

Transcriptional Regulation in Spirochetes

Karl J. Indest, Ramesh Ramamoorthy,
and Mario T. Philipp*

Department of Parasitology, Tulane Regional Primate
Research Center, Tulane University Medical Center, 18703
Three Rivers Road, Covington, La. 70433, USA

Abstract

Spirochetes belong to a widely diverse family of bacteria. Several species in this family can cause a variety of illnesses including syphilis and Lyme disease. Despite the fact that the complete genome sequence of two species, *Borrelia burgdorferi* and *Treponema pallidum*, have been deciphered, much remains to be understood about spirochetal gene regulation. In this review we focus on the environmental transitions that spirochetes undergo during their life cycles and the mechanisms of transcriptional regulation that might possibly mediate spirochetal adaptations to such changes.

Introduction

Spirochetes belong to a diverse family of bacteria, many of whose members cause a variety of human diseases. These include syphilis, Lyme disease, relapsing fever, and leptospirosis. The family Spirochaetaceae consists of six genera: *Borrelia*, *Treponema*, *Leptospira*, *Leptonema*, *Spirochaeta*, and *Cristispira*. Phylogenetic analyses based on 16S rRNA sequences indicate that spirochetes have diverged into two main clusters: the *Borrelia*-*Treponema*-*Spirochaeta* cluster and the *Leptospira* cluster (Paster, 1984). Spirochetes are only distantly related to the eubacteria (Paster, 1984). As a family, its members share a distinct morphology coupled with a unique form of motility, but are quite diverse with respect to their natural histories, metabolism and genetics (Holt, 1978; Harwood and Canale-Parola, 1984). Spirochetes of the *Borrelia* species are the only family members that cycle between vertebrate and arthropod hosts. *Treponema pallidum*, in contrast, exclusively parasitizes humans. The genus *Leptospira* includes both parasitic and aquatic free-living forms. Of the remaining genera, the genus *Spirochaeta* consists of various aerobic and anaerobic free-living bacteria, while members of the genus *Cristispira* are 10- μ m-wide giant organisms which are found associated exclusively with mollusks (Paster *et al.*, 1996). In this review we will attempt to summarize what is currently known about mechanisms of regulation of transcription in spirochetes. We will discuss these mechanisms in the context of the particular spirochete's natural history. Specifically, we will focus on the environmental transitions that spirochetes undergo during their life cycles and the mechanisms of

transcriptional regulation that might possibly mediate spirochetal adaptations to such changes. Because of the limited information on the genera *Spirochaeta*, *Leptonema*, and *Cristispira*, we will concentrate exclusively on *Borrelia*, *Treponema* and *Leptospira*.

Borrelia

B. burgdorferi, the etiologic agent of Lyme disease, alternates between two widely different hosts, a vertebrate host (usually a rodent), and ticks of the *Ixodes ricinus* complex (Anderson, 1991). The larval and nymphal stages of the tick vector feed primarily on small rodents while the adult ticks feed mostly on deer (Anderson, 1991). Larval ticks initially acquire *B. burgdorferi* when they feed on an infected rodent (Anderson, 1991; Burgdorfer *et al.*, 1991). Spirochetes are then conveyed transtadially through successive phases of feeding and molting to the nymph and adult stages. Rodents get infected by the spirochete after being fed upon by an infected nymph (Anderson, 1991; Burgdorfer *et al.*, 1991). Since larvae and nymphs have a preference for the same reservoir host within an ecological niche, the natural history of *B. burgdorferi* encompasses larvae, nymphs and, most often, rodents. As the spirochetes cycle among these hosts they undergo several environmental transitions: from the vertebrate host to the tick within a blood meal milieu, followed by a gradual deprivation of nutrients as the tick flattens, and possibly additional changes accompanying the tick molt. This process is then followed by a sudden exposure to another blood meal as the spirochete transits to the vertebrate host. Changes in temperature, quantity and quality of nutrients, cell density, and pH are some of the possible environmental cues during this cycle. In unison with these changes, *B. burgdorferi* alters the expression of some of its surface lipoproteins, and it has been postulated that these alterations are adaptive (Schwan, 1996). Several lipoproteins are now known to be differentially expressed by *B. burgdorferi* (Table 1). Two of the lipoproteins most assiduously studied are outer surface proteins (Osp) A and C.

The OspA/OspC Switch

OspA is a major outer membrane lipoprotein that is encoded by the first gene of a bicistronic operon located on *B. burgdorferi*'s 54 kb linear plasmid (Howe *et al.*, 1986; Bergstrom *et al.*, 1989). This protein is abundantly expressed by most spirochete isolates when the latter are cultivated *in vitro*. During the infection cycle, OspA is predominantly expressed by spirochetes within the tick (Burkot *et al.*, 1994; Fingerle *et al.*, 1995; Schwan *et al.*, 1995; de Silva *et al.*, 1996). OspA synthesis starts after entry of the spirochete into the tick larva. The OspA protein is retained by at least a portion of the spirochetal population that remains within the tick. The persistence of OspA in tick-borne organisms strongly suggests that this protein plays an important role in the long-term survival of the spirochete in this milieu. Likewise, OspC expression

*For correspondence. Email philipp@tpc.tulane.edu; Tel. (504) 871-6221; Fax. (504) 871-6390.

Table 1. Differentially Expressed Genes of *Borrelia burgdorferi*

Genes/ Gene Families	Gene Expression							Vertebrate	Tick	References
	Culture									
	Temperature		Growth Phase		pH					
	24°	34°	Log	Stationary	6	8				
<i>ospA</i>	+	+	+	+	+	+	-	+ ^a	(Fingerle <i>et al.</i> , 1995; Schwan <i>et al.</i> , 1995; Montgomery <i>et al.</i> , 1996; Fikrig <i>et al.</i> , 1998; Ramamoorthy and Philipp, 1998; Carrol <i>et al.</i> , 1999)	
<i>ospC</i>	-	+	+ / - ^c	+	+	+	+ / - ^c	+ ^b	(Schwan <i>et al.</i> , 1995; Stevenson <i>et al.</i> , 1995; Montgomery <i>et al.</i> , 1996; Ramamoorthy and Philipp, 1998; Carrol <i>et al.</i> , 1999)	
<i>dbpA/dbpB</i>	-	+	ND	ND	ND	ND	+	ND	(Cassatt <i>et al.</i> , 1998)	
<i>eppA</i>	ND	-	ND	ND	ND	ND	+	ND	(Champion <i>et al.</i> , 1994)	
<i>oppAV</i>	-	+	ND	ND	ND	ND	ND	ND	(Bono <i>et al.</i> , 1998)	
<i>p35-a¹</i>	ND	-	ND	ND	ND	ND	+	ND	(Fikrig <i>et al.</i> , 1997)	
<i>p35-b¹</i>	ND	+	-	+	ND	ND	+	ND	(Indest <i>et al.</i> , 1997; Ramamoorthy and Philipp, 1998)	
<i>p37</i>	ND	-	ND	ND	ND	ND	+	ND	(Fikrig <i>et al.</i> , 1997)	
<i>p6.6</i>	ND	+	-	+	ND	ND	-	ND	(Indest <i>et al.</i> , 1997; Lahdenne <i>et al.</i> , 1997; Akins <i>et al.</i> , 1998)	
<i>ospEF</i> family										
<i>ospE</i>	+ / - ^c	+	ND	ND	ND	ND	+	+	(Lam <i>et al.</i> , 1994; Nguyen <i>et al.</i> , 1994; Stevenson <i>et al.</i> , 1995; Akins <i>et al.</i> , 1998)	
<i>p21</i>	-	-	ND	ND	ND	ND	+	-	(Das <i>et al.</i> , 1997; Akins <i>et al.</i> , 1998)	
<i>ospF</i>	-	+	ND	ND	ND	ND	+	+	(Lam <i>et al.</i> , 1994; Nguyen <i>et al.</i> , 1994; Akins <i>et al.</i> , 1995; Stevenson <i>et al.</i> , 1995; Akins <i>et al.</i> , 1998)	
<i>bbk2.10</i>	-	-	ND	ND	ND	ND	?	ND	(Akins <i>et al.</i> , 1995; Akins <i>et al.</i> , 1998)	
<i>pG</i>	ND	-	ND	ND	ND	ND	+	ND	(Wallich <i>et al.</i> , 1995)	
<i>erpG</i>	+ / - ^c	+	ND	ND	ND	ND	?	ND	(Stevenson <i>et al.</i> , 1998)	
<i>erpA/I</i>	+ / - ^c	+	ND	ND	ND	ND	+	ND	"	
<i>erpB2/J</i>	+ / - ^c	+	ND	ND	ND	ND	+	ND	"	
<i>erpK</i>	+ / - ^c	+	ND	ND	ND	ND	+	ND	"	
<i>erpM</i>	+ / - ^c	+	ND	ND	ND	ND	?	ND	"	
<i>erpX</i>	+ / - ^c	+	ND	ND	ND	ND	?	ND	"	
<i>erpC</i>	ND	+	ND	ND	ND	ND	?	ND	(Stevenson <i>et al.</i> , 1996; Stevenson <i>et al.</i> , 1998)	
<i>erpT</i>	ND	+	ND	ND	ND	ND	+	+/- ^a	(Fikrig <i>et al.</i> , 1999)	
<i>mlp2.9</i> family										
<i>mlp-2.9-1</i>	ND	+	ND	ND	ND	ND	ND	ND	(Porcella <i>et al.</i> , 1996)	
<i>mlp-2.9-2</i>	ND	+	ND	ND	ND	ND	ND	ND	"	
<i>mlp-2.9-3</i>	ND	-	ND	ND	ND	ND	ND	ND	"	
<i>mlp-2.9-4</i>	ND	+	ND	ND	ND	ND	ND	ND	"	
<i>mlp-2.9-5</i>	ND	-	ND	ND	ND	ND	ND	ND	"	
<i>mlp-2.9-7</i>	-	-	ND	ND	ND	ND	+	ND	(Porcella <i>et al.</i> , 1996; Akins <i>et al.</i> , 1998)	
<i>mlp-2.9-8</i>	-	+	ND	ND	ND	ND	+	ND	(Yang <i>et al.</i> , 1999)	
<i>mlp-2.9-9</i>	-	+ / - ^c	ND	ND	ND	ND	+	ND	"	
<i>mlp-2.9-10</i>	-	+ / - ^c	ND	ND	ND	ND	+	ND	"	

