REVIEW ARTICLE

REGULATORY NETWORK IN *STREPTOCOCCUS PYOGENES*

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ABSTRACT

S. pyogenes or group A beta-haemolytic streptococcus (GAS) is an important human pathogen that causes a wide variety of diseases, ranging from throat and skin infections, such as pharyngitis and erysipelas, to severe invasive diseases, such as necrotizing fasciitis and streptococcal toxic shock syndrome. *S pyogenes* produces a large number of secreted proteins and an array of matrix-binding proteins, that all contribute to virulence by mediating adhesion to host tissues, evasion of host defense mechanisms, invasion, survival and tissue destruction. To establish a successful infection, bacterial pathogens must precisely control expression of their virulence genes in response to host signals. Virulence factor expression in GAS, like in other bacteria, is coordinately regulated via "stand-alone" response regulators (RRs) and two-component signal transduction systems (TCSs). In addition to these, three putative regulatory RNAs; *fasX*RNA, *pel*RNA and *riv*XRNA have also been discovered. An increased understanding of the complex regulatory network involved in the *S pyogenes* pathogenesis would help us in combating severe and invasive GAS diseases.

INTRODUCTION

S. pyogenes is an obligate human pathogen. It is a cause of major human morbidity and mortality worldwide. Streptococcal infections have been documented in all races, sexes and age groups. GAS causes 700 million cases each year resulting in 650,000 deaths each year (Aziz *et al.*, 2010). GAS is responsible for a number of diseases ranging from common clinical illnesses such as pharyngitis, impetigo, cellulitis and scarlet fever to severe invasive infections such as puerperal sepsis, myositis, necrotizing fasciitis (NF) (Figure 1), streptococcal toxic shock syndrome (STSS) and post infectious sequelae such as rheumatic fever and acute glomerulonephritis (Table 1). NF and STSS are rare but potentially fatal human diseases. NF also called as flesh eating disease is a disease of deeper skin layers and tissues and STSS is caused by bacterial toxin which is responsible for pyrogenicity, hypotension, shock, multi-organ failure and ultimately death.

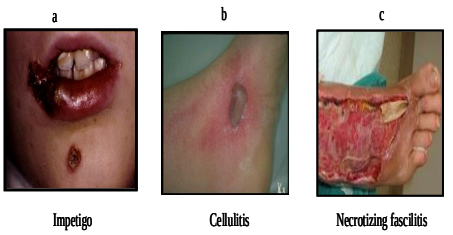

Figure- 1: GAS diseases: (a) Impetigo (b) Cellulitis (c) Necrotizing fasciitis.

Table-1: Diseases caused by GAS (Siller, 2008, Pirzada, 2009).

GAS diseases
Superficial skin and soft- tissue infections
Cellulitis
Tonsillitis
Pharyngitis
Erysipelas
Impetigo
Scarlet fever
Invasive Group A streptococcal diseases
Streptococcal toxic shock syndrome (STSS)
Necrotizing fasciitis (NF)
Bacteremia
Osteomyelitis
Septic arthritis
Pneumonia
Complications of GAS illness - nonsuppurative
Acute Rheumatic fever
Poststreptococcal glomerulonephritis
Complications of GAS illness - suppurative
Cervical lymphadenitis
Endocarditis
Fasciitis/myositis syndrome
Mastoiditis
Meningitis
Otitis media

Since the late 1980s, there has been a significant reemergence of severe forms of diseases (particularly necrotizing fasciitis and STSS) caused by group A streptococcus (GAS) worldwide (Banks *et al.*, 2002, Cunningham,2000). These outbreaks were associated with new serotypes, especially M1, M3 and M18 (Stevens *et al.*, 1989, Cleary *et al.*, 1992, Howe *et al.*, 1996, Stevens, 1992, Stevens, 2000).

S. pyogenes was considered as an extracellular organism, but studies have shown bacterial recovery from inside host cells it can also survive intra-cellularly (Medina *et al.*, 2003). In general, host defense system to bacterial infection involves many mechanisms allowing destroying the incoming pathogen like neutrophil recruitment, opsonization, bacterial entrapment and uptake, intracellular effectors mediated bacterial killing but bacteria regulate their virulence factors to interfere and impair each of these mechanisms. In the case of GAS, factors which avoid entrapment by phagocytes include peptidase ScpA, serine protease ScpC, DNAses. Capsule, M and M-like proteins and Sfb1, SIC (Streptococcal inhibitor of complement), SpeB (cysteine protease) inhibit complement and antibody functions. GAS also impairs phagocytic mechanisms with the help of the factors EndoS, Mac (1 and 2) and protein SIC. Cytolysins which promote phagocytic lysis and apoptosis are also used by GAS. Finally, GAS resist effectors of phagocytic killing by D-alanylation of LTA, SpeB, SIC, M protein and GpoA (glutathione peroxidase) (Kwinn and Nizet, 2007).

Virulence factors produced by *S. pyogenes:* GAS survival and pathogenesis is mediated by several virulence factors involved in adhesion, colonization, evasion, persistence and spread in the host (Table 2). GAS virulence factors can be divided into two groups:

Somatic virulence factors: Somatic or surface-exposed virulence factors include M protein, capsule and adhesions.

Extracellular virulence factors: Extracellular virulence factors include Streptococcal hemolysins (SLO, SLS) and Streptococcal pyrogenic exotoxins (Spes). GAS secretes two main types of hemo-lysins: Streptolysin S (SLS) and Strepto-lysin O (SLO).

Table -2: Virulence factors in *S. pyogenes* (adapted from Bisno *et al.*, 2003).

GAS virulence factors	
Antiphagocytic	
Streptococcal inhibitor of complement	
Cysteine proteinase (SpeB)	
Immunoglobulin G degrading enzyme (IdeS)	
Immunogenic secreted protein (Isp)	
M protein	
M-like protein (Mrp, Enn and others)	
Hyaluronic acid capsule	
C5a peptidase (ScpA)	
Adherence to epithelial cells	
Lipoteichoic acid (LTA)	
Fibronectin binding proteins	
M protein	
Hyaluronic acid capsule	
Lamin-binding protein (Lmb)	
Streptococcal collagen-like surface protein A (SclA)	
Streptococcal collagen-like surface protein B (SclB)	
Internalisation	
M protein	Protein F1
Inavsion	
Hyaluronic acid capsule	
M protein	
Spread through tissues	
Hyaluronidase	Streptokinase
SpeB (cysteine protease)	
DNAses A-D	
Systemic toxicity	
Streptolysin O	
Streptolysin S	
Superantigenic exotoxins	
Superantigens	
SpeA	SpeK
SpeC	SpeL
SpeG	SpeM
SpeH	SSA
SpeI	SmeZ
SpeJ	

Regulation of virulence factor expression in *S.pyogenes:* To establish a successful infection, the bacterial pathogens must precisely regulate the expression of their virulence genes in response to host signals. These signals inform the bacterium that it has reached the proper niche to begin its virulence program. Virulence gene expression in pathogenic bacteria is controlled mainly at the transcriptional level by activation or repression by proteins. Bacteria can also regulate their gene expression via regulatory RNA molecules and in response to their cell density. As in other bacteria, virulence factor expression in GAS is coordinately regulated. Regulation occurs via "stand-alone" response regulators (RRs) and two-component signal transduction systems (TCSs) (Figure 2). The complete genome sequences of 12 GAS strains corresponding to nine serotypes contain 13 TCS and about 30 stand alone for transcriptional regulators (Table 3) (Kreikemeyer *et al.*, 2007).

