The Many Faces of *Borrelia burgdorferi*

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Abstract

In this review we describe several genetic regulatory mechanisms adopted by the agent of Lyme disease, *Borrelia burgdorferi*, to sense and adapt to different host and environmental conditions either *in vitro* or *in vivo*. This review results in the increased or decreased synthesis of several proteins whose levels are believed to play key roles in the ability of *B. burgdorferi* to cycle between both arthropod and mammalian hosts. Moreover, the differential synthesis of these proteins serves to modulate the response of *B. burgdorferi* to signals in the requisite host and may also, in some cases, function as virulence determinants of this spirochete. Elucidation of these mechanisms will help in the understanding of the pathogenicity of *B. burgdorferi* as well as aid in identifying proteins that are important during different stages of infection.

Introduction

Lyme disease was first reported as an outbreak of juvenile rheumatoid arthritis (Steere et al., 1977) and is a multi-systemic, zoonotic illness that is present in most temperate parts of the Northern hemisphere. The etiologic agent is now referred to as the *Borrelia burgdorferi* sensu lato complex and comprises an expanding group of closely related *Borrelia* spp. (Barbour and Fish, 1993; Postic et al., 1994). While the isolates from North America are predominantly grouped as *B. burgdorferi* sensu stricto, European and Asian isolates also include two additional species, *B. garinii* and *B. afzelii* (Baranton et al., 1992). These are members of a growing cluster of closely related species that presently include *B. andersonii*, *B. japonica*, *B. valaisiana*, *B. lusitaniae*, *B. turdae*, *B. tanukii*, and *B. bissettii* sp. nov. (Casjens et al., 1995; Casjens et al., 2000; Wang et al., 1999). For this review most studies are restricted to *B. burgdorferi* sensu stricto, designated as *B. burgdorferi*, unless otherwise indicated.

Lyme disease is endemic in areas where there is a close ecological interaction between competent enzootic vectors, the spirochete and reservoir hosts. Transmission to humans is predominantly by *Ixodes scapularis* in the Eastern United States, *Ixodes pacificus* in Western United States, while *Ixodes ricinus* and *Ixodes persulcatus* are the primary vectors in Europe and Eurasia, respectively. Since all borrelial species are host-propagated bacteria (*i.e.*, they do not survive naturally in water, soil and are not transmitted by aerosol or fecal contamination) that shuttle between a vertebrate and an arthropod host, these spirochetes have developed strategies to sense and survive in these diverse environments (Barbour and Hayes, 1986). This is achieved by altering the level of gene expression in response to changes in temperature, pH, salts, and other host dependent factors. *B. burgdorferi* has a unique genome composed of a 910 kb linear chromosome and 21 different plasmids (9 circular and 12 linear) (Casjens et al., 2000) some of which may function as mini-chromosomes as seen in segmented genomes (Barbour, 1993). Some of the plasmids have been shown to be non-essential for *in vitro* propagation, yet required for normal infectivity, indicating a significant plasticity of its genome and implying that such a dynamic arrangement may be related to the ability of *B. burgdorferi* to occupy environments as disparate as a tick and a warm-blooded mammal (Casjens et al., 2000). In addition, *B. burgdorferi* contains multiple related genes termed paralogues (Fraser et al., 1997) encoding proteins that share significant similarity at the amino acid level with presumably similar functions that may help to compensate for the loss of some plasmids due to selective pressures encountered during infection. Alternatively, the genetic redundancy observed may provide a battery of genes that encode antigenically variable proteins with compensatory functions which are sequentially expressed via irreversible phase variation or modified further by recombination. By assessing the mechanism of gene expression in response to different growth and survival conditions encountered by *B. burgdorferi*, it may be possible to better understand the adaptive mechanisms of this spirochete and perhaps more clearly define molecules associated with the pathogenesis of Lyme disease.

Temperature

Many pathogens have been shown to exhibit a coordinately regulated synthesis of virulence determinants in response to environmental signals (Mekalanos, 1992; Miller et al., 1989). This coordinate gene regulation allows for pathogens to survive, replicate and complete their life cycles in different environmental niches as well as in different intermediate and terminal hosts. In this section we review the effect of temperature on the ability of *B. burgdorferi* to survive and grow in the host and speculate as to how these spirochetes change according to the thermal homeostasis of its hosts.

Heat Shock Response

Temperature is an important environmental cue that requires a rapid response. Dramatic increases in temperature mediate a regulatory cascade known as the heat shock response which has been extensively studied and the subject of many detailed reviews (Yura et al., 1993). In addition to the typical heat shock response, temperature changes are also known to regulate virulence determinants
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in a number of different pathogens (Konkel and Tilly, 2000). Regulation of virulence determinants in response to varying temperatures would presumably be crucial for a pathogen like B. burgdorferi, which cycles between ticks (ambient temperature) and mammalian hosts (greater than or equal to 37°C), to complete its life cycle. Sequence analysis of the genome of B. burgdorferi (Fraser et al., 1997) has identified a set of homologues of heat shock response genes (groES, groEL, grpE, dnaJ, dnaK, hslU, hslV, and htpG) and several of them have been characterized (Cluss and Boothby, 1990). Although B. burgdorferi does not have a homologue of heat shock response regulator sigma 32 (σ32), this spirochete shows a heat shock response upon exposure to elevated temperature (Cluss and Boothby, 1990; Scopio et al., 1994). Thermoregulation in B. burgdorferi was assessed using either one or two-dimensional gel electrophoresis following radiolabeling of B. burgdorferi proteins (Carreiro et al., 1990). Steady state level accumulation, as well as turnover of such proteins, was also determined (Carreiro et al., 1990). Using this approach, Cluss et al. also showed that there was an up-regulation of DnaK (Hsp70; ~72 kilodalton [kDa]) and three additional heat shock proteins (Hsp’s) of 39, 27 and 21 kDa in molecular mass (Cluss and Boothby, 1990; Cluss et al., 1996). Heat shock proteins have also been shown to play a role as chaperonins in most living systems, and, in the case of B. burgdorferi, in the molecular processing of endoflagellin critical for motility (Scopio et al., 1994). In this study, a 70 kDa heat shock protein (Hsp70 or DnaK) bound endoflagellin at all temperatures tested between 33 and 41°C in the presence of ATP; however, the binding of a 60 kDa heat shock protein (Hsp60 or GroEL) to endoflagellin was independent of ATP levels at all temperatures studied. Though these interactions were