^aflat ticks; ^bengorged ticks; ^clow expression. ¹Two unrelated genes, both encoding 35 kDa proteins

appears to be principally associated with spirochetal existence in the vertebrate host. This is based on the observation that OspC synthesis is initiated during tick feeding and appears to be maintained throughout infection of the vertebrate host (Schwan *et al.*, 1995; Montgomery *et al.*, 1996; de Silva *et al.*, 1999; Mbow *et al.*, 1999; Gilmore, Jr. and Piesman, 2000). Thus, the feeding event represents the junction at which the switch in the expression of the two lipoproteins occurs. This interphase comprises a mixed population of spirochetes which either express OspA and OspC simultaneously, or alternatively (Schwan *et al.*, 1995; Schwan and Piesman, 2000). It is likely that the OspA⁺OspC⁺ spirochetes exit the tick to invade the vertebrate host whereas the OspA⁺OspC⁻ and possibly even the OspA⁺OspC⁺ subpopulations remain in the tick (Gilmore and Piesman, 2000). Interestingly, a similar alternation in the expression of homologous surface proteins in ticks versus mammals occurs in the relapsing fever spirochete *Borrelia hermsii* (Schwan and Hinnebusch,

1998). It has been suggested that such a parallel signifies common biological functions for these proteins in tick transmission or early colonization in mammals (Schwan and Hinnebusch, 1998).

The absence of antibodies to OspA in tick-inoculated hosts in the face of a rapidly developing anti-OspC response has led to the suggestion that OspA synthesis continues to be down regulated following entry of the spirochetes into the vertebrate host (Philipp and Johnson, 1994; Barthold *et al.*, 1995; Brunet *et al.*, 1995; de Silva *et al.*, 1996). There is evidence that the down regulation of OspA and the concomitant upregulation of OspC are both controlled in part at the mRNA level. Reverse transcriptase PCR (RT-PCR) analysis of skin biopsy specimens from erythema migrans lesions of Lyme disease patients failed to detect *ospA* mRNA (Fikrig *et al.*, 1998). Similarly, RT-PCR analysis of tissue samples from needle-infected mice showed that *ospA* mRNA, while present at day 14 of infection, could no longer be detected at day 30 (Montgomery *et al.*, 1996). In

contrast, *ospC* mRNA was readily detected by RT-PCR at both time points (Montgomery *et al.*, 1996). Competitive PCR further revealed that *ospC* mRNA is present in tick-borne spirochetes prior to their entry into the vertebrate host (de Silva *et al.*, 1999). The mechanism(s) that regulate the OspA/OspC transcriptional switch is not fully understood, but studies involving cultured spirochetes indicate that various *cis* elements and *trans* factors may play a role. While most strains of *B. burgdorferi* abundantly express OspA, a clone of the strain CA-11.2A was isolated that does not synthesize this protein (Margolis and Rosa, 1993). The *ospA* gene from this strain was cloned and shown to be expressed from its own promoter in *Escherichia coli*. This indicated that the promoter was functional. The *ospAB* operon was subsequently sequenced and shown to have an intact sigma-70-like promoter, yet no *ospA* mRNA transcript was detected from CA-11.2A. Electrophoretic mobility shift assays using the *ospA* promoter region and cell-free extracts from the OspA⁻ strain revealed the presence of a specific DNA-protein complex (Margolis and Samuels, 1995). Since in *E. coli* *ospA* is readily transcribed from its own promoter it was concluded that this *ospA*-specific factor might be acting as a repressor. A more detailed analysis of the promoter region of *ospA* using a transiently expressed reporter gene indicated that in addition to the core region containing the typical -35 and -10 elements there is also a unique T-rich region (Sohaskey *et al.*, 1999). Removal of the T-rich region from the *ospAB* promoter resulted in a 6-fold reduction in promoter activity suggesting that this is a positively-acting *cis* element. The sequence and location of this element closely resembles prokaryotic upstream promoter (UP) elements (Ross *et al.*, 1993). In contrast to *ospA*, *ospC* lacks a recognizable sigma-70-like promoter and is not expressed in *E. coli* (Sohaskey *et al.*, 1997). The lack of *ospC* expression in *E. coli* suggests that additional factors specific to *B. burgdorferi* may be required for its expression. The correlation between lack of *ospC* expression and a 51 bp deletion upstream of a -35 promoter region of *ospC* suggests that essential sequences are present within this segment of the *ospC* promoter (Padula *et al.*, 1993). In addition, studies directed at curing plasmids from various strains of *B. burgdorferi* revealed an inverse relationship between OspC expression and the presence of a 16 kb linear plasmid (Sadziene *et al.*, 1993). On the basis of this observation, it was suggested that this plasmid might encode a *trans* acting factor that represses the synthesis of the *ospC* gene (Sadziene *et al.*, 1993). To date no such repressor or activator proteins have been identified for either *ospA* or *ospC*.

The *ospEF* Family and Other Paralogous Gene Families

The genome of the Lyme disease spirochete contains multiple paralogous gene families of unknown function. Included among these are the *ospEF* and the *mlp2.9* (multicopy lipoprotein genes), two 32 kb circular plasmid (cp-32) loci that are well characterized in terms of DNA sequences, gene organization and expression (Lam *et al.*, 1994; Porcella *et al.*, 1996a; Yang *et al.*, 1999). The *ospEF* family is also known by other names such as *erp* (ospEF-related proteins) (Stevenson *et al.*, 1996), UHB (upstream homology box) gene family (Marconi *et al.*, 1996), and *elp* (OspE/F-like leader peptides) family (Akins *et al.*, 1999).

Members of the *ospEF* family are expressed in cultured spirochetes (Lam *et al.*, 1994; Stevenson *et al.*, 1995; Akins *et al.*, 1998; Stevenson *et al.*, 1998), within the tick (Nguyen *et al.*, 1994; Fikrig *et al.*, 1999), and during infection of the mammalian host (Champion *et al.*, 1994; Lam *et al.*, 1994; Akins *et al.*, 1995; Suk *et al.*, 1995; Wallich *et al.*, 1995; Das *et al.*, 1997; Akins *et al.*, 1998; Stevenson *et al.*, 1998). In addition, the *ospEF* and some of the *mlp* lipoprotein genes are upregulated *in vitro* in response to an increase in temperature (Akins *et al.*, 1998; Stevenson *et al.*, 1998; Yang *et al.*, 1999). Characterization of the upstream regions of these two gene families revealed that within each family the 5' regions are highly conserved (Marconi *et al.*, 1996; Porcella *et al.*, 1996a). As a consequence, one might expect that members of each of these gene families are coregulated. Some *erp* genes appear not to be expressed *in vivo*, as indicated by the lack of specific antibody responses against these particular proteins during infection of the vertebrate host (Stevenson *et al.*, 1998). The regulation of these proteins appears inherently different from the reciprocal upregulation/downregulation process characteristic of the OspA/C switch. Recent evidence suggests that the *Erp* proteins undergo antigenic variation within the vertebrate host (Sung *et al.*, 2000). Hence the inability to detect specific serum antibody against particular *Erp* variants may have been due to the fact that such variants were not expressed prior to the time the serum sample was collected but may be expressed later, as the variation mechanism runs its course. It is possible, therefore, that all *erp* genes are expressed *in vivo*, a notion which is consistent with their shared upstream sequence and their collective upregulation at a higher temperature (34°C vs. 24°C) in culture.