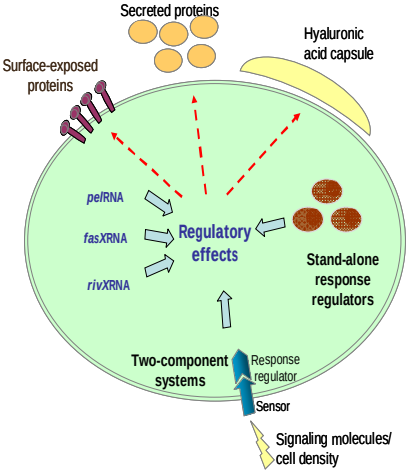

Figure- 2: Regulation of virulence factor expression in GAS (Siller, 2008, Pirzada, 2009)

Two component systems and response regulators: TCSs consist of a sensor histidine kinase, a transmembrane protein, which

senses an external signal with its extra cellular domain, and a response regulator located in the cytoplasm, which after activation acts as a transcription factor to control target gene expression. GAS has 13 common TCSs, however only some of them have been well studied, like CsrRS/ CovRS, FasBCAX, Ihk/Irr, SilA/B, VicRK and SptRS.

CsrRS/ CovRS: CsrRS/CovRS is the best described TCS in GAS. CovR controls the expression of ~15% of GAS genome transcripts (Graham *et al.*, 2002). Cov stands for control of virulence and Csr for capsule synthesis regulator. It encodes two proteins similar to response regulator and sensor components. These proteins repress the expression of the *has* operon (capsule). Apart from this CsrR repress transcription of the genes encoding streptokinase (*ska*), streptolysin S (*sagA*), mitogenic factor (*spe MF*), streptodornase (*Sda*), cysteine protease (*SpeB*) and CsrR itself (Federle *et al.*, 1999, Heath *et al.*, 1999, Darmstadt *et al.*, 2000). The system is triggered by Mg^{2+}, which is low in extracellular body fluids but high inside host cells or outside the human body. The CovRS system inactivates many invasion related GAS virulence genes outside the human body (and inside a host cell), but activates virulence factors at Mg^{2+} concentrations encountered in human mucosal secretion and extracellular fluids. If CovS is mutated, completely abolishing any activity of this system, GAS becomes significantly more invasive (Levin and Wessels, 1998, Bernish and van de Rijn, 1999, Wessels, 1999, Darmstadt *et al.*, 2000, Graham *et al.*, 2002, Gryllos *et al.*, 2003, Sumby *et al.*, 2006). A phenotypic switch from mucosal to invasive forms of GAS infection was correlated with mutations in the *covR/S* locus (Sumby *et al*., 2006).

FasBCAX: FasBCAX is the fibronectin/ fibrogen binding haemolytic activity/ streptokinase regulator. It encodes two sensor histidine kinases (FasBC) and one response regulator (FasA), integrating different signals into one adaptive response. The main effector of the Fas system is the putative untranslated 200 nt regulatory RNA encoded by fasX (Kreikemeyer *et al.*, 2001). The Fas operon upregulates the expression of *sagA* and *ska*, while down-regulates the expression of adhesin genes (*fbp54* and *mrp*). FasBCAX shows homology to quorum-sensing TCSs in *S. aureus* and *S. pneumoniae* and assist in tissue invasion and destruction (Klenk *et al.*, 2005). Expression of *fasBCA* genes changes in response to temperature change from 29ºC to 37ºC (Sitkiewicz and Musser, 2006).

Ihk/Irr: The Ihk/Irr (*isp*-adjacent histidine kinase/ response regulator) TCS has an important role in immune evasion as it controls the expression of genes that facilitate protection from PMN leukocytes mediated killing and assist in GAS survival and pathogenesis response (Federle *et al.*, 1999, Voyich *et al.*, 2003, Voyich *et al.*, 2004). It down regulates genes involved in cell wall metabolism, transcription, oxidative stress and virulence. The Ihk/ Irr mutant was found more susceptible to killing by H_2O_2 and neutrophil primary granules (Musser and DeLeo, 2005).

Sil operon: The Sil locus (Streptococcus invasive locus) is present in about 18% of clinically relevant GAS isolates. The Sil system consists of the TCS SilA/B, two putative ABC-transporters SilD and SilE and SilCR, which seems to be a quorum sensing effector molecule. Sil upregulates the transcription of its own signalling molecule and the two ABC transporters. The bacteriocin like peptide (*blp*) operon and the *spyM3_1016* transposase have

already been identified as targets (Hidalgo-Grass *et al.*, 2002, Eran *et al.*, 2007).

VicRK: VicR inactivation down-regulates the transcription of 13 genes including the putative cell wall hydrolase gene *pcsB* and a putative phosphotransferase system enzyme II for carbohydrate transport, and up-regulates the expression of five genes, including an osmoprotectant transporter OpuA. *vicR* inactivation induced osmotic stress and increased susceptibility to osmotic pressure. VicRK regulates processes involved in cell wall metabolism, nutrient uptake, and osmotic protection (Liu *et al.*, 2006).

SptRS: SptR and SptS encode two component gene regulatory systems, involved in the persistence of GAS in saliva, as the mutant of *sptR* was less able to survive in saliva. Microarray analysis of GAS grown in saliva showed that the expression of several virulence factors in *sptR* mutant was significantly decreased (Shelburne *et al.*, 2005).

TrxSR: The recently described TrxSR (two-component regulatory system X) displays homologies to a virulence related TCS in *S. pneumoniae*. A Trx response regulator mutant is defective in Mga expression and is attenuated in virulence in a murine model of GAS soft tissue infection. TrxR is the first TCS that regulates Mga expression in response to a yet unidentified signal. *trxSR* is a part of CovR-repressed virulence operon, which performs a range of different functions in translation,transcription,replication, transport and stress. As CovR can influence Mga expression, this shows that important global regulatory networks can influence each other to affect pathogenesis in GAS (Leday *et al.*, 2008).

Response regulators: Apart from TCS, GAS also relies on about 30 stand-alone response regulators, for which sensory elements have not been described. These response regulators control the expression of virulence factors in response to environmental signals.

Mga: Mga (stands for multiple gene activator), which is a ubiquitous stand-alone virulence response regulator. It is necessary for adherence, internalization and host immune evasion. Mga activates the expression of virulence genes by directly binding to their promoters. 10% of the three GAS genome transcriptome was affected when *mga* was deleted (Ribardo and McIver, 2006). Mga activates transcription of a number of virulence genes in GAS including its own gene. The Mga regulon is expressed maximally during exponential phase and activates factors involved in early stages of GAS infection. Repression of *mga* transcription is mediated by Rgg/RopB and Nra (RALP), while it is activated in response to CO_2 levels and inhibited by high salt and iron limitation conditions (Caparon *et al.*, 1992, McIver *et al.*, 1995, McIver and Scott, 1997). Mga regulates the expression of M protein (*emm* and *arp*), M-like proteins (*mrp*), C5a peptidase (*scpA*), SIC, SpeB and extracellular matrix binding proteins (*sclA* and *fba*) (Rasmussen *et al.*, 2000, Frick *et al.*, 2003, Kreikemeyer *et al.*, 2003, Roberts and Scott, 2007).

RALP (RofA like proteins): The RALP regulator family of GAS comprises four homologous members: RofA, Nra, RALP-3 and RALP-4 (Granok *et al.*, 2000, Kreikemeyer *et al.*, 2003). These are involved in the control of GAS-host cell interactions, avoidance of host-cell damage and balanced virulence factor expression ~óÿÿring stationary phase. Maximal expression of RALP occurs at stationary growth phase. RALP-regulated genes are fibronectin-binding proteins F (s*fbI*) and F2 as well as collagen-binding protein

(*cpa*),hemolysins ù*ÿÿÿA*), proteases (*speB*); superantigens (*speA*) and other virulence regulators (*mga*) (Kreikemeyer *et al.*, 2003).

A second well known RALP protein is Nra. It acts as a negative regulator of expression of *cpa*, *prtF*, *speA*, *sagA*, *mga* and *nra* itself conditions (Jaffe *et al.*, 1996, Podbielski *et al.*, 1999). Strain-specific differences are observed with Nra transcriptional circuit as Nra is a negative regulator of pilus gene transcription in M49 serotype but a positive regulator of pilus gene transcription in M53 serotype (Luo *et al.*, 2008). In contrast to RofA, Nra expression is maximal at early stationary phase and its expression is not affected due to environmental conditions (Jaffe *et al.*, 1996, Podbielski *et al.*, 1999).