<table>
<thead>
<tr>
<th>Genetic Modulation</th>
<th>Signal</th>
<th>Host</th>
<th>Borrelia Strains</th>
<th>Description of Phenomenon</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OspC ↑</td>
<td>Tick</td>
<td>Mice</td>
<td>B31</td>
<td>Antibodies to OspC in Lyme disease patients.</td>
<td>Schwan et al., (1995)</td>
</tr>
<tr>
<td>Osp A ↑, OspB ↑</td>
<td>Pre-engorgement</td>
<td>Tick</td>
<td>B31</td>
<td>Absence of antibodies to OspAB in initial stages of human infection.</td>
<td>Schwan et al., (1995)</td>
</tr>
<tr>
<td>OspC ↓</td>
<td></td>
<td></td>
<td></td>
<td>Antibodies to OspC absent in mice infected with spirochetes from unfed ticks.</td>
<td>Schwan et al., (1995):</td>
</tr>
<tr>
<td>p21 ↑</td>
<td>Active infection</td>
<td>Mouse</td>
<td>N40</td>
<td>Antibodies to P21 observed at 3 weeks post infection in mice and in sera of Lyme arthritis patients. Antibodies to P21 not seen on injection with inactivated B. burgdorferi.</td>
<td>Cassatt et al., (1998); Hanson et al., (1998); Das et al., (1997).</td>
</tr>
<tr>
<td>flaB, bpaA, p21,</td>
<td></td>
<td></td>
<td></td>
<td>Passage 75 of cN40 (high passage) is infectious but not pathogenic as several genes are repressed under in vivo growth. Correlation of pathogenicity to in vivo expression of genes.</td>
<td>Anguita et al., (2000).</td>
</tr>
<tr>
<td>erpD, gene1, gene2, bba64, bba65, bba66</td>
<td></td>
<td></td>
<td></td>
<td>Antibodies to P35, P37 only in active infections in mice; in early and late stage Lyme disease patients.</td>
<td>Fikrig et al., (1997).</td>
</tr>
<tr>
<td>p35 ↑, p37 ↑</td>
<td>Active infection</td>
<td>Mouse</td>
<td>cN40</td>
<td>Antibodies to P35, P37 only in active infections in mice; in early and late stage Lyme disease patients.</td>
<td>Nguyen et al., (1994); Stevenson et al., (1998a).</td>
</tr>
<tr>
<td>ip6.6 ↓</td>
<td>Chronic infection</td>
<td>Mice, Monkey</td>
<td>297</td>
<td>Mice and rhesus monkeys do not develop antibodies against ip6.6 after chronic infection.</td>
<td>Akins et al., (1998).</td>
</tr>
<tr>
<td>OspC1,P21, OspE/F1, 2.9-7LPa/B1, p22, ip6.6, OspA/B1, BbK2.10↓</td>
<td></td>
<td></td>
<td></td>
<td>Antibodies against OspC, P21, OspE/F in several hosts after active infection; no antibody response to ip6.6. DMC mimics host-adapted state and up-regulated determinants indicated are in response to growth in DMC. For response to other signals, refer to text.</td>
<td>Akins et al., (1998).</td>
</tr>
</tbody>
</table>

1Genes or proteins expressed differentially on cultivation of B. burgdorferi strains under in vivo growth (in comparison to in vitro growth) in different hosts or host-adapted conditions. The list does not include genes and proteins expressed differentially in response to varying environmental in vitro growth conditions such as temperature, pH, or nutrients. Refer to the text for additional information.
2The upward and downward arrows indicate increased or decreased expression or synthesis of the corresponding genes or proteins, respectively.
3Only broadly defined experimental conditions are mentioned. Infection of mice by ticks or with infectious B. burgdorferi is referred to as active infection. Refer to the text for details.
4Designations are given for strains belonging to the B. burgdorferi sensu stricto group.
analyzed under in vitro conditions, the role of chaperonins in the processing of borrelial endoflagellins is likely to be similar to what has been observed with other bacterial systems and the molecular interactions of Hsp60/Hsp70-endoflagellin may facilitate export of endoflagellin subunits across the cytoplasmic membrane as well as other protein species associated with B. burgdorferi pathogenesis.