In addition to these gene families, a third paralogous family referred to as the Bdr (*Borrelia* direct repeat) protein family, was characterized recently in two *Borrelia* species (Zuckert *et al.*, 1999; Carlyon *et al.*, 2000; Zuckert and Barbour, 2000). Members of this gene family are distributed among the 32 kb circular plasmids and various linear plasmids (Zuckert *et al.*, 1999; Carlyon *et al.*, 2000; Zuckert and Barbour, 2000). Unlike members of the OspEF and the 2.9 Mlp lipoprotein families, members of the Bdr protein family are not induced by an increase in culture temperature but rather are constitutively expressed both in culture and during infection of the vertebrate host (Zuckert *et al.*, 1999). Interestingly while these proteins themselves do not appear to be expressed differentially, it has been suggested that they may play a regulatory role in signaling or sensing (Roberts *et al.*, 2000).

Experimental Models to Study Gene Regulation

The differential *in vivo* expression of various genes and proteins has been demonstrated directly with techniques such as RT-PCR (Montgomery *et al.*, 1996; Das *et al.*, 1997; Fikrig *et al.*, 1998; Fikrig *et al.*, 1999) and immunofluorescence (Schwan *et al.*, 1995), or indirectly by assaying for the presence of antibodies to recombinant antigens in serum from infected animals (Suk *et al.*, 1995; Fikrig *et al.*, 1997). However, studies of the molecular mechanisms of spirochetal adaptations *in vivo* have been limited because of the paucity of spirochetes within the tick and vertebrate hosts. The development of models in which regulatory mechanisms of differential gene

expression can be easily studied *in vitro* has been an important step towards dissecting *B. burgdorferi* gene regulation. Several such models are now available. *B. burgdorferi* was shown to regulate the expression of several genes *in vitro* in response to changes in pH (Carroll *et al.*, 1999), temperature (Schwan *et al.*, 1995; Stevenson *et al.*, 1995; Akins *et al.*, 1998; Bono *et al.*, 1998; Cassatt *et al.*, 1998; Stevenson *et al.*, 1998; Yang *et al.*, 1999), and growth phase or cell density (Indest *et al.*, 1997; Ramamoorthy and Philipp, 1998). We showed that the expression of the lipoproteins OspC, P35 and P7.5 and numerous other *B. burgdorferi* antigens is upregulated at the onset of stationary growth phase (Indest *et al.*, 1997; Ramamoorthy and Philipp, 1998). Sequence analysis of the upstream region of *p35* revealed the presence of several potentially *cis*-acting elements that could be involved in the regulation of this gene. These elements include, in a 5'→3' order, an inverted repeat, a T-rich tract, and an AT-rich region. Electromobility shift assays showed that stationary phase cell-free extracts contain a DNA binding protein that specifically interacts with the *p35* promoter region (Indest and Philipp, 2000). The portion of the *p35* promoter that is targeted by the DNA binding protein is comprised within a segment that contains both the inverted repeat and the T-rich tract. The latter is similar to the previously mentioned T-rich sequence that was shown to positively influence OspA expression *et al.*, 1999) (Sohaskey *et al.*, 1999). Presence of a T-rich region may be necessary but is not sufficient to fully explain growth-phase-dependent regulation, for the *ospA* gene contains such a region and yet is not regulated by growth phase *in vitro* (Ramamoorthy and Philipp, 1998). We hypothesized that the DNA binding protein, as yet unidentified, activated *p35* transcription. We based this assumption on the proximity of both the T-rich sequence and the inverted repeat to the -35 promoter region, and the presence of the binding activity only in extracts obtained in stationary phase, when P35 is expressed. The recent observation that *ospC* expression is altered in response to cell density within the tick (de Silva *et al.*, 1999) further argues for the need of *in vitro* models to study gene expression, as it is possible that the same regulatory mechanisms may operate both *in vitro* and *in vivo*.

The fact that *B. burgdorferi* modulates gene expression *in vitro* in response to temperature and pH could also be utilized to develop *in vitro* models of gene regulation. The expression of many proteins, including OspC, is silent or scant in spirochetes cultivated at 24°C but is readily turned on when the culture temperature is raised to 34°C or higher (Schwan *et al.*, 1995; Stevenson *et al.*, 1995; Akins *et al.*, 1998; Bono *et al.*, 1998; Cassatt *et al.*, 1998; Stevenson *et al.*, 1998; Yang *et al.*, 1999). However, the lack of OspC expression by spirochetes in ticks incubated at 34°C indicates that in addition to temperature other cues are required for OspC expression (Schwan *et al.*, 1995). The expression of OspC and that of several other unidentified proteins also respond to changes in pH (Carroll *et al.*, 1999). Most alterations in protein expression occur between pH 7.0 and pH 8.0.

A useful complement to *in vitro* models that mimic some of the environmental transitions experienced by *B. burgdorferi* is a technique that permits recovery of large numbers of spirochetes from within the vertebrate host (Akins *et al.*, 1998). A dialysis bag is seeded at a low density

with cultured spirochetes and is surgically implanted into the peritoneal cavity of a rat. After a few days the host-adapted spirochetes are harvested and analyzed. With this technique it is possible to identify proteins whose expression is either induced or repressed *in vivo* with respect to their status *in vitro* (Akins *et al.*, 1998; Yang *et al.*, 1999).

DNA Binding Proteins in *Borrelia burgdorferi*

The transcriptional machinery of *B. burgdorferi* must have evolved to enable the organism to adapt to the diverse environments in the hosts it parasitizes. Toward this end it must correctly sense, and respond to, environmental cues that signal transitions within and between hosts. Such adaptations are possibly mediated, at least in part, by the differential expression of lipoproteins. A liberal interpretation of the current *in vitro* observations is that temperature, pH, and spirochete cell density may be some of the important *in vivo* cues responsible for modulation of lipoprotein expression. The *trans*-acting factors that mediate these processes are unknown. The recently determined sequence of the *B. burgdorferi* genome indicates that control of gene expression in this organism is different from other eubacteria (Fraser *et al.*, 1997). This can be best illustrated by the lack of orthologs of other bacterial transcriptional regulators. In addition to the house-keeping sigma RpoD (σ^{70}), *B. burgdorferi* is endowed with just two other alternative sigma factors, the stationary sigma factor RpoS and RpoN (σ^{54}).

Recently, *B. burgdorferi* spirochetes in which the *rpoS* gene was inactivated were shown to exhibit an altered stationary phase response. Two-dimensional non-equilibrium gradient gel electrophoresis of stationary phase cell lysates identified at least 11 differences between the protein profiles of the *rpoS* mutant and the wild type parent (Elias *et al.*, 2000). Six of the 11 proteins were upregulated in the *rpoS* mutant whereas the remaining 5 proteins were down regulated. These results suggest both a positive and negative role for RpoS in the regulation of gene expression. Interestingly, a candidate RpoN-dependent promoter has been identified upstream of the *B. burgdorferi* *rpoS* gene, suggesting that RpoS may be partially controlled by RpoN (Studholme *et al.*, 2000). Such an arrangement may indicate the presence of a sigma factor regulatory cascade. It has been postulated that this arrangement may allow for the rapid high-level expression of *rpoS* in response to various environmental cues (Studholme *et al.*, 2000). In contrast to other alternative sigma factors, RpoN is unique in that once it complexes with RNA polymerase it requires nucleotide hydrolysis catalyzed by an activator protein bound to an upstream enhancer element for transcription to proceed (Rombel *et al.*, 1998). The identification in the *B. burgdorferi* genome of a putative σ^{54} -dependent activator homolog of the NtrC family, namely, the response regulator protein RRP-2, is consistent with this notion (Studholme *et al.*, 2000). While the function of RpoN has yet to be determined in *B. burgdorferi*, the fact that *B. burgdorferi* lacks the flagellar specific sigma factor (σ^{28}) may also indicate that RpoN is involved in the transcriptional regulation of flagellar biosynthesis as seen in other bacteria (Kinsella *et al.*, 1997). In addition to flagellar biosynthesis, RpoN has been also shown to regulate genes involved in a variety of cellular functions, including carbon metabolism,

exopolysaccharide synthesis, and nitrogen fixation (Studholme *et al.*, 2000).