Rgg/RopB: Rgg also known as RopB, (regulation of proteinase) plays a role in host cell apoptosis, necrosis and dissemination. Rgg controls gene expression during late stationary growth phase. It is a positive regulator of the cysteine protease SpeB. It has a negative influence on the transcription of regulatory factors such as *mga* and *pel/sagA* and a positive influence on transcription of *covRS* and *fasBCA* (Neely *et al.*, 2003, Chaussee *et al.*, 2004).

Srv regulon: Srv (Streptococcal regulator of virulence) is a putative regulator protein Srv is homologous to the transcriptional regulator PrfA of *Listeria monocytogenes*. It belongs to the first GAS member of the Crp/Fnr family of transcriptional regulators. The mortality of mice injected with the *srv* mutant was significantly less as compared to the wild-type (Reid *et al.*, 2004). Another study showed that Sic levels were decreased while SpeB levels were increased in a *srv* mutant (Reid *et al.*, 2006).

CodY: CodY is a nutritional regulator mainly involved in amino-acid metabolism. This protein is highly conserved in low G+C gram positive organisms. It has been identified as a pleiotropic transcriptional regulator, activated by branched chain amino-acids. CodY controls many genes, which are operative in low nutrition conditions. To some extent CodY also regulates a RelA-independent response to amino acid (Steiner and Malke, 2001). CodY induces the expression of *pel*/*sagA* and *mga*, so this suggests a link between nutritional regulation and virulence (Malke *et al.*, 2006).

RNA regulators: As bacteria are facing different environmental conditions in their life time, hence they need to adapt according to the changing environmental conditions. Bacteria have a number of virulence factors, which are needed to be activated or repressed according to the environmental or host signals. Up to recently, it was believed that bacteria regulate mainly their gene expression by means of repressor or activator proteins at the transcriptional level. However, it has been increasingly evident that RNA can control gene expression more commonly than previously anticipated. Some of the described RNAs provide house-keeping functions like RNaseP, tmRNA and 4.5S RNA, while others are regulators of gene expression.

Regulatory RNAs: Many terms have been used for small RNAs like sRNAs (small RNAs), ncRNA (noncoding RNAs), snm-RNAs (small non messenger RNAs), fRNA (functional RNAs),regulatory RNAs and riboregulators. sRNAs are mainly confined to control biological functions as bacterial accessory elements (Wagner *et al.*, 2002). sRNAs have emerged as major regulators of adaptive responses and RNA-mediated regulation plays a main role in virulence. Most of the sRNAs are non essential i.e. their absence does not cause cell death.

The most common mode of action of regulatory RNAs is by base pairing to mostly the 5' end of target mRNAs, via a so-called antisense mechanism, thus leading to down-regulation or up-regulation of the gene expression at the post-transcriptional level. Binding of a sRNA to a target mRNA mostly inhibits translation or facilitates the decay of mRNA, however sometimes it can also activate the translation by conversion to a translationally active conformation (Storz *et al.*, 2005). Regulatory RNAs can affect the expression of multiple target mRNA or a single target mRNA encoding a virulence factor. They can also inhibit or activate the expression of single target mRNAs, which decrease or increase the synthesis of single regulatory protein, in turn affecting the expression of a great number of down stream genes. The CsrA protein, regulator of carbon storage, adhesion, cell surface properties and virulence, interacts with target mRNAs and either facilitates their rapid degradation or activates translation (Liu *et al.*, 1997). The third mechanism involves binding of a metabolite to the nascent mRNA thus inducing a structural change to a terminator or a translationally inactive state, called riboswitch (Winkler and Breaker, 2005).

Regulatory antisense RNAs can be of two types: cis-encoded or trans-encoded. Cis-encoded RNAs can bind to the genes downstream from the same locus and are fully complementary to their target RNAs. In contrast trans-encoded RNAs and their targets are encoded in separate loci and the regions of complementarity are generally short and often non-contagious (Delihas, 1995, Altuvia and Wagner, 2000). Most of the chromosomally encoded antisense sRNAs belong to this class. In gram-negative bacteria, many sRNAs that form only a limited complementarity to their targets require the hexameric Sm-like protein Hfq (Valentin-Hansen *et al.*, 2004). Hfq can help in stabilizing sRNAs by promoting antisense-target RNA pairing or by acting in an unfolding or playing chaperone role. Hfq can also act as an adapter between sRNAs and RNase E for degradation (Romby *et al.*, 2006). Many of the sRNAs discovered are stable when induced, exhibiting a half life of 20-60 minutes. Many sRNAs are unprocessed primary transcripts e.g. DsrA, Spot42, MicF, OxyS, while several others are generated from longer precursors and are finally cleaved to their actual sizes e.g. 4.5S RNA, tmRNA, 6S RNA.

Regulatory RNAs regulate gene expression in different growth conditions. For example, in *Listeria monocytogenes*, the transcriptional regulator PrfA is thermo-regulated i.e. its translation initiation only happens when temperature is increased to 37^{o}C (Johansson *et al.*, 2002). This occurs through a riboswitch-like mechanism. A typical non-coding sRNA can bind to several different targets, as it usually forms only a limited number of basepairs to its target mRNA. This limited complementarity not only allows single sRNA to act on multiple targets but also allows the sRNAs to be induced under different growth conditions, allowing multiple sRNAs to act on single targets (Romby *et al.*, 2006).

Small RNAs in bacterial pathogenecity: More than 80 non-coding RNAs have been found in *Escherichia coli*. The majority of these RNAs is present in pathogenic strains and has a role in stress-response regulation and bacterial pathogenicity (Table 4). The first trans-encoded antisense RNA identified is MicF in *E. coli* (Andersen *et al.*, 1987). Expression of MicF is induced by different environmental stresses like osmolarity and temperature (Delihas and

Forst, 2001). MicF binds to the translation initiation region (TIR) of *ompF* mRNA, blocks the translation of the OmpF protein (porin protein) and induces rapid RNase E dependent *ompF* mRNA degradation (Rasmussen *et al.*, 2005).

The most studied example of a regulatory RNA controlling bacterial virulence is the 512-nucleotide long RNAIII of *S. aureus*, which affects both transcription and translation of virulence genes. It acts as multi-gene regulator, repressing the expression of cell surface proteins like protein A and fibronectin-binding proteins. This activates the expression of extra-cellular toxins and proteases like α-toxin, β-haemolysin, TSST-1, enterotoxin B, leucocidin, staphylokinase, serine protease, metalloproteases (Janzon and Arvidson, 1990, Wolz *et al.*, 1996). In totality, RNAIII regulates the expression of ~100 genes (Dunman *et al.*, 2001). Consequently, the *agr* system functions as a switch from a localized infection to invasive and systemic dissemination (Novick and Muir, 1999). Apart from regulatory functions, RNAIII also acts as an mRNA encoding δ–hemolysin. Unlike most of the antisense RNAs, RNAIII can activate the expression of its target e.g. *hla* mRNA (Morfeldt *et al.*, 1995) instead of inhibiting its expression.

OxyS RNA of *E. coli* is yet another example of multigene regulator, regulating the expression of ~40 genes. It has a role in defense against oxidative damage. It accumulates at high levels when bacteria are treated with hydrogen peroxide (Altuvia *et al.*, 1997). OxyS negatively affects the expression of two target genes: *fhlA* (a transcriptional activator protein) and *rpoS* (stress/ stationary phase σ subunit of RNA polymerase).

DsrA is 87nt long sRNA which affects polysaccharide capsule synthesis. It is induced at low temperature. mRNA encoding global transcriptional proteins RpoS and hns are targets of DsrA. One segment of nucleotides binds to the TIR of *hns* mRNA while another one basepairs to nucleotides near the 5' end of the *rpoS* mRNA (Lease and Belfort, 2000).