OspA/OspC Switch
Heat shock proteins modulate the host’s response to a rapid increase in temperature, however they are not the only class of proteins up-regulated under these conditions. In B. burgdorferi, for example, outer surface protein A (OspA), but not OspC (Schwan et al., 1995), is expressed in the tick midgut prior to feeding. However, once ticks have fed to repletion there is an up-regulation of OspC and a down regulation of OspA (see Table 1). Even though this differential expression of OspC was partly mimicked in response to in vitro growth of B. burgdorferi at 32 or 37 °C instead of 24 °C, the lower levels of OspC induced by temperature induction alone demonstrated that other factors in the blood are required for maximal OspC expression (Schwan et al., 1995). These studies revealed that different environmental cues, like tick midgut temperature and their contents following feeding, could serve as signals to alter gene expression in B. burgdorferi. The derepression of ospC and concomitant repression of ospA was also observed upon co-cultivation of B. burgdorferi with mammalian cells at different ambient temperatures (Obonyo et al., 1999). The down regulation of ospA was not observed when the spirochetes were cultivated in axenic medium at 37 °C indicating that a combination of additional host factors that are not present in the in vitro cell culture, presumably limited metal ion availability, contribute to the differential expression of ospA and ospC. The presence of antibodies specific to OspC during the initial stages of human infection indicated that OspC is expressed during transmission from the tick vector and the resulting primary infection (Aguero-Rosenfeld et al., 1993; Engstrom et al., 1995; Fung et al., 1994; Padula et al., 1993). Moreover, Borrelia extracted from the unfed ticks are noninfectious, indicating that there is a correlation of ospC expression and mammalian infection (Piesman, 1993). Recent studies on temporal changes in the levels of OspA and OspC indicated that OspC synthesis occurred during the first 48 hours of attachment in feeding Ixodes scapularis ticks (Schwan and Piesman, 2000). In contrast, tick larvae and unfected nymphs that fed to repletion on infected mice acquired B. burgdorferi cells with higher levels of OspA and little or no OspC. These results, taken together, suggest that the OspA/OspC switch in B. burgdorferi correlates with an increase in spirochetal infectivity for mammalian hosts and implies that the OspC switch observed is involved in the transmission from ticks to mammals, but is not involved in the movement of B. burgdorferi from the reservoir host to the naïve tick vector.

OspE/F-Related (Erp) Proteins
In addition to OspC, immunoblotting of bacterial lysates with sera from infected mice indicated that several additional proteins were induced when B. burgdorferi cultures were shifted from 23 °C to 35 °C (see Table 1; Stevenson et al., 1995). A couple of these proteins were identified as OspE (a 19 kDa, surface-exposed lipoprotein) and OspF (a 27 kDa lipoprotein) which are co-expressed in an operon (Lam et al., 1994). Antibodies against the aforementioned temperature-regulated antigens were prevalent in the serum of patients with late stage Lyme disease. Furthermore, B. burgdorferi were substantially destroyed in ticks that engorged on either OspE- or OspF-immunized mice indicating that these antigens were expressed in the ticks and could be useful targets for spirochete killing (Nguyen et al., 1994). Apart from OspE/F, additional homologous antigens in B. burgdorferi strain B31 were also identified that mapped to the 32 kilobase circular plasmids (cp32 plasmids) and a closely related 56 kb linear plasmid (Ip56) (Casjens et al., 1997; Stevenson et al., 1998a; Stevenson et al., 1996).

Low passage, infectious B. burgdorferi strain B31 contain cp32 in seven copies and all, along with Ip56, contain at least one ospE/F-related or erp family gene (Casjens et al., 2000; Casjens et al., 1997). This degree of plasmid-associated genetic redundancy is unique and implies that there could be an evolutionary advantage in having several copies of related plasmids in low copy number as opposed to having a single plasmid in high copy number. One possible explanation for this may be that OspE/F-related (Erp’s) proteins are antigenically distinct yet retain an as yet unknown compensatory function(s) that is (are) required for normal transmission and infectivity. Additional studies indicated that homologues of the OspE/F family (Erp proteins) are up-regulated both transcriptionally and translationally when low passage B. burgdorferi strain B31 cultures are shifted from 23 °C to 35 °C (Stevenson et al., 1998a). Several of the OspE/F-related (Erp) proteins from B31 isolates were recognized by both sera from Lyme disease patients and tick-infected mice (Stevenson et al., 1998a). Since the OspE/F family (Erp) of proteins are induced in response to a temperature increase and appear to be antigenic during the early stages of mammalian infection, these proteins may also play a role in the transmission of B. burgdorferi from ticks to mammals. Several authors have speculated that the presence of numerous ospE/F-related (erp) genes on the different cp32 plasmids and Ip56 may provide a mechanism to avoid immune clearance (Das et al., 1997; Marconi et al., 1996) akin to variable membrane proteins (Vmp proteins) of the relapsing fever Borrelia (Barbour et al., 1982). The presence of cross-reactive OspE/F-related protein (Erp) antibodies during the early stages of infection that may neutralize these proteins expressed at later stages of infection, coupled with the lack of structurally distinct promoter elements that may aid in the temporal expression of ospE/F-related (erp) genes, would seem to contradict the aforementioned hypothesis regarding immune evasion. Nevertheless, the ubiquity of the plasmids carrying the ospE/F-related (erp) genes suggest that they may be important in the natural life cycle of B. burgdorferi (Casjens et al., 1997). Further, the evidence of past recombination events at ospE/F loci (erp) suggests that there are possibly other subtle but unknown mechanisms that allow the spirochete to exploit the heterogeneity of the ospE/F (erp) paralogues (Stevenson et al., 1998a; Stevenson et al., 1998b). Along these lines, Sung et al., have recently shown that ospE/F-related genes are subject to antigenic variation during infection in the mouse (Sung et al., 2000), not unlike the other B. burgdorferi antigenic variant visE (Zhang et al., 1997).
Other Infection Associated Antigens

More recently, it has been shown that the infection-associated antigen VraA (virulent strain associated repetitive antigen A), whose gene (bbi16) maps to the linear plasmid 28-4 (lp28-4) (Skare et al., 1999), was up-regulated in *in vitro* at 37°C relative to growth at either 32°C or 23°C in *B. burgdorferi* strain B31 (M. Labandeira-Rey and J. T. Skare, unpublished observations). Interestingly, VraA contains a unique 9 amino acid structure repeated 21 consecutive times that may confer adhesin-like properties with increased avidity for its target due to its repetitive structure. However, this hypothesis remains to be determined.

