion by Group B Streptococcus depends upon proper cell-surface anchoring of lipoteichoic acid. J Clin Invest 2005;115:2499–2507. [PubMed: 16138192]

168. Lin B, Hollingshead SK, Coligan JE, Egan ML, Baker JR, Pritchard DG. Cloning and expression of the gene for Group B Streptococcal hyaluronate lyase. J Biol Chem 1994;269:30113–30116. [PubMed: 7982914]

169. Gase K, Ozegowski J, Malke H. The *Streptococcus agalactiae hylB* gene encoding hyaluronate lyase: completion of the sequence and expression analysis. Biochimica Biophysica Acta 1998;1398:86–98.

170. Laurent TC, Fraser JR. Hyaluronan. FASEB J 1992;6:2397–2404. [PubMed: 1563592]

171. Lindahl U, Hook M. Glycosaminoglycans and their binding to biological macromolecules. Annu Rev Biochem 1978;47:385–417. [PubMed: 354500]

172. Li S, Jedrzejas MJ. Hyaluronan binding and degradation by *Streptococcus agalactiae* hyaluronate lyase. J Biol Chem 2001;276:41407–41416. [PubMed: 11527972]

173. Pritchard DG, Lin B, Willingham TR, Baker JR. Characterization of the Group B Streptococcal hyaluronate lyase. Arch Biochem Biophys 1994;315:431–437. [PubMed: 7986088]

174. Akhtar MS, Krishnan MY, Bhakuni V. Insights into the mechanism of action of hyaluronate lyase: role of C-terminal domain and Ca^{2+} in the functional regulation of enzyme. J Biol Chem 2006;281:28336–28344. [PubMed: 16854993]

175. Milligan TW, Baker CJ, Straus DC, Mattingly SJ. Association of elevated levels of extracellular neuraminidase with clinical isolates of type III Group B Streptococci. Infect Immun 1978;21:738–746. [PubMed: 361576]

176. Juul SE, Kinsella MG, Truog WE, Gibson RL, Redding GJ. Lung hyaluronan decreases during Group B Streptococcal pneumonia in neonatal piglets. American Am J Respir Crit Care Med 1996;153:1567–1570.

177▪. Johri AK, Margarit I, Broenstrup M, et al. Transcriptional and proteomic profiles of Group B Streptococcus type V reveal potential adherence proteins associated with high-level invasion. Infect Immun 2007;75:1473–1483. [PubMed: 17210664]Describes changes in GBS gene and protein expression under conditions that mimic invasion

178. Clancy A, Loar JW, Speziali CD, Oberg M, Heinrichs DE, Rubens CE. Evidence for siderophore-dependent iron acquisition in Group B Streptococcus. Mol Microbiol 2006;59:707–721. [PubMed: 16390461]

179▪▪. Mereghetti L, Sitkiewicz I, Green NM, Musser JM. Extensive adaptive changes occur in the transcriptome of *Streptococcus agalactiae* (Group B Streptococcus) in response to incubation with human blood. PLoS ONE 2008;3:e3143. [PubMed: 18769548]Adaptation of GBS to human blood

180. Gryllos I, Grifantini R, Colaprico A, et al. Mg^{2+} signalling defines the Group A Streptococcal CsrRS (CovRS) regulon. Mol Microbiol 2007;65:671–683. [PubMed: 17608796]

181. Mascher T, Helmann JD, Unden G. Stimulus perception in bacterial signal-transducing histidine kinases. Microbiol Mol Biol Rev 2006;70:910–938. [PubMed: 17158704]

182. Li M, Cha DJ, Lai Y, Villaruz AE, Sturdevant DE, Otto M. The antimicrobial peptide-sensing system aps of *Staphylococcus aureus*. Mol Microbiol 2007;66:1136–1147. [PubMed: 17961141]