Other global bacterial regulators present in *B. burgdorferi*, as inferred from the genome sequence, are transcriptional regulators of the helix-turn-helix (HTH) class which include two response regulators, two sugar kinase transcriptional regulators, and a Fur homologue. Fur may play a role in the metabolism of *B. burgdorferi*. The addition of increasing concentrations of iron to the culture medium has been shown to stimulate spirochetal growth (Sambri *et al.*, 1991). A transferrin-binding protein also was described in *B. burgdorferi*. However, the role of this protein in iron acquisition remains to be determined (Carroll *et al.*, 1996). A recent comparison of the *B. burgdorferi* and *T. pallidum* genomes further revealed the presence of two unusual HTH-containing proteins specific to the spirochetes (Subramanian *et al.*, 2000). These predicted proteins are unusual in that they contain a C-terminal HTH domain similar to the paired domains of resolvases, while their N-terminal region resembles that of a signal peptide (Subramanian *et al.*, 2000). In addition to the HTH class of transcriptional regulators, a putative member of the MetJ/Arc family of beta-sheet-containing transcriptional factors is present on a linear plasmid (Subramanian *et al.*, 2000).

Thus far only one DNA binding protein whose identity is known, the 34 kDa C-terminal polypeptide of *gyrA*, has been described (Knight and Samuels, 1999). This protein was identified in a biochemical screen for telomere-binding proteins in *B. burgdorferi*. It was demonstrated that the C-terminal polypeptide of *gyrA* is able to complement an HU-deficient strain of *E. coli*. The importance of this finding is somewhat diminished by the observation that a *B. burgdorferi* mutant deficient in this protein exhibits a wild type phenotype (Knight *et al.*, 2000). A putative DNA-binding protein, an HU/IHF homologue encoded by the *hbb* gene, also has been described in *B. burgdorferi*; its function is unknown (Tilly *et al.*, 1996). The nature of the *cis*-acting elements that facilitate binding of these regulatory factors is not known. However, it does appear that T-rich sequences are important for activation of promoters and recruitment of protein factors (Sohaskey *et al.*, 1999; Indest and Philipp, 2000).

It still remains to be determined how environmental signals are detected and transduced to the genome to affect gene expression. DNA supercoiling may be responsible for relaying temperature fluctuations. The fact that a majority of the *B. burgdorferi* genes responsive to temperature reside on supercoiled plasmids may indicate that superhelical density is involved in temperature regulation (Porcella *et al.*, 1996a). Temperature change has been shown in other bacteria to result in the activation of genes through its effect on DNA supercoiling (Hurme and Rhen, 1998). Responses to other environmental cues such as changes in cell-density or pH likely involve one or more signal-response systems.

Quorum Sensing in *Borrelia burgdorferi*

The observation that cell-density appears to affect gene expression in *B. burgdorferi* is suggestive of a specific signal-response system known as quorum sensing. Quorum sensing, or density-dependent regulation of gene expression, is a specific type of cell-cell communication found in several bacteria. It is mediated by the synthesis,

secretion, and detection of small diffusible signaling molecules (Dunny and Winans, 1999). Quorum sensing, previously known as autoinduction, was first described in the marine bacteria *Vibrio fischeri* and *Vibrio harveyi* (Nealson *et al.*, 1970; Eberhard, 1972). In these organisms quorum sensing controls bioluminescence. Similar sensing systems are now known to exist in numerous genera of bacteria including several human pathogens. In addition to bioluminescence, quorum sensing has been implicated in a number of diverse processes such as conjugation, antibiotic production, biofilm formation, and pathogenesis (Dunny and Winans, 1999). Recently, a new family of genes responsible for quorum sensing has been described in *V. harveyi*, *E. coli*, and *Salmonella typhimurium* (Surette *et al.*, 1999). The gene, *luxS*, responsible for the synthesis of this autoinducer (AI-2) is homologous to a gene in *B. burgdorferi*. The nature of the signaling molecule that this gene product synthesizes is unknown. It has been proposed that in *V. harveyi* the product of the *luxS* gene interacts with a two-component response regulator resulting in the activation of genes involved in bioluminescence (Bassler *et al.*, 1994). This two component response regulator bears similarity with putative regulators encoded by homologous genes in the *B. burgdorferi* genome. This suggests that *B. burgdorferi* has the molecular circuitry required for a functional quorum sensing pathway. We have evidence that the *B. burgdorferi luxS* homolog is expressed in cultured spirochetes and upregulated in stationary growth phase (our unpublished data). Evidence is now mounting that there may be some interplay between components of the quorum sensing pathway and the alternative sigma factors RpoS and RpoN. Recently, components of the quorum sensing cascade and RpoS were both found to regulate genes of the type III secretion apparatus in an enteropathogenic strain of *E. coli* (Sperandio *et al.*, 1999). In *V. cholerae* a σ^{54} -dependent transcriptional activator required for host colonization was identified that has high sequence identity with one of the quorum sensing response regulators, *luxO* (Klose *et al.*, 1998). The notion that *B. burgdorferi* utilizes quorum sensing is especially attractive when considering the natural history of this organism. *B. burgdorferi* could upregulate proteins required for transmission and infection only after spirochete numbers are sufficiently high in the tick to ensure a successful transition to the vertebrate host.

Treponema

Treponema pallidum, the etiologic agent of syphilis, is the best known species of this genus. Like *B. burgdorferi*, *T. pallidum* has a small genome (~1Mb) (Fraser *et al.*, 1998) and causes a chronic disseminated infection. Despite such similarities, these organisms differ with respect to the transitions they encounter during their natural histories. *T. pallidum* only parasitizes humans, and is thus not exposed to the more extreme environmental changes encountered by *B. burgdorferi*. However, it is possible that these spirochetes share at least a subset of the transitions encountered in the human host as suggested, for example, by their common predilection for the central nervous system. The inability to propagate *T. pallidum in vitro* and the (consequent) lack of a genetic exchange system have hindered the study of this spirochete's genetics and physiology. RT-PCR has been used to assess expression

of *T. pallidum* genes *in vivo* (Stamm et al., 1998) but there are no studies of differential gene expression in this organism. Most of the information on gene regulation in *Treponema* has come from studies involving *Treponema denticola* and *Treponema hyodysenteriae*. *T. denticola* is an oral spirochete associated with human periodontal disease. *T. hyodysenteriae* is the etiologic agent of swine dysentery and has recently been placed in the new genus *Serpulina* (Pettersson et al., 1996). *T. denticola* and *T. hyodysenteriae* have been less refractory to genetic manipulations than *T. pallidum* due in part to the fact that they are cultivable. Genetic studies in the treponemes have centered primarily on the cloning and characterization of genes that encode major antigens or proteins involved in motility (Hsu et al., 1989; Fenno et al., 1996; Li et al., 1996; Limberger et al., 1996; Porcella et al., 1996b; Hardham, 1997; Greene et al., 1997; Hagman et al., 1997; Heinzerling et al., 1997). The fact that some *Treponema* genes are readily expressed in *E. coli* suggests that *Treponema* regulatory signals may be similar to those found in *E. coli* (Stamm et al., 1988). Alternative sigma factors like σ^{28} also may play a role in gene regulation. This supposition is based on the fact that a subset of *Treponema* motility genes have σ^{28} -like promoters (Champion et al., 1990; Limberger et al., 1992; Limberger et al., 1996; Heinzerling et al., 1997; Stamm, 1999). Now that the complete genome sequence of *T. pallidum* is available, comparative genome analyses with *B. burgdorferi* are feasible (Subramanian et al., 2000). Such analyses have revealed that these organisms differ in their repertoire of transcriptional regulators (Subramanian et al., 2000). For example, unlike *B. burgdorferi*, *T. pallidum* possesses σ^{24} , σ^{28} , and σ^{43} . The functions of σ^{24} and σ^{43} may partially substitute for that of RpoS, which is not present in *T. pallidum* (Fraser et al., 1998). Both σ^{24} and σ^{43} are necessary for growth and survival of *E. coli* at higher temperatures (Chang et al., 1994; Hiratsu et al., 1995). In addition, σ^{24} is critical for virulence in *Salmonella typhimurium* (Humphreys et al., 1999). The presence of σ^{28} confirms previous observations of σ^{28} -like promoter sequences in *Treponema*. The absence of the heat shock sigma factor σ^{32} and the constitutive expression of GroEL and Dna K in *T. pallidum* have led to the suggestion that this organism does not undergo a heat-shock response (Stamm et al., 1991). This is a sensible idea, considering that this organism exclusively parasitizes humans. In a manner consistent with the presence of multiple sigma factors, *T. pallidum* encodes sigma factor regulators. These include two phosphatases of the PP2C family and the anti-sigma factor RsbV (Yang et al., 1996; Subramanian et al., 2000).

