# Temporal regulation of outer surface proteins of the Lyme-disease spirochaete *Borrelia burgdorferi*

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## Abstract

**In the 20 years since the first agent of Lyme disease was discovered, much interest has focused on the possible biological roles of a few outer surface proteins (Osps) in the alternating life cycle that includes ticks and vertebrate hosts. Two major proteins, OspA and OspC, are differentially regulated by the spirochaete *Borrelia burgdorferi* during the several days when ticks feed. The reciprocal decrease in OspA with the rapid up-regulation of OspC by the spirochaetes when ticks are feeding suggests that OspA aids in spirochaete attachment while OspC assists in the dissemination of spirochaetes from tick to vertebrate. Future experiments in ticks with mutant spirochaetes that lack these proteins should clarify the speculative functions currently given to these proteins.**

## Introduction

In 1981, the first agent of Lyme disease was discovered in ticks, isolated in pure culture, and shown to be immunologically reactive with convalescent serum samples from Lyme-disease patients [1]. Reports soon followed that confirmed the spirochaetal aetiology of the disease [2,3], and the organism was named *Borrelia burgdorferi* [4]. During the last 20 years, many important contributions have been made towards understanding the diversity of *Borrelia* species that cause this disease and the transmission cycles with ixodid ticks, mammals and birds [5]. The complex genome of *B. burgdorferi* has been determined [6], and a vaccine for human use in the U.S. has been licensed and removed from the market [7]. Between 1991 and 2000, the incidence of Lyme disease in the U.S. nearly doubled, and the 17 730 cases reported for 2000 is more than that reported for any previous year [8]. In this current post-vaccine era of Lyme disease, controlling the infection will continue to rely on reducing the exposure of humans to infected ticks, prompt diagnosis of those individuals infected and their antibiotic treatment. Whether or not a new vaccine is considered for future control will depend, in part, on identifying new suitable candidate antigens, most probably one or more outer surface proteins (Osps), and determining when these proteins are produced by Lyme-disease spirochaetes during their complex life cycle in ticks and vertebrates. In the present paper, I will consider two major Osps of *B. burgdorferi* and discuss their possible role for infection and transmission by ticks.

**Key words:** *Ixodes scapularis*, phenotypic changes, temperature regulation, tick feeding.

**Abbreviations used:** Osp, outer surface protein.

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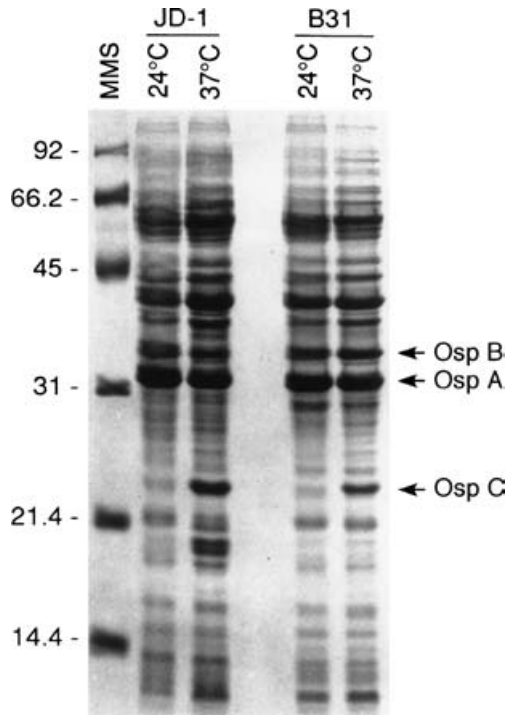
## First Osps identified

Shortly after Lyme-disease spirochaetes were first observed in the midgut of *Ixodes scapularis* ticks [1], several reports demonstrated protein profiles of whole-cell lysates of spirochaetes grown *in vitro* [9–11]. This work was facilitated by Kelly's development, 10 years earlier, of a liquid culture medium that grew the relapsing fever spirochaete, *Borrelia hermsii* [12], and which also allowed the newly discovered spirochaete to be grown to high cell densities [13].

The first Osp identified in the culture-derived *B. burgdorferi* was approx. 31 kDa, and a monoclonal antibody (H5332) produced against this protein showed that it was present in spirochaetes that originated from various tick and mammalian sources from different geographic locations [11]. Indirect immunofluorescence with this antibody also visualized spirochaetes with this protein in infected ticks [11]. Thus this specific protein–antibody binding that was demonstrated with fluorescence microscopy became a rapid method for identifying Lyme-disease spirochaetes and assaying tick tissues for spirochaete infection. This 31 kDa protein was soon designated OspA and a 34 kDa protein was named OspB [14], with the genes that encoded these proteins being adjacent and co-transcribed [15]. A year later, Wilske et al. [16] described a 22 kDa protein, designated pC, that was prevalent in Lyme-disease spirochaetes isolated from ticks and patients in Europe. DNA-sequence analysis and cell localization studies showed pC was a putative lipoprotein on the spirochaete's outer surface and the protein was renamed OspC [17,18] to be consistent with the nomenclature of the previously described Osps [14] (Figure 1). Initially, European investigators thought that OspC was primarily restricted to Lyme-disease spirochaetes found in Eurasia, but this is not the case. Many studies have since shown that the OspC gene or protein is present in all North American isolates of *B. burgdorferi* [19,20]. Also, the OspC gene is located on a

**Figure 1 | OspA, B and C of *B. burgdorferi* JD-1 (left) and B31 (right) grown *in vitro* at 24°C and 37°C**

Whole-cell lysates of the spirochaetes were examined by SDS/PAGE. OspA, B and C are indicated with arrows. MMS, molecular mass standards (in kDa). Proteins were stained with Coomassie Brilliant Blue. Note the up-regulation of OspC in both isolates when grown at 37°C.