The RhyB sRNA, 90nt long, is regulated by iron. At high iron concentration, it is repressed by the ferric uptake protein Fur. Fur dependent activation of *sdh*, genes required for growth on succinate, occurs by repression of RhyB transcription.

Small RNAs in *S. pyogenes:* In *S. pyogenes*, so far, only three putative regulatory RNAs have been discovered: *fasX*RNA, *pel*RNA and *rivX*RNA.

***fasX* RNA:** Fas stands for fibronectin/ fibrinogen binding/ haemolytic activity/ streptokinase regulator. Downstream of *fasBCA* is a small 200 nucleotide RNA *fasX*. The *fas* operon consists of two genes encoding histidine kinases, FasB and FasC and the single response regulator FasA. Presence of two sensor kinase genes might mean that the sensors are bind to different environmental stimuli. These genes were identified on the basis of homology to the *S. aureus agr* two-component system and *S pneumoniae com* operons, however a quorum-sensing driven regulation process was not demonstrated for *fasX*. Rather, *fasX* controls the growth phase associated expression of GAS virulence factors. It regulates surface fibronectin and fibrinogen binding proteins and certain GAS virulence factors like *mrp*, *fbp45*, streptolysin A (*pel*) and streptokinase (*ska*) transcription (Figure 3). Both *fasX* RNA and the fasA response regulator have a similar regulatory effect on the transcription of virulence factors; however complementation studies showed that the small *fasX* RNA mainly controls virulence factor expression (Kreikemeyer *et al.*,

2001). Another study shows that the wild-type strain adhered to and was internalized by Hep2 cells at a higher degree compared to the *fasX* mutant. In addition, a *fasX* mutant showed reduced aggressiveness of strain as the mutant displayed decreased cytokine production, apoptosis induction and cytotoxicity. These results show that *fasX* expression is important for GAS in tissue colonization and intracellular persistence (Klenk *et al.*, 2005).

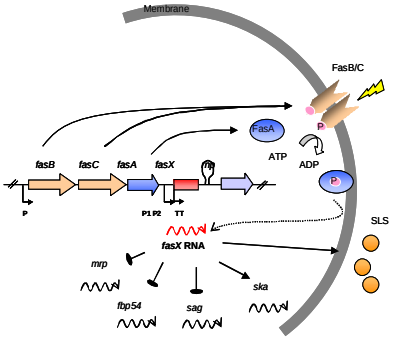

Figure-3: The *fas* operon and *fasX* RNA. P1 and P2: putative promoter sequence, TT: putative transcription terminator adapted from Kreikemeyer *et al.*, 2001 (Siller, 2008).

***pel* RNA:** *pel* (pleiotropic effect locus) includes the *sagA* gene, structural gene for SLS, and functions as an effector of virulence factor expression (Nizet *et al.*, 2000). Downstream of the *sagA* gene are *sagB* to *sagI* genes, which are involved in chemical modification, processing and secretion of SLS (Figure 4). Creation of isogenic *sagBC*, *sagDEF* and *sagGHI*-deficient strains and complementation studies proved that the phenotype is due to the *pel* locus and not due to polar effects of downstream genes. *pel* RNA regulates the expression of virulence factors like M protein, SpeB, Ska (Li *et al.*, 1999, Biswas *et al.*, 2001) and SIC (Mangold *et al.*, 2004). *pel* RNA is regulated in a growth-phase dependent manner and is induced by condition media. TCSs or genes encoding signaling molecules have not been identified in the vicinity of the *pel* locus, however addition of conditioned media to early logarithmic cells induced transcription of *pel*.

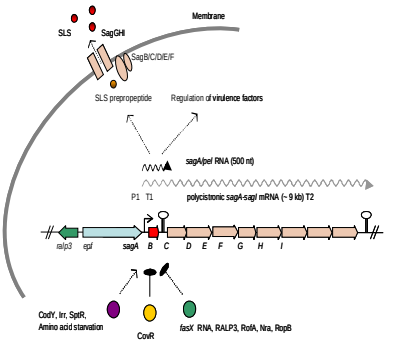

Figure- 4: The genetic locus for *pel* RNA and SLS production in *S. pyogenes* (M1 serotype) (Siller, 2008). P1: transcriptional start, T1: putative transcriptional terminator 1, T2: putative transcriptional terminator 2

***rivX* RNA:** RivR, a RofA-like protein, activates the expression of *mga*. RivX is a novel small RNA, encoded within an operon with *rivR*. *rivX* also activates the expression of the Mga regulon via a RivR-independent mechanism. *rivX* is conserved in all GAS genomes sequenced to date. Furthermore, RivRX is directly repressed by CovR (Roberts *et al.*, 2007). This show a link between two major regulators of GAS virulence, Mga and CovR; where RivR and RivX play intermediaries in pathogenic regulatory circuits (Figure 5). A *rivRX* mutant in the *covR* deletion background was strongly attenuated for virulence in a murine model of invasive soft tissue infection (Roberts and Scott, 2007). *rivRX*-activated virulence factors include M protein, ScpA, Fba and Sic (Okada *et al.*, 1994, Ashbaugh *et al.*, 2000,

Lukomski *et al*., 2000, Terao *et al*., 2001). The *rivRX* locus does not autoregulate its own expression. Further, the analysis of *rivX* containing non sense mutations proved that the sRNA *rivX* itself, rather than a peptide encoded by *rivX* is responsible for *rivX* activity. However, *rivX* dependent *mga*-activated gene expression requires Mga protein.

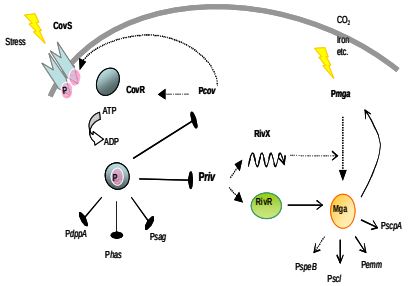

Figure-5: Model for regulation of virulence by RivR and *rivX* RNA adapted from Roberts and Scott, 2007 (Siller, 2008)

The presence of a number of "stand-alone" response regulators, two-component signal transduction systems and putative regulatory RNAs prove that the virulence factor expression in GAS, like in other bacteria, is coordinately regulated. The aim of this review was to identify all the components discovered so far involved in the regulatory network that *S pyogenes* uses to cause a successful infection. An increased understanding of the complex regulatory network involved in the *S pyogenes* pathogenesis would help us in combating severe and invasive GAS diseases in an effective way.

Table- 3: Regulators in GAS (Siller, 2008, Pirzada, 2009)

Stand-alone regulators	
Regulator	**Targets**
CodY	UR: *pel/sagA, nga-slo, mga, grab, scl, prtS, scpA, speH, ideS, hasA* DR: *covRS, ropB, opp, ska, sda*
LacD.1	UR: *manL, slo, sagH, ntpK* DR: *speB, salA, scrA*
MalR	DR: transcripts of polysaccharide utilization proteins. MalR contributes to the persistence of GAS in the oropharynx.
Mga	UR: *arp, emm, enn, scpA, fcrA, nra, mga, ska, speB, scnA/salA, opp, fbA, sic, lbp, scl1/scl-A, sof, mrp* DR: genes for sugar utilization
MsmR	UR: *prtF2, cpa, nra, spy0128, nga, spy0166, slo, spy0170, spy2006, sof, sfbx, hasA*
MtsR	UR: *htsA* DR: *sia* operon, *mtsA* The *mtsR* mutant is hypersensitive to streptonigrin and hydrogen peroxide.
RALP3	DR: *hasA, spn, sdal, lsap, speB, mga, slo, covR, sic, sagA, grab, emm, eno* (at logarithmic growth phase), *ska, scpC* UR: *scpA, cpa, eno* (at stationary growth phase), *sic* RALP3 contributes to epithelial cell inavasion and bloodstream survival. Counteracts Nra and MsmR regulation.
RALP4 (RivR)	UR: *mga, emm, scpA, fba, sic, scl, grm* (Mga regulated genes), *speB,* spy*1508*/NT01sp*1245*, M5005_spy*0190*/NT01sp*0244* and NT01sp*1815* (hypot. proteins); required for virulence in *covR* deleted mutant.
Nra	DR: cpa, mga, nra, prtF2, operon orf5-nifR3L-kinL, operon cpa-lepAL-egfflSL-orf2, sof/sfbII, sagA, speA, speB, ralp3
RofA	UR: prtF, rofA, rpsL, hasB, emm2 DR: emm6, mga, sagA, speA, speB, ska
Rgg/RopB	UR: speB, autolysin, clpB, lysozyme, covR, covS, fasBCA, isp1, isp2, ihk, irr DR: mf, DNA entry nuclease (orf226), orf953, emm, grab, hasAB, orfX, sagA, scl1, scpA, ska, slo, speH, mac, mga, cpsX, yufM, lytR, spy0875, ClpE, ClpL
PerR	UR: *csp, sod, czcD* DR: *mtsA, mrgA, pmtA, phtY, phtD, lsp, lrpsN2*

