Transcriptional Regulation in Spirochetes

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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. Because of the limited information on the genera *Spirochaeta, Leptotena*, and *Cristispira*, we will concentrate exclusively on *Borrelia, Treponema* and *Leptotena*.

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 transstadially 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...
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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., 1996; Gilmore, Jr. and Piesman, 2000). Thus, the feeding event represents the juncture 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 lipoproteins exit the tick to invade the vertebrate host whereas the OspA-OspC- 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 osp4mRNA (Fikrig et al., 1998). Similarly, RT-PCR analysis of tissue samples from needle-injected mice showed that ospA mRNA, while present at day 14 of infection, could no longer be detected at day 30 (Montgomery et al., 1996).

### Table 1. Differentially Expressed Genes of Borrelia burgdorferi

<table>
<thead>
<tr>
<th>Gene Families</th>
<th>Culture</th>
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\(^a\)Flat ticks; \(^b\)Engorged ticks; \(^c\)Low expression. \(^d\)Two unrelated genes, both encoding 35 kDa proteins.
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 ospA operon was subsequently sequenced and shown to have an intact sigma-70-like promoter, yet no ospAmRNA 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 immuno-fluorescence (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; Cassett 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 (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 spirochete cultures 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; Cassett 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 (σ70), *B. burgdorferi* is endowed with just two other alternative sigma factors, the stationary sigma factor RpoS and RpoN (σ54).

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 (Elías 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 σ54-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 (σ28) 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 telomer-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 α^{+}-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 palidum, 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 characterized 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 σ26 also may play a role in gene regulation. This supposition is based on the fact that a subset of *Treponema* motility genes have σ26-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 σ24, σ26, and σ43. The functions of σ24 and σ43 may partially substitute for that of RpoS, which is not present in *T. pallidum* (Fraser et al., 1998). Both σ24 and σ43 are necessary for growth and survival of *E. coli* at higher temperatures (Chang et al., 1994; Hiratsu et al., 1995). In addition, σ24 is critical for virulence in *Salmonella typhimurium* (Humphreys et al., 1999). The presence of σ26 confirms previous observations of σ26-like promoter sequences in *Treponema*. The absence of the heat shock sigma factor σ32 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).
Transcriptional Regulation in Spirochetes

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 as antigenic variation. However, the fact that antigenic dependence on other mechanisms for adaptation, such as transcriptional regulators needed to invade and colonize the spirochetes, likely harbor a complete array of adaptive mechanisms differ from those of *Borrelia 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.

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References


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