T. pallidum also possesses a minimal set of regulator genes that includes two response-regulator two-component systems and various putative transcriptional repressors. One of these repressors, TroR, is an HTH protein which was predicted to be involved in iron-dependent transcriptional regulation (Fraser et al., 1998). This protein was recently characterized in detail (Posey et al., 1999). The gene encoding TroR is part of a transport-related operon (*tro*), in which the gene encoding TroR is preceded by four genes that encode a putative ABC metal transport system. Electromobility-shift assays using purified TroR indicated that TroR bound to the *tro* promoter/operator region. Interestingly, the binding of TroR was manganese dependent, not iron dependent. Deoxyribonuclease I

footprint analysis revealed that TroR protected a 22 nt region that included a region of dyad symmetry. This sequence was 88% identical to the consensus binding sequence of FNR protein from *E. coli* and 68% identical to that of Fur. Evidence that TroR binds and represses *tro* expression was obtained in *E. coli*. Strains of this organism that contained *troPO/lacZ* transcriptional fusions did not express β -galactosidase in the presence of a second plasmid harboring the *troR* gene. It was concluded that TroR represses the *tro* operon in a manganese dependent fashion (Posey et al., 1999). The authors indicate that the significance of this finding may lie in the fact that manganese levels are progressively elevated in the transitions from skin, to blood, to central nervous system. A similar role for manganese in *B. burgdorferi* has yet to be established but its existence is possible considering that this spirochete also parasitizes the central nervous system.

Leptospira

The genus *Leptospira* contains pathogenic (*Leptospira interrogans*) and aquatic free-living organisms (*Leptospira biflexa*). *L. interrogans*, the causal agent of Weil's disease, is transmitted by both wild and domestic animals through contact of the skin or mucous membranes with contaminated urine. Humans are accidental hosts in the transmission cycle. After *L. interrogans* is shed in the urine, the now free-living organisms can survive under favorable conditions for as long as 6 months (Kelly, 1998). The ability to exist both as parasitic and free-living forms probably requires regulatory mechanisms more complex than those of *B. burgdorferi* or *T. pallidum*. This is perhaps reflected in the size of the *Leptospira* genome, which is roughly five times that of the *Borrelia* or *Treponema* genomes (Zuerner et al., 1991). The fact that *L. interrogans* can be cultured has facilitated studies of its physiology. Unlike the spirochetes described previously, *Leptospira* has the ability to synthesize amino acids (Johnson, 1976), possesses cytochromes and catalase, and has enzymes of the citric acid cycle (Smibert, 1973). Very little is known about the genetics of this organism. As with other spirochetes, the lack of a genetic exchange system has hindered progress in this area. Expression of cloned *Leptospira* genes from native promoters has been demonstrated in *E. coli* and sequences resembling that of sigma 70 promoters have been identified (Yamaguchi et al., 1988; Zuerner, 1988; Lin et al., 1999). In addition, molecular analyses of the *hsp(groE)* (Ballard et al., 1993) and *dnaK* operons (Ballard et al., 1998) have revealed the presence of *cis* elements known as CIRCE (Controlling Inverted Repeat of Chaperone Expression) elements. The CIRCE is a conserved inverted repeat found in Gram positive organisms which is located in the transcriptional control region of the *groE* and *dnaK* operons. This sequence acts as a binding site for the transcriptional repressor HrcA. An HrcA homolog is present in the *Leptospira dnaK* operon. Studies of differential gene expression in this organism have focused thus far on a 36 kDa lipoprotein that is downregulated in stationary phase and during mammalian infection (Haake et al., 1998; Haake et al., 2000).

Conclusions

Despite the fact that spirochetes share a distinct morphology coupled with a unique form of motility, there is much diversity in these organisms' natural histories, metabolism and genetics. Members of the genus *Borrelia* are the only microorganisms that cycle between vertebrates and arthropods. Because of this alternating life style *Borrelia* has had to develop regulatory strategies to cope with changes in variables such as temperature, cell-density and pH, which likely involve modulation of lipoproteins. Exploitation of differential lipoprotein expression is perhaps not surprising considering that nearly 10% of the *B. burgdorferi* genome codes for lipoproteins. In contrast, only 1.4% of the *T. pallidum* genome and 0.2% of the *E. coli* genome encode putative lipoproteins. The paucity of transcriptional regulators in *B. burgdorferi* may reflect a dependence on other mechanisms for adaptation, such as antigenic variation. However, the fact that antigenic variation occurs in the vertebrate host but not *in vitro* suggests that the factors that mediate this process are themselves subjected to some kind of regulation (Zhang *et al.* 1997). *T. pallidum*, in contrast, has likely developed different strategies to divide and persist in the human host. The observation that *T. pallidum* has very little protein associated with its outer membrane suggests that its adaptive mechanisms differ from those of *B. burgdorferi* (Radolf *et al.*, 1994). The Leptospirae, the most ancient of the spirochetes, likely harbor a complete array of transcriptional regulators needed to invade and colonize the host as well as survive as a free-living organism. By further studying *Leptospira* genetics it may be possible to reconstruct the regulatory adaptations that allowed both *Borrelia* and *Treponema* to occupy their respective niches.

Acknowledgements

This work was supported in part by grant RR00164-39 of the National Institutes of Health.

References

- Akins, D.R., Porcella, S.F., Popova, T.G., Shevchenko, D., Baker, S.I., Li, M., Norgard, M.V., and Radolf, J.D. 1995. Evidence for *in vivo* but not *in vitro* expression of a *Borrelia burgdorferi* outer surface protein F (OspF) homologue. *Mol. Microbiol.* 18: 507-520.
- Akins, D.R., Bourell, K.W., Caimano, M.J., Norgard, M.V., and Radolf, J.D. 1998. A new animal model for studying Lyme disease spirochetes in a mammalian host-adapted state. *J. Clin. Invest.* 101: 2240-2250.
- Akins, D.R., Caimano, M.J., Yang, X., Cerna, F., Norgard, M.V., and Radolf, J.D. 1999. Molecular and evolutionary analysis of *Borrelia burgdorferi* 297 circular plasmid-encoded lipoproteins with OspE- and OspF-like leader peptides. *Infect. Immun.* 67: 1526-1532.
- Anderson, J.F. 1991. Epizootiology of Lyme borreliosis. *Scand. J. Infect. Dis. Suppl* 77: 23-34.
- Ballard, S.A., Segers, R.P., Bleumink-Pluym, N., Fyfe, J., Faine, S., and Adler, B. 1993. Molecular analysis of the *hsp* (*groE*) operon of *Leptospira interrogans* serovar copenhageni. *Mol. Microbiol.* 8: 739-751.
- Ballard, S.A., Go, M., Segers, R.P., and Adler, B. 1998. Molecular analysis of the *dnaK* locus of *Leptospira interrogans* serovar Copenhageni. *Gene* 216: 21-29.
- Barthold, S. W., Fikrig, E., Bockenstedt, L. K. and Persing, D. H. 1995. Circumvention of outer surface protein A immunity by host-adapted *Borrelia burgdorferi*. *Infect. Immun.* 63: 2255-2261.
- Bassler, B.L., Wright, M., and Silverman, M.R. 1994. Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol. Microbiol.* 13: 273-286.
- Bergstrom, S., Bundoc, V.G., and Barbour, A.G. 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete *Borrelia burgdorferi*. *Mol. Microbiol.* 3: 479-486.