P-Ser-HPr	UR: *mga*
Srv	UR: *sir*, *spy0044* (zinc containing dehydrogenase), *spy0285* (ATP binding protein), *spy0714* (zinc binding protein), *spy2007*, *sic*, *speB*
Regulator	**Targets**
CovRS	UR: spy0138, spy1062, spy1680, spy1755, spy533 DR: covR, grab, has-operon, dppA, ideS, ihk/irr, isp2, lmb, mac, mspA, ralp3, ralp4, sagA, sda, ska, speB, speF, speMF
fasBCAX	UR: *ska*, SLS activity DR: *fbp45*, *mrp*, *sagA*
Ihk/Irr	UR: cytokine genes, *fbp*, *gidB*, *mf/mf3*, *mryY*, *sagA*, *spy0510*, *spy1035*, *spy1093*, *spy1205*, *spy1311*
SilA/B	UR: *silE/D/CR*, *spyM3-1016* (transposase), bacteriocin like peptide (*blp*) DR: *silC*
SptRS	UR: carbyhydrate metabolism enzymes, *emm*, *hasA*, *perR*, *rofA*, *sagA*, *sic*, *spd*, *speB*, *spy0470*
VicRK	UR: putative cell wall hydrolase gene *pcsB*, putative phosphotransferase system enzyme II for carbohydrate transport. DR: *spy0183*, *spy0184* (osmoprotectant transporter OpuA) VicRK regulates processes involved in cell wall metabolism, nutrient uptake and osmotic protection.
TrxSR	UR*: emm1*, *sic*, *fba*, *scpA*, *sclA*, *grm* (Mga regulated genes), *mga* DR: *rbfA*, *nusA*, *aroB*, *secA*, *dnaJ*, *miaA*, *nagB*, *rplT*, *polA*, *lacE*, *flaR*, *aroD*, *phnA*, *clpL*, *opuAA*, *opuABc*
Regulator	**Targets**
fasX	UR: *ska*, SLS activity DR: *fbp45*, *mrp*, *sagA*
pel	UR: *emm*, *nga*, *sic*, SpeB activity
RivX	UR: *mga*, *emm*, *scpA*, *fba*, *sic*, *scl*, *grm* (Mga regulated genes), *speB*, spy*1508*/NT01sp*1245*, M5005_spy*0190*/NT01sp*0244* and NT01sp*1815* (hypot. proteins); required for virulence in *covR* deleted mutant.
UR: up-regulation	DR: down-regulation

Table- 4: Regulatory RNAs in bacterial pathogenicity (Siller, 2008, Pirzada, 2009)

RNA (Species)	**Target genes**	**Mechanism of action**
CsrB (*Salmonella typhimurium*)	UR: genes of the salmonella pathogenicity island 1	Protein-targeting CsrB RNA functions as an antagonist of CsrA by sequestering this protein and preventing its action.
CsrB (*Escherichia coli*)	UR: *glgC* (ADP-glucose pyrophosphorylase)	Protein-targeting CsrB RNA functions as an antagonist of CsrA by sequestering this protein and preventing its action.
CsrB/C/D (*Vibrio cholerae*)	Interact via CsrA with the expression of Qrr sRNAs.	Protein-targeting The three sRNAs control the activity of CsrA, which interacts with the expression of Qrr sRNAs, therefore regulating the entire quorum-sensing system.
csRNA (1-5) (*Streptococcus pneumoniae*)	DR: (csRNA4 and csRNA5) stationary-phase autolysis	Most likley inhibit initiation of translation by RNA-RNA base pairing.
DsrA (*Escherichia coli*)	UR: *rpoS* DR: Global regulator H-NS	RNA-RNA basepairing Basepairing to the *rpoS* mRNA leader sequence, opens secondary structure formation so that translation can occur. Inhibits *hnS* mRNA translation by base pairing.
fasX (*Streptococcus pyogenes*)	UR: SLS, *ska* DR: *mrp*, *fbp54*, *sagA*	unknown
gadY (*Escherichia coli*)	UR: *gadX*, *gadW* (acid response genes)	RNA-RNA basepairing Basepairing with the 3´end of the *gadX* mRNA leads to stabilization of the target transcript.

GcvB (*Salmonella typhimurium*)	DR: Seven target mRNAs that encode periplasmic substarte-binding proteins of ABC uptake systems for amino acids and peptides.	RNA-RNA basepairing Represses target mRNAs by binding to extended C/A-rich enhancer motifs. In some cases GcvB masks directly the S-D-motif to prevent 30 S subunit binding.
IstR1/ltsR2 (*Escherichia coli*)	DR: *tisAB*	RNA-RNA basepairing Base pairing of lstR-1 to the *tisAB* mRNA promotes cleavage of the toxic transcript by RNAseIII.
MicA, MicF, MicC (*Escherichia coli*)	DR: *ompA, ompF, ompC*	RNA-RNA basepairing MicA, MicC and MicF bind regions in their respective target mRNAs leading to repression of translation and induction of rapid RNase E-dependent mRNA decay.
MicX (*Vibrio cholerae*)	DR: *vc0972* (uncharacterized OMP), *vc0620* (ABC transporter)	RNA-RNA basepairing Primary transcripts of MicX are processed in an RNase E- and Hfq-dependent fashion. Processed MicX downregulates transcripts of *vc0972* and *vc0620*.
OmrA, OmrB (*Escherichia coli*)	DR: several genes encoding outer membrane poteins, including *cirA*, *fecA*, *fepA* and *ompT*.	RNA-RNA base pairing Because OmrA and OmrB bind Hfq, they were predicted to act by pairing with their target mRNAs; thus changing stability and/or translation potential.
OxyS (*Escherichia coli*)	Represses translation of *fhlA and rpoS*	RNA-RNA basepairing Blocks translation of *fhlA* mRNA by binding across the S-D sequence. Mechanism is Hfq dependent.
Pel (*Streptococcus pyogenes*)	UR: *emm*, *sic*, *nga*, SpeB activity	unknown
PrfA (*Listeria monocytogenes*)	Listeriolysin O, phospholipases PlcA and PlcB	Thermosensor At low temperature the *prfA*-UTR forms a secondary structure wich masks the RBS. At higher temperature the structure melts, permitting binding to the ribosome.
PrrB (*Pseudomonas fluorescens*)	UR: of 2, 4-diacetylphloroglucinol (Phl) and hydrogencyanide HCN	Protein targeting The structure is similar to the CsrB and RsmB regulatory RNAs in *E. coli and E. carotovora.*
PrrF1 PrrF2 (*Pseudomonas aeruginosa*)	DR: *sodB* (superoxide dismutase), *sdh* (succinate dehydrogenase), and bacterioferritin.	RNA-RNA base pairing Functional homologs of RyhB in *E. coli*; Expression is regulated by iron.
Qrr1-4 (*Vibrio cholerae; Vibrio harveyi*)	DR: *luxR*/*hapR*	RNA-RNA base pairing The sRNAs act downstram of LuxO-P to destabilize *luxR*/*hapR* mRNA and regulate quorum-sensing dependent gene expression in *V. harveyi* and *V. cholerae*.
RatA (*Bacillus subtilis*)	DR: *txpA* (toxic peptide A)	Toxin:antitoxin module *ratA* is an antisense RNA that overlaps the *txpA* mRNA. Hybridisation results in degradation of the ds-RNA complex.
RivX (*Streptococcus pyogenes*)	UR: *mga* regulon	unknown
RNAalpha (*Vibrio anguillarum*)	DR: iron-uptake complex (*fat* operon)	RNA-RNA basepairing The expression of *fatA* and *fatB* is repressed under iron-rich conditions, in which RNAalpha is induced. RNAalpha is homologous to two-thirds of the coding region of *fatB.*
RNAIII (*Staphylococcus aureus*)	UR: toxins and enzymes DR: surface proteins, *spa*, rot	RNA-RNA basepairing Binding to the anti-S-D region of the *hla* mRNA leads to disruption of the intramolecular base-pairing, so that the ribosome can bind. 3´end of RNAIII binds to the *spa* mRNA, sequesters the RBS and further destabilizes the *spa* mRNA. Binding to *rot* mRNA S-D sequence inhibits translation, thus leading to *rot* mRNA procession.