Another set of temperature regulated proteins are the decorin binding proteins A and B (DbpA and DbpB) (Cassatt et al., 1998). These borrelial proteins are adhesins that bind decorin, a proteoglycan associated with type III collagen (Guo et al., 1995). The skin is the primary site of infection and since collagen is abundant at this locale, the up-regulation of these adhesins in response to temperature would presumably increase the avidity of *B. burgdorferi* to decorin, aiding in colonization at this site. It remains to be seen if individual mutations in dbpA or dbpB, or a double mutant in dbpAB, will result in the loss of adherence of *B. burgdorferi* to decorin and whether this loss of binding correlates with a reduction in infectivity.

Even though it is possible to observe the *de novo* synthesis of some *B. burgdorferi* proteins under controlled experimental conditions (like temperature), the mechanism(s) by which these genes are regulated is not yet known. Moreover, the regulatory mechanism(s) involved in the OspA/OspC switch may either be mediated by a global effector or sensitive to many different environmental signals independent of temperature. As the genetic tools become available for making knockout mutations in infectious isolates, it will be critical to use isogenic mutants to assess the requirement of a defined gene (or genes) for infectivity in experimental models of Lyme borreliosis. Furthermore, the global effects of temperature on gene expression seen in other bacteria (Richmond et al., 1999) will be facilitated when DNA microarrays of the entire *B. burgdorferi* genome become readily available.

**pH and Nutrients**

In addition to temperature, there are additional environmental signals that are unique to both the tick and mammalian hosts that *B. burgdorferi* must adapt to transiently. These include differences in the pH, osmolarity and availability of nutrients within these diverse microenvironments that *B. burgdorferi* inhabits. For example, prior to a blood meal, *B. burgdorferi* resides within the midgut of *Ixodes* ticks where the pH is alkaline (Munderloh and Kurtti, 1995). Upon exposure to the tick blood meal, *B. burgdorferi* is transmitted into the mammalian host where the pH encountered is approximately 7.4. Rapid adjustment to the approximate 100-fold change in proton concentration (*i.e.*, pH 9.5 to 7.4) would thus be a critical compensatory mechanism that provides an evolutionary advantage to *B. burgdorferi*. When the spirochetes are subjected to similar changes in the pH *in vitro* growth conditions, there is either an up-regulation or exclusive expression of at least six membrane proteins at pH 6.0 or 7.0 but not at pH 8.0 (Carroll et al., 1999). One of the major proteins that is down-regulated at a more alkaline pH (pH 8.0) is OspC, whose synthesis is decreased 10-fold under these conditions (Table 1). Conversely, there is an up-regulation of a 42 kDa protein at pH 8.0 when compared to pH 6.0 or 7.0. The reduction in the levels of OspC at alkaline pH is consistent with the previous observations that ospC is down regulated in the midgut of unfed ticks (Schwan et al., 1995). It has also been suggested that the expression of ospC could be under coordinate regulation of both pH and temperature and, in part, explain the lack of ospC up-regulation in the midguts of infected unfed ticks exposed to higher temperature alone (Schwan et al., 1995). A total of 37 changes to membrane protein profiles were observed by two-dimensional nonequilibrium pH gradient gel electrophoresis (2D-NEPHGE) analysis when *B. burgdorferi* cells were incubated at pH 6.0, 7.0 or 8.0. (Carroll et al., 1999), suggesting that there is an extensive adaptive mechanism in *B. burgdorferi* in response to changes in pH that accompanies mobilization of the spirochetes from the midgut to the salivary glands and ultimately to the mammalian host. In addition to pH, there may be other uncharacterized factors that aid in the migration of *B. burgdorferi* from ticks to mammals. The migration of spirochetes within the tick vector is blocked if the ticks are fed on mice immunized with OspC, suggesting that OspC is involved in movement of *B. burgdorferi* from the midgut to the salivary glands (Gilmore and Piesman, 2000). Anti-OspC antibodies in the blood meal in conjunction with other unknown factors/nutrients were able to down regulate *ospC* expression even though these same OspC antibodies were not borrellicidal under *in vitro* conditions (Gilmore and Piesman, 2000). It is tempting to speculate that *B. burgdorferi* may be able to sense the status of the mammalian host based on the contents of the blood meal and alter its gene expression in order to either escape killing in the midgut or migrate to the salivary gland for transmission to the mammalian host.

In addition to the ability of spirochetes to rapidly adapt to changes in temperature and pH, the differences in the nutrient contents of the tick and mammalian hosts may also play a role in borrelial survival in these disparate environments. Interestingly, the levels of purines in the tick may be sufficient for DNA synthesis in *B. burgdorferi* through the use of purine salvage pathway (Munderloh and Kurtti, 1995). Unlike other prokaryotes, *Borrelia* species carry genes involved in purine synthesis (*guaA* and *guaB*) on plasmids (the 26 kb circular plasmid [cp26] in *B. burgdorferi*) instead of the chromosome (Margolis et al., 1994). The presence of these genes would allow for *de novo* purine biosynthesis and as such, would presumably be beneficial in terms of survival within mammalian host inasmuch as the levels of extracellular purines and pyrimidines are extremely low (Margolis et al., 1994). Although it is not known if there is a preferential up-regulation of *gua* genes when *B. burgdorferi* shuttles between ticks and mammals, there is evidence that the copy number of a *B. hermsii* linear plasmid is lower in rich medium rather than in mice (Kitten and Barbour, 1992). Environmental levels of guanine may regulate *guaA* and *guaB* genes and the requirement of these genes for *de novo* purine synthesis may be in part responsible for the maintenance of the cp26 plasmid despite extensive *in vitro* cultivation and passage of *B. burgdorferi* (Barbour, 1988; Hinnebusch and Barbour, 1992; Schwan et al., 1988).
In vivo Versus in vitro Gene Expression in B. burgdorferi