- Bono, J.L., Tilly, K., Stevenson, B., Hogan, D., and Rosa, P. 1998. Oligopeptide permease in *Borrelia burgdorferi*: putative peptide-binding components encoded by both chromosomal and plasmid loci. *Microbiol.* 144: 1033-1044.
- Brunet, L.R., Sellitto, C., Spielman, A., and Telford, S.R., III. 1995. Antibody response of the mouse reservoir of *Borrelia burgdorferi* in nature. *Infect. Immun.* 63: 3030-3036.
- Burgdorfer, W., Anderson, J.F., Gern, L., Lane, R.S., Piesman, J., and Spielman, A. 1991. Relationship of *Borrelia burgdorferi* to its arthropod vectors. *Scand. J. Infect. Dis. Suppl* 77: 35-40.
- Burkot, T.R., Piesman, J., and Wirtz, R.A. 1994. Quantitation of the *Borrelia burgdorferi* outer surface protein A in *Ixodes scapularis*: fluctuations during the tick life cycle, doubling times, and loss while feeding. *J. Infect. Dis.* 170: 883-889.
- Carlyon, J.F., Roberts, D.M., and Marconi, R.T. 2000. Evolutionary and molecular analyses of the borrelia *bdr* super gene family: delineation of distinct sub-families and demonstration of the genus wide conservation of putative functional domains, structural properties and repeat motifs. *Microb. Pathog.* 28: 89-105.
- Carroll, J.I., Dorward, D.W., and Gherardini, F.C. 1996. Identification of a transferrin-binding protein from *Borrelia burgdorferi*. *Infect. Immun.* 64: 2911-2916.
- Carroll, J.A., Garon, C.F., and Schwan, T.G. 1999. Effects of environmental pH on membrane proteins in *Borrelia burgdorferi*. *Infect. Immun.* 67: 3181-3187.
- Cassatt, D.R., Patel, N.K., Ulbrandt, N.D., and Hanson, M.S. 1998. DbpA, but not OspA, is expressed by *Borrelia burgdorferi* during spirochetemia and is a target for protective antibodies. *Infect. Immun.* 66: 5379-5387.
- Champion, C.I., Miller, J.N., Lovett, M.A., and Blanco, D.R. 1990. Cloning, sequencing, and expression of two class B endoflagellar genes of *Treponema pallidum* subsp. pallidum encoding the 34.5- and 31.0-kilodalton proteins. *Infect. Immun.* 58: 1697-1704.
- Champion, C.I., Blanco, D.R., Skare, J.T., Haake, D.A., Giladi, M., Foley, D., Miller, J.N., and Lovett, M.A. 1994. A 9.0-kilobase-pair circular plasmid of *Borrelia burgdorferi* encodes an exported protein: evidence for expression only during infection. *Infect. Immun.* 62: 2653-2661.
- Chang, B.Y., Chen, K.Y., Wen, Y.D., and Liao, C.T. 1994. The response of a *Bacillus subtilis* temperature-sensitive *sigA* mutant to heat stress. *J. Bacteriol.* 176: 3102-3110.
- Das, S., Barthold, S.W., Giles, S.S., Montgomery, R.R., Telford, S.R., III, and Fikrig, E. 1997. Temporal pattern of *Borrelia burgdorferi* p21 expression in ticks and the mammalian host. *J. Clin. Invest.* 99: 987-995.
- de Silva, A.M., Telford, S.R., III, Brunet, L.R., Barthold, S.W., and Fikrig, E. 1996. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. *J. Exp. Med.* 183: 271-275.
- de Silva, A.M., Zeidner, N.S., Zhang, Y., Dolan, M.C., Piesman, J., and Fikrig, E. 1999. Influence of outer surface protein A antibody on *Borrelia burgdorferi* within feeding ticks. *Infect. Immun.* 67: 30-35.
- Dunny, G.M., and Winans, S.C. 1999. Cell-Cell Signaling in Bacteria., G.M.Dunny and S.C.Winans, eds. Washington D.C.: ASM Press, p.1-5.
- Eberhard, A. 1972. Inhibition and activation of bacterial luciferase synthesis. *J. Bacteriol.* 109: 1101-1105.
- Elias, A.F., Bono, J.L., Carroll, J.A., Stewart, P., Tilly, K., and Rosa, P. 2000. Altered stationary-phase response in a *Borrelia burgdorferi* *rpoS* mutant. *J. Bacteriol.* 182: 2909-2918.
- Fenno, J.C., Muller, K.H., and McBride, B.C. 1996. Sequence analysis, expression, and binding activity of recombinant major outer sheath protein (Msp) of *Treponema denticola*. *J. Bacteriol.* 178: 2489-2497.
- Fikrig, E., Barthold, S.W., Sun, W., Feng, W., Telford, S.R., III, and Flavell, R.A. 1997. *Borrelia burgdorferi* P35 and P37 proteins, expressed *in vivo*, elicit protective immunity [published erratum appears in *Immunity* 1998 Nov;9(5):following 755]. *Immunity.* 6: 531-539.
- Fikrig, E., Feng, W., Aversa, J., Schoen, R.T., and Flavell, R.A. 1998. Differential expression of *Borrelia burgdorferi* genes during erythema migrans and Lyme arthritis. *J. Infect. Dis.* 178: 1198-1201.
- Fikrig, E., Chen, M., Barthold, S.W., Anguita, J., Feng, W., Telford, S.R., III, and Flavell, R.A. 1999. *Borrelia burgdorferi* *erpT* expression in the arthropod vector and murine host. *Mol. Microbiol.* 31: 281-290.
- Fingerle, V., Hauser, U., Liegl, G., Petko, B., Preac-Mursic, V., and Wilske, B. 1995. Expression of outer surface proteins A and C of *Borrelia burgdorferi* in *Ixodes ricinus*. *J. Clin. Microbiol.* 33: 1867-1869.
- Fraser, C.M., Casjens, S., Huang, W.M., Sutton, G.G., Clayton, R., Lathigra, R., White, O., Ketchum, K.A., Dodson, R., Hickey, E.K., Gwinn, M., Dougherty, B., Tomb, J.F., Fleischmann, R.D., Richardson, D., Peterson, J., Kerlavage, A.R., Quackenbush, J., Salzberg, S., Hanson, M., van Vugt, R., Palmer, N., Adams, M.D., Gocayne, J., and Venter, J.C. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 390: 580-586.
- Fraser, C.M., Norris, S.J., Weinstock, G.M., White, O., Sutton, G.G., Dodson, R., Gwinn, M., Hickey, E.K., Clayton, R., Ketchum, K.A., Sodergren, E.,