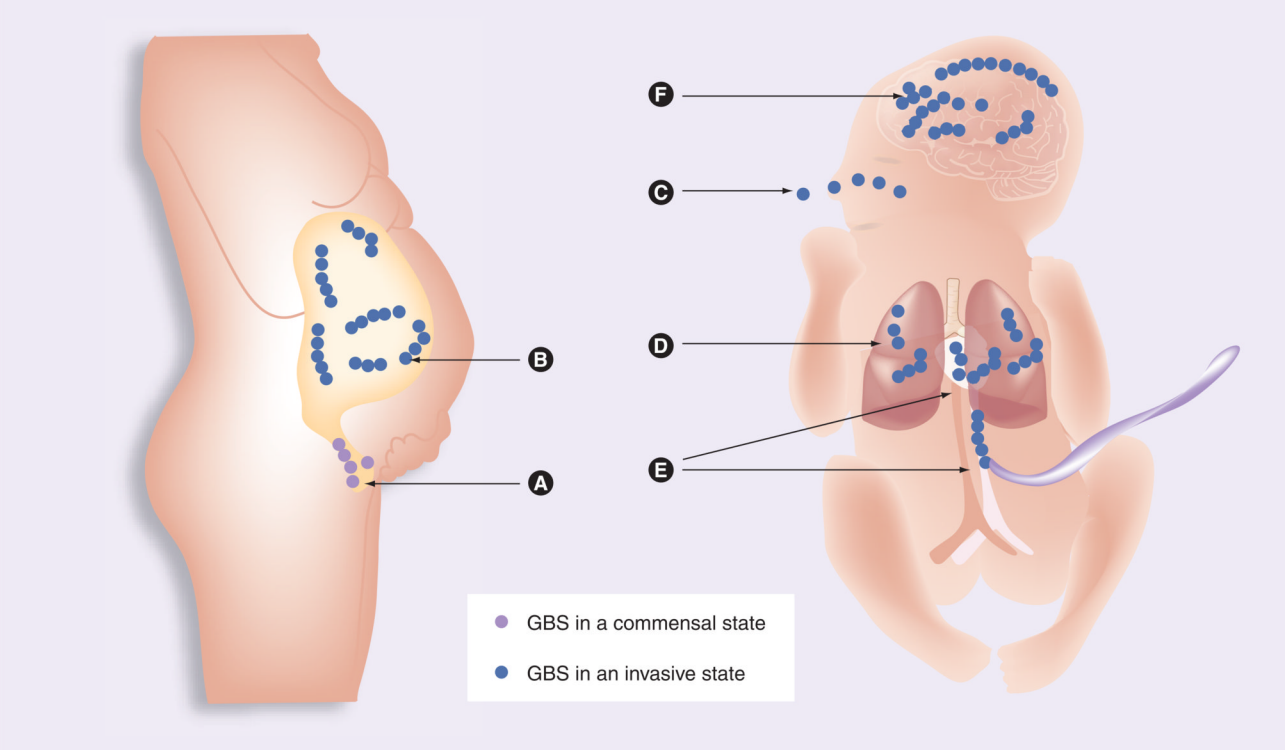

Figure 1. Lifecycle of Group B Streptococci as a neonatal pathogen

(A) GBS reside as a commensal in genital and lower gastrointestinal tracts of women. **(B)** GBS can infiltrate the intrauterine compartment in pregnant mothers who are asymptomatic carriers. **(C)** Newborn aspirate GBS in utero or during birth. **(D)** GBS invades the neonatal lung causing pneumonia. **(E)** From the lung, GBS gains access into the bloodstream of the neonate causing sepsis and invades multiple neonatal organs including the heart **(F)** GBS penetrates the blood–brain barrier causing meningitis.

GBS: Group B Streptococci.