The fate of pathogenic bacteria to infect and adapt to the demanding conditions imposed by eukaryotic hosts is dependent on the ability of the bacterium to modulate gene expression in a manner appropriate for colonization and survival. Numerous studies have shown that gene expression in vitro is distinct from gene expression in vivo in a wide variety of pathogenic bacteria (Chiang et al., 1999; Mahan et al., 1993; Shea et al., 1996; Valdivia and Falkow, 1997). Over the past several years, various studies have shown that B. burgdorferi is also subject to differential gene expression in vivo relative to in vitro cultivation, indicating that B. burgdorferi responds to host specific factors to express genes accordingly (Akins et al., 1998; Schwan et al., 1995; Suk et al., 1995). Along these lines, Barthold and colleagues demonstrated that mice immunized with OspA, a protein known to be down-regulated in infected mammals, were resistant to needle inoculated B. burgdorferi yet were sensitive to challenge with transplanted skin from mice infected with B. burgdorferi (Barthold et al., 1995). This result indicated that B. burgdorferi within the host did not express ospA and was consistent with the previous observation that infected humans have little or no antibody titer to OspA during early infection (Barthold et al., 1995). Golde and co-workers also demonstrated that a passage 6 isolate of B. burgdorferi strain B31, when maintained in the natural zoonotic cycle of transmission between laboratory mice and laboratory-reared Ixodes ticks, exhibited limited genetic and antigenic variation after 5 cycles of transmission (Golde and Dolan, 1995). This study, along with others, (Barbour, 1988; Norris et al., 1995; Schwan et al., 1988), revealed that changes in the plasmid profiles of B. burgdorferi correlate with a change in infectivity in animal models of Lyme borreliosis. These results suggest that genes on plasmids contribute either directly or indirectly to the infectious phenotype and indicate that there is a tolerance for a dynamic genome, at least in vitro. Recent studies have shown that plasmid content also varies from clonal isolates obtained from infected mouse tissue, indicating that plasmid heterogeneity is also tolerated in vivo (M. Labandeira-Rey and J. T. Skare, unpublished observations; R. T. Marconi, personal communication).

Selection in the Arthropod and Mammalian Hosts

Sequential passage of B. burgdorferi between either an arthropod vector or a mammalian host indicated that selective pressures imposed by each eukaryotic host results in a population of spirochetes with a defined, unique phenotype (Ryan et al., 1998). The transition of B. burgdorferi from one host to another produced a striking series of alternating phenotypic signatures resulting in the reciprocal expression of different antigenic forms of OspB and OspC. Sequence analysis of the ospB and ospC genes derived from both mammal and tick clonal isolates revealed two allelic forms of ospC. Interestingly, one form of ospC was specific for mammals and the other for ticks. Though there was no dramatic change in the amino acid sequence encoded by the ospB alleles, there was a marked difference in the antigenicity of the OspC variants as determined by differential reactivity with an OspC monoclonal antibody. These observations suggest the possibility of recombinatorial activation, reminiscent of Neisseria gonorrhoeae pilin antigenic variation (Seifert, 1996), to generate a full-length allelic variant of ospC. In addition to these reciprocal antigenic variations, Ryan and co-workers also observed a differential selection for the 9.0 kilobase supercoiled plasmid in the tick isolates (Ryan et al., 1998). Taken together, these data indicate that there is a selection for specific B. burgdorferi populations during in vivo propagation of this spirochete through the arthropod vector and mammalian host resulting in distinct antigenic changes. These changes could contribute to functions selected for within the different hosts, via recombinational mechanisms, similar to what is seen in several other eukaryotic and prokaryotic pathogens (Pays, 1991; Reeder and Brown, 1996; Restrepo and Barbour, 1994; Seifert, 1996).

In vivo Expressed Antigens

An exported plasmid protein A (EppA) encoded on the 9.0 kb circular plasmid (cp9 according to TIGR; Fraser et al., 1997) of B. burgdorferi was the first protein purported to be induced preferentially during the infection of the mammalian host (Champion et al., 1994). This interpretation was based on the paradoxical observation that EppA was antigenic in some patients with Lyme disease and in rabbits experimentally infected with B. burgdorferi strain B31, but did not appear to be expressed by in vitro grown B. burgdorferi (see Table 1). Similar results were obtained for a genetic locus designated lp6.6, encoding a 6.6 kDa lipoprotein, which was not antigenic in either chronically infected rhesus monkeys or mice, implying that this gene was not expressed in either warm-blooded species (Lahdenne et al., 1997), not unlike ospA (Montgomery et al., 1996; Schwan et al., 1995). Based on these observations, the authors speculated that lp6.6 was expressed preferentially in the tick vector; however, to date, no tick-specific transcript or protein species for lp6.6 has been detected.