RprA (*Escherichia coli*)	UR: *rpoS*	RNA-RNA basepairing, Translational regulation of the stationary phase sigma factor RpoS is mediated by the formation of a double-stranded RNA stem-loop structure in the upstream region of the *rpoS* mRNA, occluding the translation initiation site.
RsmB (*Pseudomonas aeruginosa*)	UR: production of AHL C4-HSL and C6-HSL	Protein-targeting AHL production increases when the RsmB RNA binds the repressor protein RsmA, which therfore becomes inactivated.
RsmB´ (*Erwinia caratovora*)	UR: proteoloytic enzymes, proteases and cellulases	Protein-targeting Inhibits target mRNA degradation by binding to the RNA binding protein RsmA.
RsmY, RsmZ (*Pseudomonas fluoresecens*)	UR: biocontrol traits	Protein-targeting RsmZ sequesters the RNA-binding protein RsmA, a translational regulator of genes involved in biocontrol. Relieve RsmA-mediated regulation of secondary metabolism and biocontrol traits.
RyaA/SgrS (*Escherichia coli*)	DR: *ptsG* (encodes the glucose transporter of the phosphoenolpyruvate phosphotransferase system (PTS))	RNA-RNA base pairing RyaA is required for posttranscriptional regulation of *ptsG* in response to phosphoglucose stress. In addition, *ryaA* transcription is activated by YabN, the member of transcriptional regulators.
RyhB (*Escherichia coli*)	DR: *sodB* (encoding superoxide dismutase), *ftn* and *bfr* (encoding ferritin and bacterioferritin) and several iron-sulfur cluster -containing TCA cycle enzyme genes, including the *sdh* operon (encoding succinate dehydro-genase) and *acnA* (encoding aconitase)	RNA-RNA basepairing Base pairing between RyhB and its mRNA target and subsequent Rnase E-mediated degradation of the RyhB-mRNA duplex. Because RyhB is itself negatively regulated by Fur, the net effect is positive regulation of these genes under high-iron conditions.
RyhB (*Vibrio cholerae*)	DR: iron storage and utilization genes; motility, chemotaxis and biofilm formation.	RNA-RNA basepairing *V. cholerae* may use a system analogous to the *E. coli* RyhB mechanism for regulating genes encoding iron-containing proteins and those involved in iron metabolism.
SR1 (*Bacillus subtilis*)	unknown	RNA-RNA basepairing Glucose mediated repression of SR1 transcription; regulated by CcpN.
SurA, SurC (*Bacillus subtilis*)	*yndL* (involved in porulation)	
VR-RNA (*Clostridium perfringens*)	UR: *colA, plc, ptp, cpd* DR: *ycgJ, metB, cysK, ygaG*	unknown

UR: up-regulation DR: down-regulation

REFERENCES

Altuvia,S. and E.G.Wagner, Switching on and off with RNA. *Proc. Natl. Acad. Sci USA* **97**: 9824-9826 (2000)

Altuvia,S., D.Weinstein-Fischer, A. Zhang, L.Postow and G. Storz, A small, stable RNA induced by oxidative stress: role as a pleiotropic regulator and anti-mutator. *Cell* **90**: 43-53 (1997)

Andersen,J., N.Delihas, K.Ikenaka, P.J. Green, O.Pines, O.Ilercil and M. Inouye, The isolation and characterization of RNA coded by the micF gene in Escherichia coli.*Nucleic Acids Res.***15**:2089 (1987)

Ashbaugh,C.D., T.J.Moser, M.H.Shearer, G.L.White, R.C.Kennedy and M.R. Wessels, Bacterial determinants of persistent throat colonization and the associated immune response in a primate model of human group A

streptococcal pharyngeal infection. *Cell Microbiol* **2**: 283-292 (2000)

Aziz,R.K., R.Kansal, B.J.Aronow, W.L. Taylor, S.L.Rowe, M.Kubal,G.S.Chhatwal, M. J.Walker and M.Kotb, Microevolution of group A streptococci in vivo: capturing regulatory networks engaged in sociomicrobiology, niche adaptation, and hypervirulence. *PLoS One* **5**: e9798 (2010)

Banks, D. J., S. B. Beres and J. M. Musser, The fundamental contribution of phages to GAS evolution, genome diversification and strain emergence. *Trends Microbiol* **10**: 515-521 (2002).

Bernish,B. and I.van de Rijn, Characterization of a two-component system in Streptococcus pyogenes which is involved in regulation of hyaluronic acid production. *J Biol Chem* **274**: 4786-4793 (1999)

Biswas,I., P.Germon, K.McDade and J. R. Scott, Generation and surface localization of intact M protein in *Streptococcus pyogenes* are dependent on *sagA*. *Infect.Immun.***69**:7029-38 (2001)

Bisno,A.L., et al., Molecular basis of group A streptococcal virulence. *Lancet Infect. Dis.* **3**(4):191-200 (2003)

Caparon,M.G.,R.T.Geist,J.Perez-Casal and J.R.Scott, Environmental regulation of virulence in group A streptococci: transcription of the gene encoding M protein is stimulated by carbon dioxide. *J. Bacteriol.* **174**: 5693-5701 (1992)

Chaussee,M.A., E.A.Callegari and M. S. Chaussee, Rgg regulates growth phase dependent expression of proteins associated with secondary metabolism and stress in *Streptococcus pyogenes*. *J. Bacteriol.* **186**: 7091-7099 (2004)

Cleary, P.P., E.L.Kaplan, J. P. Handley, A. Wlazlo, M.H.Kim, A.R.Hauser and P. M.Schlievert, Clonal basis for resurgence of serious *Streptococcus pyogenes* disease in the 1980s. *Lancet* **339**: 518-521 (1992)

Cunningham,M.W., Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.* **13**: 470-511 (2000)

Darmstadt,G. L., L. Mentele, A. Podbielski and C.E. Rubens, Role of group A streptococcal virulence factors in adherence to keratinocytes. *Infect Immun* **68**: 1215-1221 (2000)

Delihas, N., Regulation of gene expression by trans-encoded antisense RNAs. *Mol Microbiol* **15**: 411-414 (1995)

Delihas,N. and S. Forst, MicF: an antisense RNA gene involved in response of *Escherichia coli* to global stress factors. *J. Mol. Biol.* **313**: 1-12 (2001)