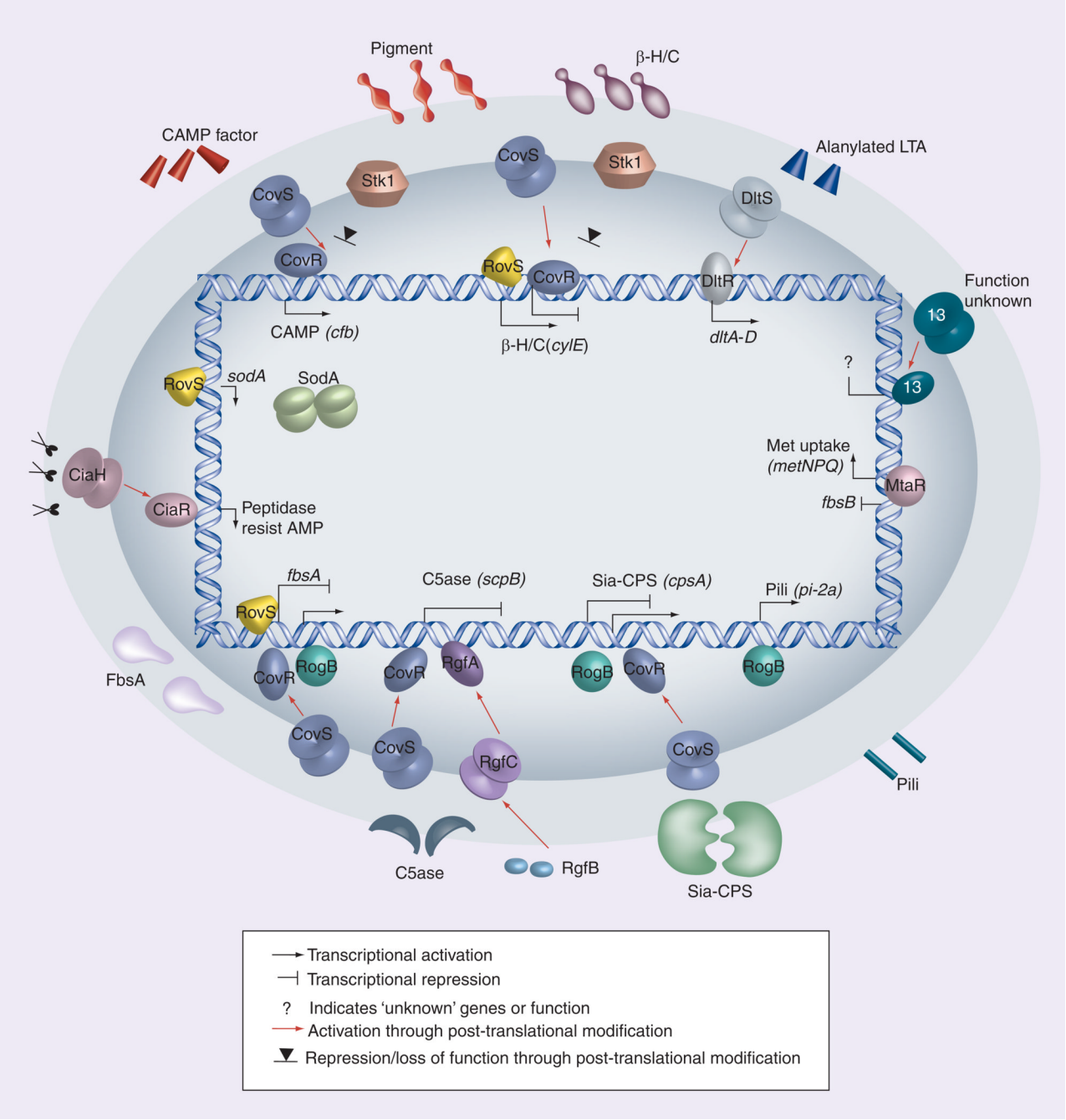

Figure 2. Regulation of factors important for Group B Streptococci disease pathogenesis

Two-component system (TCS) comprising the response regulators CovR, RgfA, CiaR and DltR, and their cognate sensor histidine kinases CovS, RgfC, CiaH and DltS, regulate the transcription of toxins and other factors that contribute to GBS virulence. These include the pore-forming toxins and factors that promote adherence, immune evasion and resistance to host defences. The genes regulated by 13 other TCS that are conserved in GBS serotypes are not known. Apart from TCS, GBS encodes signaling enzymes commonly found in eukaryotes, such as a serine threonine kinase Stk1 and its cognate phosphatase Stp1 that also regulates the expression of pore-forming toxins. Of the six standalone regulators, the function of only three

(MtaR, RovS and RogB) have been examined. The genome sequence indicates that GBS encodes up to 107 regulatory molecules; the function of only a few are known.
β-H/C: β-hemolysin/cytolysin; AMP: Antimicrobial peptide; C5ase: C5a peptidase; CAMP: Christie Atkins Munch Peterson; FbsA: Fibrinogen-binding protein A; GBS: Group B Streptococci LTA: Lipotechoic acid; Met: Methionine; Sia-CPS: Sialic acid capsular polysaccharide; SodA: Superoxide dismutase.

Table 1
Regulation of virulence factor expression.