- Hardham, J.M., McLeod, M.P., Salzberg, S., Peterson, J., Khalak, H., Richardson, D., Howell, J.K., Chidambaram, M., Utterback, T., McDonald, L., Ariach, P., Bowman, C., Cotton, M.D., and Venter, J.C. 1998. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 281: 375-388.
- Gilmore, R.D., Jr. and Piesman, J. 2000. Inhibition of *Borrelia burgdorferi* migration from the midgut to the salivary glands following feeding by ticks on OspC-immunized mice. *Infect. Immun.* 68: 411-414.
- Greene, S.R., Stamm, L.V., Hardham, J.M., Young, N.R., and Frye, J.G. 1997. Identification, sequences, and expression of *Treponema pallidum* chemotaxis genes. *DNA Seq.* 7: 267-284.
- Haake, D.A., Chao, G., Zuerner, R.L., Barnett, J.K., Barnett, D., Mazel, M., Matsunaga, J., Levett, P.N., and Bolin, C.A. 2000. The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infect. Immun.* 68: 2276-2285.
- Haake, D.A., Martinich, C., Summers, T.A., Shang, E.S., Pruetz, J.D., McCoy, A.M., Mazel, M.K., and Bolin, C.A. 1998. Characterization of leptospiral outer membrane lipoprotein LipL36: downregulation associated with late-log-phase growth and mammalian infection. *Infect. Immun.* 66: 1579-1587.
- Hagman, K.E., Porcella, S.F., Popova, T.G., and Norgard, M.V. 1997. Evidence for a methyl-accepting chemotaxis protein gene (*mcp1*) that encodes a putative sensory transducer in virulent *Treponema pallidum*. *Infect. Immun.* 65: 1701-1709.
- Hardham, J.M. 1997. Identification and sequences of the *Treponema pallidum* *flhA*, *flhF*, and *orf304* genes. *DNA Seq.* 7: 107-116.
- Harwood, C.S., and Canale-Parola, E. 1984. Ecology of spirochetes. *Annu. Rev. Microbiol.* 38: 161-192.
- Heinzerling, H.F., Olivares, M., and Burne, R.A. 1997. Genetic and transcriptional analysis of *flgB* flagellar operon constituents in the oral spirochete *Treponema denticola* and their heterologous expression in enteric bacteria. *Infect. Immun.* 65: 2041-2051.
- Hiratsu, K., Amemura, M., Nashimoto, H., Shinagawa, H., and Makino, K. 1995. The *rpoE* gene of *Escherichia coli*, which encodes sigma E, is essential for bacterial growth at high temperature. *J. Bacteriol.* 177: 2918-2922.
- Holt, S.C. 1978. Anatomy and chemistry of spirochetes. *Microbiol. Rev.* 42: 114-160.
- Howe, T.R., LaQuier, F.W., and Barbour, A.G. 1986. Organization of genes encoding two outer membrane proteins of the Lyme disease agent *Borrelia burgdorferi* within a single transcriptional unit. *Infect. Immun.* 54: 207-212.
- Hsu, P.L., Chamberlain, N.R., Orth, K., Moomaw, C.R., Zhang, L.Q., Slaughter, C.A., Radolf, J.D., Sell, S., and Norgard, M.V. 1989. Sequence analysis of the 47-kilodalton major integral membrane immunogen of *Treponema pallidum*. *Infect. Immun.* 57: 196-203.
- Humphreys, S., Stevenson, A., Bacon, A., Weinhardt, A. B., and Roberts, M. 1999. The alternative sigma factor, sigma E, is critically important for the virulence of *Salmonella typhimurium*. *Infect. Immun.* 67: 1560-1568.
- Hurme, R. and Rhen, M. 1998. Temperature sensing in bacterial gene regulation—what it all boils down to. *Mol. Microbiol.* 30: 1-6.
- Indest, K.J., Ramamoorthy, R., Sole, M., Gilmore, R.D., Johnson, B.J., and Philipp, M.T. 1997. Cell-density-dependent expression of *Borrelia burgdorferi* lipoproteins in vitro. *Infect. Immun.* 65: 1165-1171.
- Indest, K.J. and Philipp, M.T. 2000. DNA-binding proteins possibly involved in regulation of the post-logarithmic-phase expression of lipoprotein P35 in *Borrelia burgdorferi*. *J. Bacteriol.* 182: 522-525.
- Johnson, R.C. 1976. Comparative spirochete physiology and cellular composition. In *The Biology of Parasitic Spirochetes*, R.C. Johnson, ed. New York: Academic Press, p. 39-48.
- Kelly, P.W. 1998. Leptospira. In *Infectious Diseases*, S. L. Gorbach, J.G. Bartlett, and N.R. Blacklow, eds. Philadelphia: W.B. Saunders Company, p. 1948-1952.
- Klose, K. E., Novik, V., and Mekalanos, J. J. 1998. Identification of multiple sigma 54-dependent transcriptional activators in *Vibrio cholerae*. *J. Bacteriol.* 180: 5256-5259.
- Kinsella, N., Guerry, P., Cooney, J., and Trust, T.J. 1997. The *flgE* gene of *Campylobacter coli* is under the control of the alternative sigma factor sigma 54. *J. Bacteriol.* 179: 4647-4653.
- Knight, S.W. and Samuels, D.S. 1999. Natural synthesis of a DNA-binding protein from the C-terminal domain of DNA gyrase A in *Borrelia burgdorferi*. *EMBO J.* 18: 4875-4881.
- Knight, S.W., Kimmel, B.J., Eggers, C.H., and Samuels, D.S. 2000. Disruption of the *Borrelia burgdorferi* *gac* gene, encoding the naturally synthesized GyrA C-terminal domain. *J. Bacteriol.* 182: 2048-2051.
- Lahdenne, P., Porcella, S.F., Hagman, K.E., Akins, D.R., Popova, T.G., Cox, D.L., Katona, L.I., Radolf, J.D., and Norgard, M.V. 1997. Molecular characterization of a 6.6-kilodalton *Borrelia burgdorferi* outer membrane-associated lipoprotein (lp6.6) which appears to be downregulated during mammalian infection. *Infect. Immun.* 65: 412-421.
- Lam, T.T., Nguyen, T.P., Montgomery, R.R., Kantor, F.S., Fikrig, E., and Flavell, R.A. 1994. Outer surface proteins E and F of *Borrelia burgdorferi*, the agent of Lyme disease. *Infect. Immun.* 62: 290-298.
- Li, H., Ruby, J., Charon, N., and Kuramitsu, H. 1996. Gene inactivation in the oral spirochete *Treponema denticola*: construction of a *flgE* mutant. *J. Bacteriol.* 178: 3664-3667.
- Limberger, R.J., Sliwinski, L.L., Yelton, D.B., and Charon, N.W. 1992. Molecular genetic analysis of a class B periplasmic-flagellum gene of *Treponema phagedenis*. *J. Bacteriol.* 174: 6404-6410.
- Limberger, R.J., Sliwinski, L.L., El Afandi, M.C., and Dantuono, L.A. 1996. Organization, transcription, and expression of the 5' region of the *fla* operon of *Treponema phagedenis* and *Treponema pallidum*. *J. Bacteriol.* 178: 4628-4634.
- Lin, M., Bughio, N., and Surujballi, O. 1999. Expression in *Escherichia coli* of *flaB*, the gene coding for a periplasmic flagellin of *Leptospira interrogans* serovar pomona. *J. Med. Microbiol.* 48: 977-982.
- Marconi, R.T., Sung, S.Y., Hughes, C.A., and Carlyon, J.A. 1996. Molecular and evolutionary analyses of a variable series of genes in *Borrelia burgdorferi* that are related to *ospE* and *ospF*, constitute a gene family, and share a common upstream homology box. *J. Bacteriol.* 178: 5615-5626.
- Margolis, N., and Rosa, P.A. 1993. Regulation of expression of major outer surface proteins in *Borrelia burgdorferi*. *Infect. Immun.* 61: 2207-2210.
- Margolis, N. and Samuels, D.S. 1995. Proteins binding to the promoter region of the operon encoding the major outer surface proteins OspA and OspB of *Borrelia burgdorferi*. *Mol. Biol. Rep.* 21: 159-164.
- Mbow, M.L., Gilmore, R.D., Jr., and Titus, R.G. 1999. An OspC-specific monoclonal antibody passively protects mice from tick-transmitted infection by *Borrelia burgdorferi* B31. *Infect. Immun.* 67: 5470-5472.
- Montgomery, R.R., Malawista, S.E., Feen, K.J., and Bockenstedt, L.K. 1996. Direct demonstration of antigenic substitution of *Borrelia burgdorferi* *ex vivo*: exploration of the paradox of the early immune response to outer surface proteins A and C in Lyme disease. *J. Exp. Med.* 183: 261-269.
- Nealson, K. H., Platt, T., and Hastings, J. W. 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. *J. Bacteriol.* 104: 313-322.
- Nguyen, T.P., Lam, T.T., Barthold, S.W., Telford, S.R., III, Flavell, R.A., and Fikrig, E. 1994. Partial destruction of *Borrelia burgdorferi* within ticks that engorged on OspE- or OspF-immunized mice. *Infect. Immun.* 62: 2079-2084.
- Padula, S.J., Sampieri, A., Dias, F., Szczepanski, A., and Ryan, R.W. 1993. Molecular characterization and expression of p23 (OspC) from a North American strain of *Borrelia burgdorferi*. *Infect. Immun.* 61: 5097-5105.
- Paster, B.J. 1984. The phylogeny of the spirochetes. *Syst. Appl. Microbiol.* 5: 337-351.
- Paster, B.J., Pelletier, D.A., Dewhirst, F.E., Weisburg, W.G., Fussing, V., Poulsen, L.K., Dannenberg, S., and Schroeder, I. 1996. Phylogenetic position of the spirochetal genus *Cristispira*. *Appl. Environ. Microbiol.* 62: 942-946.
- Petersson, B., Fellstrom, C., Andersson, A., Uhlen, M., Gunnarsson, A., and Johansson, K.E. 1996. The phylogeny of intestinal porcine spirochetes (*Serpulina* species) based on sequence analysis of the 16S rRNA gene. *J. Bacteriol.* 178: 4189-4199.
- Philipp, M. T. and Johnson, B. J. 1994. Animal models of Lyme disease: pathogenesis and immunopathology. *Trends Microbiol.* 2: 431-437.
- Porcella, S.F., Popova, T.G., Akins, D.R., Li, M., Radolf, J.D., and Norgard, M.V. 1996a. *Borrelia burgdorferi* supercoiled plasmids encode multicopy tandem open reading frames and a lipoprotein gene family. *J. Bacteriol.* 178: 3293-3307.
- Porcella, S.F., Popova, T.G., Hagman, K.E., Penn, C.W., Radolf, J.D., and Norgard, M.V. 1996b. A *mgl*-like operon in *Treponema pallidum*, the syphilis spirochete. *Gene* 177: 115-121.
- Posey, J.E., Hardham, J.M., Norris, S.J., and Gherardini, F.C. 1999. Characterization of a manganese-dependent regulatory protein, TroR, from *Treponema pallidum*. *Proc. Acad. Sci. USA.* 96: 10887-10892.
- Radolf, J.D., Bourell, K.W., Akins, D.R., Brusca, J.S., and Norgard, M.V. 1994. Analysis of *Borrelia burgdorferi* membrane architecture by freeze-fracture electron microscopy. *J. Bacteriol.* 176: 21-31.
- Ramamoorthy, R. and Philipp, M.T. 1998. Differential expression of *Borrelia burgdorferi* proteins during growth in vitro. *Infect. Immun.* 66: 5119-5124.
- Roberts, D. M., Carlyon, J. A., Theisen, M., and Marconi, R. T. 2000. The *bdr* gene families of the Lyme disease and Relapsing fever spirochetes: Potential influence on biology, pathogenesis, and evolution. *Emerg. Infect. Dis.* 6: 110-121.
- Rombel, I., North, A., Hwang, I., Wyman, C., Kustu, S. 1998. The bacterial enhancer-binding protein NtrC as a molecular machine. *Cold Spring Harbor Symp. Quant. Biol.* 63: 157-166.
- Ross, W., Gosink, K.K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K., and Gourse, R.L. 1993. A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase.