Subsequently, Suk et al. used sera from mice infected with B. burgdorferi to identify genes expressed only in the host (Suk et al., 1995). Several genes (p21, p35 and p37) that were specifically expressed in vivo were identified in this screen (Table 1). Further characterization of these genes revealed that p21 encoded a 20.7 kDa protein that was closely related to, but distinct from, ospE while p35 and p37 each encoded for B. burgdorferi lipoproteins. Later studies indicated that P35 and P37 (now designated as BBK32 and BBK50 by TIGR, respectively (Fraser et al., 1997)), elicited protective immunity in mice (Fikrig et al., 1997). Additional experimentation has shown that p21 is specifically derepressed in vivo and under host-adapted conditions described below (Akins et al., 1998). Studies on the temporal expression of p21 in mice infected with B. burgdorferi indicated that p21 mRNA and anti-P21 antibodies were detectable at 21-28 days post infection, whereas expression of ospE mRNA and anti-OspE antibodies were present at day 7 (Das et al., 1997). Even though p21 and ospE are homologous genes, there are well defined signals, as yet unknown, that regulate their expression. While ospE is expressed in both the mammalian host and arthropod vector, p21 is expressed only in the mammalian host and, moreover, appears to be constitutively expressed for several weeks following infection (Das et al., 1997). Therefore, spirochetes from engorged ticks appear to differ from spirochetes in
vertebrate hosts, and the differential expression of related homologues in different hosts and at different times of infection may allow for successful colonization and dissemination of *B. burgdorferi*. A differential immunoscreening strategy was also used to identify a 22 kDa lipoprotein related to OspF, designated pG, induced in vivo in *B. burgdorferi* strain ZS7 (Table 1; Wallich et al., 1995). Inasmuch as pG is an *ospE/F*-related locus, like *p21*, *pG* may also exhibit delayed expression. More importantly, Sung *et al.* have recently demonstrated that OspE/F–related proteins (Erp’s) are subject to antigenic variation only in vivo (Sung *et al.*, 2000), implying that these additional homologues (i.e., *P21*, *pG*) may contribute to immune evasion (see below for details).

Additional evidence for tissue specific differential gene expression comes from the study of Fikrig *et al.*, who showed that p35 and p37 specific mRNA were detected in the erythema migrans biopsy specimens from 2 human patients and in the synovium of patients with Lyme arthritis (Fikrig *et al.*, 1998). In contrast, mRNA encoding *ospA* was undetectable in these tissues, consistent with the known repression of *ospA* in mammalian infection (Akins *et al.*, 1998; Barthold *et al.*, 1995; Montgomery *et al.*, 1996; Schwan *et al.*, 1995).

Another strategy to identify genes uniquely expressed in infectious *B. burgdorferi* involved using antisera from infection immune rabbits that was adsorbed against non-infectious *B. burgdorferi* strain B31 (Skare *et al.*, 1999). A total of 18 different immunoreactive phage clones from an expression library containing DNA from infectious *B. burgdorferi* were characterized. Sequence analysis indicated that these 18 clones were defined by 9 genetic loci. All of the 9 genes mapped to plasmids and included decorin binding proteins A and B (*dbpAB*), a rev homologue present on the 9 kb circular plasmid (cp9), a rev homologue from 32-kb circular plasmid (cp32-6), *erpLM, erpX* and 4 previously uncharacterized loci designated *bbi16, bbk19, bbi34 and bbbk45* by TIGR (Fraser *et al.*, 1997). Since these antigens were identified using serum from infection immune rabbits (Foley *et al.*, 1995), these antigens may represent targets for killing antibody that are preferentially expressed in the mammalian host (i.e., a rabbit), and by analogy to *dbpAB* identified in this screen, may be involved in pathogenic mechanisms. Along these lines, *dbpAB* and *vraA* (*bbi16*) appear to be temperature inducible loci (see above section, “Other Infection Associated Antigens”). Furthermore, VraA, like DbaP, provides protection against infectious challenge in the mouse model of Lyme borreliosis (Cassatt *et al.*, 1998) (M. Labandeira-Rey and J. T. Skare, unpublished observations) and, in the case of DbaP, can confer passive immunity (Table 1; Hanson *et al.*, 1998) confirming that *dbpA* is expressed during infection.

**Dialysis Membrane Chambers (DMC’s): Host-Adapted Spirochetes**

Determination and comparison of mRNA and protein levels induced under in vitro and in vivo conditions has also been used in several infectious disease models to identify genes and their products that are critical for conferring virulence (Finlay and Falkow, 1997). In an attempt to apply such an approach to *B. burgdorferi*, dialysis membrane chambers (DMC’s) containing strain 297 spirochetes were implanted into the peritoneal cavities of rats (Akins *et al.*, 1998). This approach eliminates the limitations inherent to studying host-adapted *B. burgdorferi*, notably the inability to recover spirochetes from infected tissues in appreciable numbers. These host-adapted spirochetes exhibited profound and reversible alterations when compared with in vitro cultivated *B. burgdorferi* grown at either 23°C, 34°C, or 37°C (see Table 1). While OspA and Lp6.6 were not expressed in host-adapted spirochetes, there was a dramatic increase in levels of OspC and P21 consistent with previous results indicating that these proteins are preferentially repressed or derepressed during mammalian infection, respectively (Lahdenne *et al.*, 1997; Schwan *et al.*, 1995; Suk *et al.*, 1995). OspE, OspF, and an OspF-related protein termed BbK2.11, were produced at similar levels in either in vitro or host-adapted organisms. A considerably lower amount of these proteins were made when *B. burgdorferi* was cultivated at 23°C as opposed to 34°C indicating that, as seen in previous in vivo studies, OspE, OspF, or BbK2.11 were not synthesized to the level observed for P21. These observations are difficult to reconcile inasmuch as *p21* and related *ospE/F* loci (erp genes) contain nearly identical sequences upstream from their translational start codon, termed upstream homology boxes (UHB). As such, one would predict that these loci would be coordinately regulated. Clearly other factors, perhaps involving transcriptional activators that bring unlinked upstream regions of DNA into apposition via DNA bending, like the AraC protein in *Escherichia coli* (Lee and Schleif, 1989), may explain this apparent regulatory dilemma.