Virulence factor	Mode of action	Genetic basis	Mechanism of regulation	Activating signal for regulation
Pore-forming toxins				
β-hemolysin/cytolysin (β-H/C, CylE)	Promotes invasion of host cells and triggers host-cell lysis	*cylE* and other genes in the *cyl* locus	Repressed by CovR/CovS	Unknown
	Impairs cardiac and liver function		Activated by Stk1 through CovR	Unknown
	Induces inflammatory responses and apoptosis		Activated by RovS independent of CovS/CovR and Stk1	Growth rate‡
CAMP factor (Cfb)	Forms pores in host-cell membrane	*cfb*	Activated by CovR/CovS	Unknown
	Binds to GPI anchored proteins		Repressed by Stk1 through CovR	Unknown
Factors for immune evasion				
Sialic acid capsular polysaccharide (CPS)	Prevents recognition of GBS through molecular mimicry of host-cell surface glycoconjugates	*cpsA–L*, *neuA–D*	Repressed by RogB (strain-dependent)	Growth rate‡
	Masks pro-inflammatory cell wall components		Activated by CovR/CovS in a strain-dependent manner	Unknown
Superoxide dismutase (SodA)	Detoxifies singlet oxygen and superoxide	*soda*	Activated by RovS	Unknown
Pigment (rhamno-polyene)	Detoxifies singlet oxygen and superoxide	*cyl* locus	Repressed by CovR/CovS	Unknown
			Activated by Stk1 through CovR	Unknown
			Role of RovS is unclear	Unknown
C5a peptidase (ScpB)	Prevents neutrophil recruitment due to cleavage of complement C5a	*scpB*	Repressed by RgfC/RgfA and CovR/CovS in a strain-dependent manner	RgfB for RgfC/RgfA regulation‡
	Promotes adherence by binding to ECM fibronectin and epithelial cells			Signal for CovR/S regulation unknown
Serine protease (CspA)	Cleaves fibrinogen and chemokines	*cspA*	Unknown	Unknown
	Impairs neutrophil recruitment and phagocytic killing of GBS			
Resistance to AMPs				
Alanylation of lipotechoic acid	Decreases net negative charge on cell surface, repels AMPs	*dltA–D*	Activated by DltR/DltS	Unknown

Virulence factor	Mode of action	Genetic basis	Mechanism of regulation	Activating signal for regulation
Penicillin-binding protein 1a (PBP1a)	Promotes resistance to AMPs through an unknown mechanism	*ponA*	Unknown	Unknown
Pili	Promotes resistance to AMPs through an unknown mechanism	PI-1 and PI-2	Expression of PI-2a is activated by	Unknown
	Also promotes adherence of GBS to host cells	(PI-2a or PI-2b)	RogB (strain-dependent)	
Host-cell adherence & invasion				
Fibrinogen-binding protein A (FbsA)*	Promotes adherence of GBS to host cells by binding to ECM fibrinogen	*fbsA*	Repressed by RovS	Unknown
			Activated by CovR/CovS and RogB in a strain-dependent manner	Growth phase-dependent regulation by RogB
Fibrinogen-binding protein B (FbsB)	Promotes entry of GBS into host cells	*fbsB*	Repressed by MtaR	Unknown
Laminin-binding protein (Lmb)	Promotes adherence of GBS to host cells by binding to ECM laminin	*lmb*	Unknown	Unknown
Serine-rich repeat proteins (Srr)	Srr-1 promotes adherence of GBS to human keratin and epithelial (HEp2) cells	*srr-1*	Unknown	Unknown
	Srr-2 enhances virulence of GBS	*srr-2*		
Immunogenic bacterial adhesin (BibA)	Promotes adherence of GBS to host cells and binds complement regulatory protein C4bp	*bibA*	Unknown	Unknown
αC protein*	Facilitates GBS adherence to epithelial cells	*bca*	Unknown	Unknown
Invasion-associated gene (*IagA*)	Membrane anchoring of lipotechoic acid; important for blood–brain barrier invasion	*iagA*	Unknown	Unknown
Other virulence functions				
Hyaluronate lysase	Cleaves hyaluron and promotes spread of GBS during infection	*hlyB*	Unknown	Unknown
Methionine transport regulator (MtaR)	Methionine transport/uptake	*mtaR*	Unknown	Unknown

*Represents virulence factors that are not conserved in all GBS strains.

‡These are suggested signals, but they have not yet been proven.

AMPs: Antimicrobial peptides; CAMP: Christie Atkins Munch Peterson; ECM: Extracellular matrix; GPI: Glycosylphosphotidylinositol; GBS: Group B Streptococcus; PI: Pilus island.