Dunman,P.M.,E.Murphy,S.Haney,D.Palacios, G.Tucker-Kellogg, S.Wu, E.L.Brown, R.J.Zagursky,D.Shlaes and S.J.Projan, Transcription profiling based identification of Staphylococcus aureus genes regulated by the agr and/or sarA loci. *J. Bacteriol.* **183**: 7341-7353 (2001)

Eran,Y., Y.Getter, M.Baruch, I. Belotserkovsky, G. Padalon, I. Mishalian, A. Podbielski, B. Kreikemeyer and E. Hanski, Transcriptional regulation of the *sil* locus by the SilCR signalling peptide and its implications on group A streptococcus virulence. *Mol. Microbiol.* **63**: 1209-1222 (2007)

Federle,M. J., K. S. McIver and J. R. Scott, A response regulator that represses transcription of several virulence operons in the group A streptococcus. *J. Bacteriol.* **181**: 3649-3657 (1999)

Frick,I.M., P. Akesson, M. Rasmussen, A. Schmidtchen and L.Bjorck, SIC, a secreted protein of *Streptococcus pyogenes* that inactivates antibacterial peptides. *J. Bio.l Chem.* **278**: 16561-16566 (2003)

Graham,M.R.,L.M.Smoot, C.A.Migliaccio, K. Virtaneva, D. E. Sturdevant, S.F. Porcella, M.J.Federle, G.J.Adams,J. R. Scott and J.M.Musser, Virulence control in group A Streptococcus by a two-component gene regulatory system: global expression profiling and in vivo infection modeling. *Proc. Natl. Acad. Sci. USA* **99**: 13855-13860 (2002)

Granok,A.B., D. Parsonage, R. P. Ross and M.G.Caparon, The RofA binding site in Streptococcus pyogenes is utilized in multiple transcriptional pathways. *J Bacteriol* **182**: 1529-1540 (2000)

Gryllos,I., J.C.Levin and M.R.Wessels, The CsrR/CsrS two-component system of group A Streptococcus responds to environmental Mg2+. *Proc Natl Acad Sci U S A* **100**: 4227-4232 (2003)

Heath,A., V.J.DiRita, N.L.Barg and N. C. Engleberg, A two-component regulatory system, CsrR-CsrS, represses expression of three *Streptococcus pyogenes* virulence factors, hyaluronic acid capsule, streptolysin S, and pyrogenic exotoxin B. *Infect.Immun.* **67**:5298-5305 (1999)

Hidalgo-Grass,C., M.Ravins, M.Dan-Goor, J. Jaffe, A. E. Moses and E. Hanski, A locus of group A Streptococcus involved in invasive disease and DNA transfer. *Mol. Microbiol.* **46**: 87-99 (2002)

Howe,R.A., N.M.Brown and R.C. Spencer, The new threats of Gram positive pathogens: re-emergence of things past. *J.Clin.Pathol.* **49**:444-449 (1996)

Jaffe,J., S.Natanson-Yaron, M.G.Caparon and E.Hanski, Protein F2, a novel fibronectin binding protein from Strep-to-coccus pyogenes, possesses two binding domains. *Mol Microbiol* **21**: 373-384 (1996)

Janzon, L. and S. Arvidson, The role of the delta-lysin gene (hld) in the regulation of virulence genes by the accessory gene regulator (agr) in Staphylococcus aureus. *Embo J* **9**: 1391-1399 (1990)

Johansson,J.,P.Mandin,A.Renzoni,C.Chiar-uttini, M. Springer and P. Cossart, An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. *Cell* **110**: 551-561 (2002)

Klenk,M., D.Koczan, R.Guthke, M.Nakata, H. J.Thiesen,A.Podbielski and B. Kreikemeyer, Global epithelial cell transcriptional responses reveal *Streptococcus pyogenes* Fas regulator activity association with bacterial aggressiveness. *Cell Microbiol.* **7**:1237-1250 (2005)

Kreikemeyer,B., M.D.Boyle, B.A. Buttaro, M.Heinemann and A.Podbielski, Group A streptococcal growth phase-associated virulence factor regulation by a novel operon (Fas) with homologies to two-component-type regulators requires a small RNA molecule. *Mol. Microbiol.* **39**: 392-406 (2001)

Kreikemeyer, B., K. S. McIver and A. Podbielski, Virulence factor regulation and regulatory networks in *Streptococcus pyogenes* and their impact on pathogen-host interactions. *Trends Microbiol.* **11**: 224-232 (2003)

Kreikemeyer, B., M. Nakata, T. Koller, H. Hildisch, V. Kourakos, K. Standar, S. Kawabata, M.O.Glocker and A.Podbielski, The Streptococcus pyogenes serotype M49 Nra-Ralp3 transcriptional regulatory network and its control of virulence factor expression from the novel *eno ralp3 epf sagA* pathogenicity region. *Infect. Immun.* **75**: 5698-5710 (2007)

Kwinn,L.A. and V.Nizet, How group A Streptococcus circumvents host phagocyte defenses. *Future Microbiol* **2**: 75-84 (2007)

Lease, R. A. and M. Belfort, A trans-acting RNA as a control switch in E.coli: DsrA modulates function by forming

alternative structures. *Proc Natl Acad Sci U S A* **97**: 9919-9924 (2000)

Leday,T.V., K.M.Gold, T.L.Kinkel, S. A. Roberts, J. R. Scott and K.S.McIver, TrxR, a new CovR-repressed response regulator that activates the Mga virulence regulon in the Group A Streptococcus. *Infect.Immun.***76**: 4659-4668 (2008)

Levin,J.C. and M.R.Wessels, Identification of *csrR/csrS*, a genetic locus that regulates hyaluronic acid capsule synthesis in group A Streptococcus. *Mol. Microbiol.* **30**: 209-219 (1998)

Li, Z., D. D. Sledjeski, B. Kreikemeyer, A. Podbielski and M. D. Boyle, Identification of *pel*, a *Streptococcus pyogenes l*ocus that affects both surface and secreted proteins. *J. Bacteriol.* **181**: 6019-6027 (1999)

Liu,M., T.S.Hanks, J.Zhang, M.J. Mc Clure, D.W.Siemsen, J. L. Elser, M. T. Quinn and B. Lei, Defects in ex vivo and in vivo growth and sensitivity to osmotic stress of group A Streptococcus caused by interruption of response regulator gene *vicR*. *Microbiology* **152**: 967-978 (2006)

Liu,M.Y., G.Gui, B.Wei, J.F.Preston, 3rd, L. Oakford, U. Yuksel, D. P. Giedroc and T. Romeo, The RNA molecule CsrB binds to the global regulatory protein CsrA and antagonizes its activity in *Escherichia coli*. *J. Biol. Chem.* **272**: 17502-17510 (1997)

Lukomski, S., K. Nakashima, I. Abdi, V. J. Cipriano, R. M. Ireland, S. D. Reid, G. G.Adams and J.M.Musser, Identification and characterization of the scl gene encoding a group A Streptococcus extracellular protein virulence factor with similarity to human collagen. *Infect Immun* **68**: 6542-6553 (2000)

Luo,F., S.Lizano and D.E.Bessen, Heterogeneity in the polarity of Nra regulatory effects on Streptococcal pilus gene transcription and virulence. *Infect Immun* **76**: 2490-2497 (2008)

Malke,H.,K.Steiner, W.M.McShan and J.J. Ferretti, Linking the nutritional status of *Streptococcus pyogenes* to alteration of transcriptional gene expression: the action of CodY and RelA. *Int. J.Med. Microbiol.***296**: 259-75 (2006)

Mangold, M., M.Siller, B. Roppenser, B. J. Vlaminckx, T.A.Penfound, R.Klein, R.Novak, R.P.Novick and E. Charpentier, Synthesis of group A streptococcal virulence factors is controlled by a regulatory RNA molecule. *Mol. Microbiol.* **53**: 1515-1527 (2004)

McIver, K. S., A. S. Heath, B. D. Green and J. R. Scott, Specific binding of the activator Mga to promoter sequences of the *emm* and *scpA* genes in the group A *Streptococcus. J. Bacteriol.* **177**: 6619-6624 (1995)