Differences in gene expression between in vitro grown and DMC grown spirochetes were further analyzed by differential display RT-PCR and this analysis revealed that two additional borrelial lipoproteins, designated 2.9-7lpA and 2.9-7lpB, are transcriptionally linked and induced in chamber grown spirochetes (Akins *et al.*, 1998). These related lipoproteins were previously shown to belong to a paralogous family of differentially expressed genes located at a loci termed 2.9 that is found on homologous 32 kilobase circular plasmids of *B. burgdorferi* strain 297 (Porcella *et al.*, 1996). These observations indicated that differential gene expression of *B. burgdorferi* within a mammalian host could be simulated in implanted DMC’s. These studies have provided a foundation for assessing in vivo expression in mammalian hosts, yet have some clear limitations.

By analogy with other pathogens, it is conceivable that differential gene expression may involve contact dependent interaction of *B. burgdorferi* with host cells, something that cannot be mimicked by this procedure. Furthermore, detection of proteins (or transcripts) low in abundance that may be important in the regulation of the observed response have yet to be identified using this methodology. Clearly once the genetic tools become available to make stable isogenic mutants in infectious *B. burgdorferi*, identification of the genes and their products that are essential for normal infectivity will no longer be limited to issues pertaining to biochemical sensitivity.

**Antigenic Variation, Diversity and Cloaking**

Antigenic variation of virulence determinants has been shown to be an important, rapid adaptation strategy that pathogens have evolved to escape and survive detrimental host immune responses. Antigenic variation could be confined to regions of protein(s) that are critical for host–pathogen interactions and changes acquired through
several genetic mechanisms may result in hypervariable regions in these molecules. A complex mechanism of antigenic variation was first characterized in *Borrelia* species using the relapsing fever spirochete *Borrelia hermsii* as a model (Barbour, 1990; Barbour et al., 1983; Barbour et al., 1982; Kitten and Barbour, 1990; Plasterk et al., 1985). Surface exposed lipoproteins termed variable major proteins (Vmp proteins) are encoded by homologous genes located on the 28-32 kb linear plasmid with covalently closed telomere-like sequences (Kitten and Barbour, 1990). Each organism has at least 40 vmp genes most of which are located in the storage plasmids in the silent or unexpressed form (Hinnebusch et al., 1998). Only one vmp is expressed in each organism (Barbour et al., 1991; Restrepo and Barbour, 1994; Restrepo et al., 1994) and antigenic variation is generated when the expressed vmp is replaced partially or completely by one of the silent vmp genes. Several genetic mechanisms, including interplasmic recombination (Barbour et al., 1991; Plasterk et al., 1985), intraplasmic recombination (Restrepo et al., 1994), and post-switch rearrangement (Restrepo and Barbour, 1994), have been attributed to the antigenic variation. Antigenic variation in relapsing fever *Borrelia* occurs at a frequency of $10^{-3}$ to $10^{-4}$ per generation in *B. hermsii* (Stoenner et al., 1982) during both *in vitro* cultivation and *in vivo* indicating that immune selection does not mediate vmp recombination.

**The VlsE Antigenic Variant**

A similar yet distinct genetic system that promotes antigenic variation was reported for *B. burgdorferi* at a genetic loci called vls for vmp-like sequence that closely resembles the vmp system of *B. hermsii* (Zhang et al., 1997). A vls expression site (vlsE) and 15 additional silent vls cassettes were identified on a 28 kilobase linear plasmid, subsequently designated as lp28-1 (Fraser et al., 1997). The presence of lp28-1 correlates with a high infectivity phenotype in *B. burgdorferi* and the vlsE locus, located near a telomere of lp28-1, encodes a surface–exposed lipoprotein which exhibits promiscuous recombination upon infection of mice (Norris et al., 1995; Zhang et al., 1997). VlsE variants exhibited differential reactivity to antiserum generated against the parental vls1 cassette region, providing evidence of antigenic variation at this loci in a mammalian host which could facilitate evasion of the host immune response. This mechanism is not unique to *B. burgdorferi* in that several pathogens utilize genetic variation in multi-gene families as a way to escape the host response due to the resulting antigenic variation (Borst et al., 1995). The genetic variation at the vlsE site in *B. burgdorferi* is similar to the combinatorial recombination of the pilin-encoding genes of *Neisseria gonorrhoeae* (Seifert and So, 1988) inasmuch as it involves recombination to generate antigenic variants. However, unlike most antigenically variable loci, vlsE only exhibits variation *in vivo* indicating that the signal for recombination is host specific (Zhang et al., 1997).

The mechanism of genetic variation induced at the vlsE locus has been shown to occur through an unidirectional process where segments of the silent vls cassettes recombine in the vlsE cassette region without affecting the sequence and organization of the silent vls loci (Zhang et al., 1997; Zhang and Norris, 1998). This strategy allows *B. burgdorferi* to have a nearly inexhaustible reservoir of vlsE sequence variation much like the repertoire of mammalian germline immunoglobulin and T cell receptor loci. The observation that vlsE has been found in numerous human and tick isolates suggests that antigenic variation at the vlsE locus is important, in part, for *B. burgdorferi* pathogenesis (Iyer et al., 2000). It should be re-emphasized that although both *B. burgdorferi* and *B. hermsii* exhibit antigenic variation, the vlsE variation in *B. burgdorferi* occurs only under *in vivo* selection.