- Science 262: 1407-1413.
- Sadziene, A., Wilske, B., Ferdows, M.S., and Barbour, A.G. 1993. The cryptic *ospC* gene of *Borrelia burgdorferi* B31 is located on a circular plasmid. *Infect. Immun.* 61: 2192-2195.
- Sambri, V., Cevenini, R., and La Placa, M. 1991. Susceptibility of iron-loaded *Borrelia burgdorferi* to killing by hydrogen peroxide and human polymorphonuclear leucocytes. *FEMS Microbiol. Lett.* 65: 67-71.
- Schwan, T.G., Piesman, J., Golde, W.T., Dolan, M.C., and Rosa, P.A. 1995. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc. Acad. Sci. USA.* 92: 2909-2913.
- Schwan, T.G. 1996. Ticks and *Borrelia*: model systems for investigating pathogen-arthropod interactions. *Infect. Agents Dis.* 5: 167-181.
- Schwan, T.G., and Hinnebusch, B.J. 1998. Bloodstream- versus tick-associated variants of a relapsing fever bacterium. *Science* 280: 1938-1940.
- Schwan, T.G., and Piesman, J. 2000. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete *Borrelia burgdorferi* during the chain of infection in ticks and mice. *J. Clin. Microbiol.* 38:382-388.
- Smibert, R.M. 1973. Spirochaetales, a review. *Crit. Rev. Microbiol.* 2: 491.
- Sohaskey, C.D., Arnold, C., and Barbour, A.G. 1997. Analysis of promoters in *Borrelia burgdorferi* by use of a transiently expressed reporter gene. *J. Bacteriol.* 179: 6837-6842.
- Sohaskey, C.D., Zuckert, W.R., and Barbour, A.G. 1999. The extended promoters for two outer membrane lipoprotein genes of *Borrelia* spp. uniquely include a T-rich region. *Mol. Microbiol.* 33: 41-51.
- Sperandio, V., Mellies, J.L., Nguyen, W., Shin, S., and Kaper, J.B. 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Acad. Sci. USA.* 96: 15196-15201.
- Stamm, L.V., Dallas, W.S., Ray, P.H., and Bassford, P.J., Jr. 1988. Identification, cloning, and purification of protein antigens of *Treponema pallidum*. *Rev. Infect. Dis.* 10 Suppl 2: S403-S407.
- Stamm, L.V., Gherardini, F.C., Parrish, E.A., and Moomaw, C.R. 1991. Heat shock response of spirochetes. *Infect. Immun.* 59: 1572-1575.
- Stamm, L.V., Greene, S.R., Bergen, H.L., Hardham, J.M., and Barnes, N.Y. 1998. Identification and sequence analysis of *Treponema pallidum tprJ*, a member of a polymorphic multigene family. *FEMS Microbiol. Lett.* 169: 155-163.
- Stamm, L.V. 1999. Molecular characterization of a flagellar (*fla*) operon in the oral spirochete *Treponema denticola* ATCC 35405. *FEMS Microbiol. Lett.* 179: 31-36.
- Stevenson, B., Schwan, T.G., and Rosa, P.A. 1995. Temperature-related differential expression of antigens in the Lyme disease spirochete, *Borrelia burgdorferi*. *Infect. Immun.* 63: 4535-4539.
- Stevenson, B., Tilly, K., and Rosa, P.A. 1996. A family of genes located on four separate 32-kilobase circular plasmids in *Borrelia burgdorferi* B31. *J. Bacteriol.* 178: 3508-3516.
- Stevenson, B., Bono, J.L., Schwan, T.G., and Rosa, P. 1998. *Borrelia burgdorferi* Erp proteins are immunogenic in mammals infected by tick bite, and their synthesis is inducible in cultured bacteria. *Infect. Immun.* 66: 2648-2654.
- Studholme, D. J. and Buck, M. 2000. Novel roles of σ^N in small genomes. *Microbiol.* 146: 4-5.
- Studholme, D. J. and Buck, M. 2000. The biology of enhancer-dependent transcriptional regulation in bacteria: insights from genome sequences. *FEMS Microbiol. Lett.* 186: 1-9.
- Subramanian, G., Koonin, E.V., and Aravind, L. 2000. Comparative genome analysis of the pathogenic spirochetes *Borrelia burgdorferi* and *Treponema pallidum*. *Infect. Immun.* 68: 1633-1648.
- Suk, K., Das, S., Sun, W., Jwang, B., Barthold, S.W., Flavell, R.A., and Fikrig, E. 1995. *Borrelia burgdorferi* genes selectively expressed in the infected host. *Proc. Acad. Sci. USA.* 92: 4269-4273.
- Sung, S.Y., McDowell, J.V., Carlyon, J.A., and Marconi, R.T. 2000. Mutation and recombination in the upstream homology box-flanked *ospE*-related genes of the Lyme disease spirochetes result in the development of new antigenic variants during infection. *Infect. Immun.* 68: 1319-1327.
- Surette, M.G., Miller, M.B., and Bassler, B.L. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Acad. Sci. USA.* 96: 1639-1644.
- Tilly, K., Fuhrman, J., Campbell, J., and Samuels, D.S. 1996. Isolation of *Borrelia burgdorferi* genes encoding homologues of DNA-binding protein HU and ribosomal protein S20. *Microbiol.* 142: 2471-2479.
- Wallich, R., Brenner, C., Kramer, M.D., and Simon, M.M. 1995. Molecular cloning and immunological characterization of a novel linear-plasmid-encoded gene, pG, of *Borrelia burgdorferi* expressed only *in vivo*. *Infect. Immun.* 63: 3327-3335.
- Yamaguchi, T., Ono, E., and Yanagawa, R. 1988. Expression of antigen genes of *Leptospira interrogans* serovar canicola in *Escherichia coli*. *Microbiol. Immunol.* 32: 1179-1187.
- Yang, X., Kang, C.M., Brody, M.S., and Price, C.W. 1996. Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. *Genes Dev.* 10: 2265-2275.
- Yang, X., Popova, T.G., Hagman, K.E., Wikel, S.K., Schoeler, G.B., Caimano, M.J., Radolf, J.D., and Norgard, M.V. 1999. Identification, characterization, and expression of three new members of the *Borrelia burgdorferi* Mlp (2.9) lipoprotein gene family. *Infect. Immun.* 67: 6008-6018.
- Zhang, J. R., Hardham, J. M., Barbour, A. G., and Norris, S.J. 1997. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* 89: 275-285.
- Zuckert, W.R., Meyer, J., and Barbour, A.G. 1999. Comparative analysis and immunological characterization of the *Borrelia* Bdr protein family. *Infect. Immun.* 67: 3257-3266.
- Zuckert, W.R., and Barbour, A.G. 2000. Stability of *Borrelia burgdorferi* bdr loci *in vitro* and *in vivo*. *Infect. Immun.* 68: 1727-1730.
- Zuerner, R.L. 1988. Nucleotide sequence analysis of a gene cloned from *Leptospira biflexa* serovar patoc which complements an *argE* defect in *Escherichia coli*. *J. Bacteriol.* 170: 4548-4554.
- Zuerner, R.L. 1991. Physical map of chromosomal and plasmid DNA comprising the genome of *Leptospira interrogans*. *Nucleic Acids Res.* 19: 4857-4860.