McIver,K.S. and J.R.Scott, Role of *mga* in growth phase regulation of virulence genes of the group A streptococcus. *J. Bacteriol.* **179**: 5178-5187. (1997)

Medina,E., O.Goldmann, A.W.Toppel and G.S.Chhatwal, Survival of *Streptococcus pyogenes* within host phagocytic cells: a pathogenic mechanism for persistence and systemic invasion. *J. Infect. Dis.* **187**: 597-603 (2003)

Morfeldt,E., D.Taylor, A.von Gabain and S. Arvidson, Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded antisense RNA, RNAIII. *Embo.J.***14**:4569-4577 (1995)

Musser,J.M. and F.R.DeLeo, Toward a genome wide systems biology analysis of host-pathogen interactions in group A Streptococcus. *Am J Pathol* **167**: 1461-1472 (2005)

Neely, M. N., W. R. Lyon, D. L. Runft and M. Caparon, Role of RopB in growth phase expression of the SpeB cysteine protease of *Streptococcus pyogenes*. *J. Bacteriol.* **185**: 5166-5174 (2003)

Nizet,V., B.Beall, D.J.Bast,V.Datta, L. Kilburn, D.E.Low and J.C.De Azavedo, Genetic locus for streptolysin S production by group A streptococcus. *Infect. Immun.* **68**: 4245-4254 (2000)

Novick,R.P. and T.W.Muir, Virulence gene regulation by peptides in staphylococci and other Gram positive bacteria. *Curr.Opin. Microbiol.* **2**: 40-45 (1999)

Okada, N., A. P. Pentland, P. Falk and M. G. Caparon, M protein and protein F act as important determinants of cell-specific tropism of Streptococcus pyogenes in skin tissue. *J Clin Invest* **94**: 965-977 (1994)

Pirzada,Z.A., Ph.D. Thesis. Virulence factors in *Streptococcus pyogenes*: horizontal transfer and regulatory aspects submitted to University of Vienna, Vienna, Austria (2009)

Podbielski, A., M. Woischnik, B. A. Leonard and K. H. Schmidt, Characterization of *nra*, a global negative regulator gene in group A streptococci. *Mol. Microbiol.* **31**: 1051-1064 (1999)

Rasmussen,A.A., M.Eriksen, K.Gilany, C. Udesen, T.Franch, C.Petersen and P. Valentin-Hansen, Regulation of ompA mRNA stability: the role of a small regulatory RNA in growth phase-dependent control. *Mol. Microbiol.* **58**: 1421-1429 (2005)

Rasmussen,M., A.Eden and L.Bjorck, SclA, a novel collagen-like surface protein of Streptococcus pyogenes. *Infect. Immun.* **68**: 6370-6377 (2000)

Reid,S.D., M.S.Chaussee, C.D.Doern, M. A.Chaussee, A.G.Montgomery, D.E. Sturdevant and J.M.Musser, Inactivation of the group A Streptococcus regulator *srv* results in chromosome wide reduction of transcript levels, and changes in extracellular levels of Sic and SpeB. *FEMS Immunol. Med. Microbiol.* **48**: 283-292 (2006)

Reid,S.D., A.G.Montgomery and J.M. Musser, Identification of *srv*, a PrfA-like regulator of group A streptococcus that influences virulence. *Infect. Immun.* **72**: 1799-1803 (2004)

Ribardo, D. A. and K. S. McIver, Defining the Mga regulon: Comparative transcriptome analysis reveals both direct and indirect regulation by Mga in the group A streptococcus. *Mol. Microbiol.* **62**: 491-508 (2006)

Roberts, S. A., G. G. Churchward and J. R. Scott, Unraveling the regulatory network in *Streptococcus pyogenes*: the global response regulator CovR represses *rivR* directly. *J. Bacteriol.* **189**: 1459-1463 (2007)

Roberts, S. A. and J. R. Scott, RivR and the small RNA RivX: the missing links between the CovR regulatory cascade and the Mga regulon. *Mol. Microbiol.* **66**: 1506-1522 (2007)

Romby,P.,F.Vandenesch and E.G. Wagner, The role of RNAs in the regulation of virulence-gene expression. *Curr Opin Microbiol* **9**: 229-236 (2006)

Shelburne,S.A.,3rd, P.Sumby,I. Sitkiewicz, C.Granville, F.R.DeLeo and J.M. Musser, Central role of a bacterial two-component gene regulatory system of previously unknown function in pathogen persistence in human saliva. *Proc. Natl. Acad. Sci. U S A* **102**: 16037-16042 (2005)

Siller,M., Ph.D. Thesis. Regulatory processes in the pathogenicity of Group A streptococcus submitted to University of Vienna, Vienna, Austria (2008)

Sitkiewicz,I. and J.M.Musser,Expression microarray and mouse virulence analysis of four conserved two-component gene regulatory systems in group a streptococcus.*Infect.Immun.***74**:1339-51 (2006)

Steiner,K. and H.Malke, *relA*-Independent amino acid starvation response network of *Streptococcus pyogenes*. *J. Bacteriol.* **183**: 7354-7364 (2001)

Stevens,D.L., Invasive group A streptococcus infections. *Clin Infect Dis* **14**: 2-11 (1992)

Stevens,D.L., Streptococcal toxic shock syndrome associated with necrotizing fasciitis. *Ann Rev Med* **51**:271-288 (2000)

Stevens,D.L., M.H. Tanner, J. Winship, R. Swarts, K.M.Ries, P.M.Schlievert and E.Kaplan, Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N Engl J Med* **321**: 1-7 (1989)

Storz,G., S. Altuvia and K. M. Wassarman, An abundance of RNA regulators. *Annu.Rev.Biochem.***74**:199-217 (2005)

Sumby,P., A.R.Whitney, E.A.Graviss, F.R. DeLeo and J. M. Musser, Genome-wide analysis of group a streptococci reveals a mutation that modulates global phenotype and disease specificity. *PLoS. Pathog.* **2**:e5 (2006)

Terao,Y.,S.Kawabata,E.Kunitomo, J.Murakami, I.Nakagawa and S.Hamada, Fba, a novel fibronectin-binding protein from Streptococcus pyogenes, promotes bacterial entry into epithelial cells, and the fba gene is positively transcribed under the Mga regulator. *Mol. Microbiol.* **42**: 75-86 (2001)

Valentin-Hansen, P., M.Eriksen and C. Udesen, The bacterial Sm-like protein Hfq: a key player in RNA transactions. *Mol. Microbiol.* **51**: 1525-1533 (2004)

Voyich,J.M., K.R.Braughton, D.E. Sturdevant, C.Vuong, S.D.Kobayashi, S.F. Porcella,M. Otto, J.M.Musser and F.R. DeLeo, Engagement of the pathogen survival response used by group A *Streptococcus* to avert destruction by innate host defense. *J. Immunol.* **173**: 1194-1201 (2004)

Voyich,J.M., D.E.Sturdevant, K.R. Braughton, S.D.Kobayashi,B.Lei,K.Virtaneva, D.W. Dorward, J.M.Musser and F.R. DeLeo, Genome-wide protective response used by group A Streptococcus to evade destruction by human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* **100**:1996-2001 (2003)

Wagner,E.G., S.Altuvia and P.Romby, Antisense RNAs in bacteria and their genetic elements. *Adv. Genet.* **46**: 361-398 (2002)

Wessels,M.R., Regulation of virulence factor expression in group A streptococcus. *Trends Microbiol* **7**: 428-430 (1999)

Winkler,W.C. and R.R.Breaker, Regulation of bacterial gene expression by riboswitches. *Annu.Rev.Microbiol.* **59**: 487-517 (2005)

Wolz,C.,D.McDevitt, T.J.Foster and A.L.Cheung, Influence of agr on fibrinogen binding in Staphylococcus aureus Newman. *Infect. Immun.* **64**: 3142-3147 (1996)