**The OspE/F-Related (Erp) Antigenic Variants**

Recently, the ospEF-related antigenic family of *B. burgdorferi* has also been shown to be antigenically variable (Sung et al., 2000). The antigenic variation is presumably mediated by DNA repeats flanking the hypervariable domain (Sung et al., 2000). As with vlsE, recombination at the ospEF alleles occurs only during infection and not during *in vitro* cultivation, suggesting again that the mammalian environment leads to or selects for the accumulation of the observed genetic changes. The genetic redundancy of the plasmid components of the *Borrelia* genome (Fraser et al., 1997) could serve as an excellent substrate for recombination and rearrangements and this, coupled with differential gene expression strategies, may allow *B. burgdorferi* to escape the host immune clearance mechanisms leading to chronic, persistent infection associated with Lyme borreliosis. It is tempting to speculate that additional mechanisms, such as phase variation, may be operative in *B. burgdorferi* to evade host defenses and would partly explain the retention of genetic redundancy one observes within the *B. burgdorferi* genome sequence. The plethora of paralogous gene families identified in *B. burgdorferi* may be a gene organization pattern where individual members are sequentially expressed in order to not only maintain a compensatory function but to also provide a unique antigenic target that prevents clearance of the spirochetes by the host immune response. Further studies to address this hypothesis appear to be warranted and may explain the advantage of the redundancy and plasticity of the borrelial genome.

**Plasticity of the *B. burgdorferi* Genome**

In order to establish a link between pathogenicity and infectivity of *B. burgdorferi*, the role of several known differentially expressed determinants were examined after infection of C3H/HeN mice with either an attenuated or infectious isolate of *B. burgdorferi* strain N40. Anguita et al., showed that strain N40 passage 75 (N40-75) isolate was capable of infecting C3H/HeN mice but did not cause arthritis and carditis as seen with infection by a low-passage clonal isolate of strain N40 (designated cN40) (Anguita et al., 2000). N40-75 did not express *dbpAB* (*bb*24/25; *bb* designations assigned by TIGR (Fraser et al., 1997)), *bb*A4, *bb*A65, *bb*A66, *p*21, *erp*D, and loci designated *gene-1* and *gene-2*, whereas cN40 expressed all of these loci except *p*21, consistent with its delayed expression *in vivo* (Das et al., 1997). Presumably N40-75 lacks a genetic component (*i.e.*, plasmid(s)?) that precludes this isolate from expressing these *in vivo* genes, thereby adapting to the mammalian host in a manner commensurate with the infectious cN40 isolate. Although the authors determined that the protein and antigenic profiles were essentially the same for cN40 and N40-75, they did not exhaustively account for the loss of plasmid species which may
contribute to the defect in adaptation observed. Along these lines, several investigators have shown that the loss of plasmids in B. burgdorferi correlates with a loss in infectivity (Schwan et al., 1988; Barbour, 1988; Norris et al., 1995), suggesting that a subset of genes on the lost plasmids contribute to pathogenesis. With the advent of the B. burgdorferi genome sequence, it is now possible to identify which plasmids are present in clonal isolates through the use of oligonucleotide primers specific for each plasmid coupled with PCR. Subsequently, these clones can then be tested for infectivity deficits in animal models of Lyme borreliosis. It is possible that the loss of plasmid(s) not only results in the direct loss of genes and their products, but may also lead to more indirect effects that manifest as differential expression defects, i.e., inability to express a subset of antigens and/or ectopic expression of antigens in mutant (plasmidless) isolates, due to the loss of a global regulatory locus. To date, no studies pertaining to B. burgdorferi either support or refute this hypothesis.

Plasminogen Binding/Activation
In addition to changing its antigenic face, B. burgdorferi also cloaks itself with host factors. B. burgdorferi binds to these host factors at the primary site of infection, namely the skin, and uses them to penetrate normal tissue barriers such as vascular basement membranes and other organized extracellular matrices. Specifically, B. burgdorferi uses endogenous or host derived factors, particularly proteinases, to penetrate to other tissues of the mammalian hosts (Coleman et al., 1995; Fuchs et al., 1994; Klempner et al., 1995). A 70 kDa protein and, to a lesser extent, OspA of B. burgdorferi bind to plasminogen resulting in the activation of plasmin in the presence of host-derived plasminogen activator (Hu et al., 1995; Hu et al., 1997). The binding of this host derived proteinase (plasminogen) has been proposed to aid in the dissemination of the spirochete (Coleman et al., 1995; Fuchs et al., 1996; Klempner et al., 1995) and could contribute to cloaking B. burgdorferi such that it would be hidden from the host immune system. As such, this interaction could potentially contribute to the pathogenesis of B. burgdorferi and facilitate persistent infection due to immune evasion mechanisms.

Concluding Remarks
The survival and transmission of B. burgdorferi in tick and mammalian hosts impose conditions that require these spirochetes to express genes in accordance with their new microenvironments. The ability of these spirochetes to quickly adapt is essential for survival of B. burgdorferi and highlights their dynamic nature. Although details pertaining to the biochemical and genetic regulatory pathways that allow for this differential gene expression are unknown, a number of determinants that are either de-repressed or repressed have been identified in conjunction with the signal(s) that is (are) most predominant in inducing these proteins. In addition to variations in environmental conditions encountered by the spirochetes relative to the arthropod and mammalian hosts, it is becoming apparent that tissue tropism and the eventual pathogenesis of B. burgdorferi may very well depend on the temporal expression of host-adapted genes. It is tempting to speculate that the two-component regulatory systems identified in the genome sequence may be involved in sensing and modulating gene expression in order to adapt to the host. The role of antigenic variation in B. burgdorferi has been established, however the potential for phase variation and the mechanisms mediating such a response have yet to be determined. Identification of such variable determinants may shed light on additional escape variants selected for by the host and would help explain the chronic infection associated with Lyme borreliosis. Recent advances in gene knockout systems for B. burgdorferi (Bono et al., 2000; Elias et al., 2000; Rosa et al., 1996; Tilly et al., 1997), as well as the wealth of genome sequence information available, should aid in defining the host-spirochete interactions at the molecular level and define which loci are involved in the pathogenesis of B. burgdorferi.

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