26 kb circular plasmid [21,22], which is stably maintained among isolates of the spirochaete [23].

For the 10 years subsequent to the discovery of OspC [16], much work was focused on the possible roles for OspA, OspB and OspC in the pathogenesis of Lyme disease, the interaction of spirochaetes with eukaryotic host cells (Figure 2) and their potential use as immunogens for vaccine development. OspA was chosen for the primary antigen in the first recombinant, acellular Lyme-disease vaccine for human use in the U.S. [7]. However, none of the earlier studies addressed the hypothesis that Lyme-disease spirochaetes may differentially regulate Osps, which altered their phenotype during specific times in the bacterium's complex life cycle with alternating hosts. The possibility that spirochaetes that are grown *in vitro* may not express the same proteins compared with natural infections was also not yet fully appreciated even though the influence of sustained cultivation on protein expression was known [24].

## Differential regulation of surface proteins

OspA is a major surface protein that is abundant in spirochaetes that are grown *in vitro* and is detectable in spirochaetes that infect ticks [11]. This led Burkot and co-workers to develop an OspA antigen-capture ELISA, first to detect *B. burgdorferi* in ticks [25], and then to quantify spirochaetes, based on the amount of OspA measured, in unfed ticks and ticks at various stages of feeding [26]. These investigators observed a 54% decrease in the amount of OspA during the last 12 h of feeding of infected nymphal *I. scapularis*, followed by an increase in the protein after the ticks had completed feeding [26]. Because there was no precedent for OspA being temporally regulated by *B. burgdorferi*, they concluded that the drop in the quantity of OspA detected was most likely to be due to the loss of spirochaetes associated with their transmission from the ticks to mammals on which the ticks were feeding.

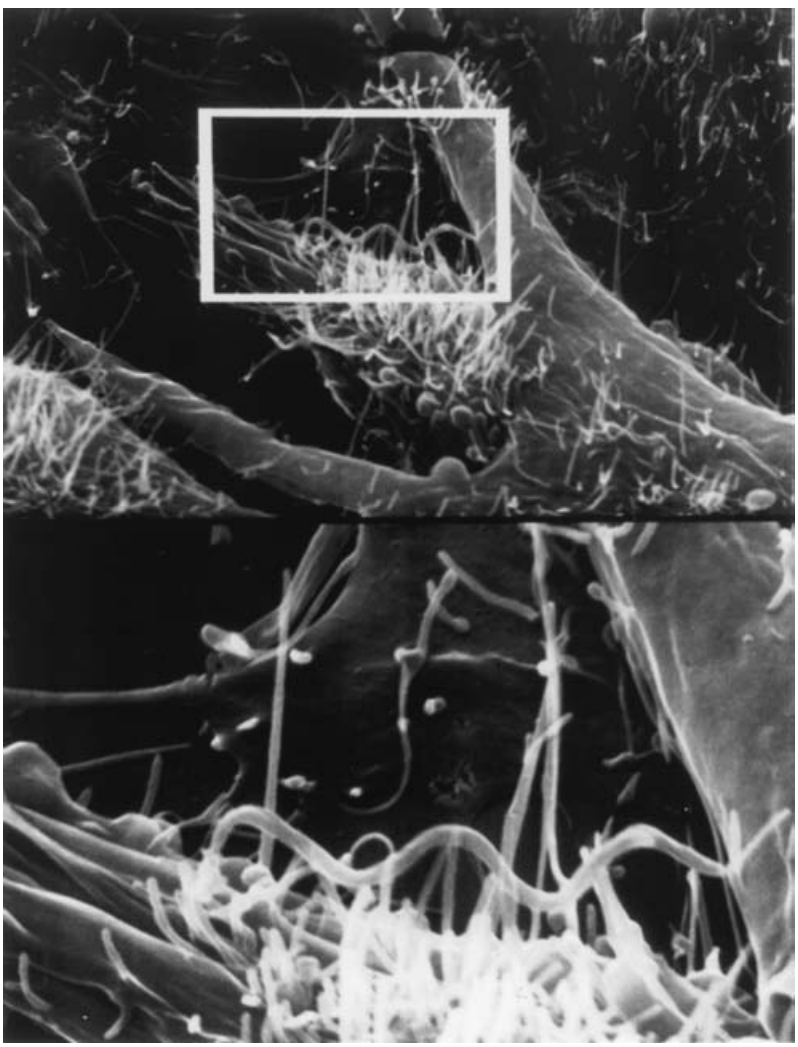
Antibody responses in animals that were experimentally infected with cultured Lyme-disease spirochaetes differ from animals infected by tick bite [27–29]. Many studies demonstrated that infection in rodents with *B. burgdorferi* resulting from tick bite rarely stimulates an antibody response to OspA [28–30], whereas inoculation with cultured spirochaetes does. Humans with Lyme disease also rarely seroconvert to OspA [31]. Yet, authors of the earlier studies concluded that the variability observed in the presence of anti-OspA antibody probably resulted from the different routes of inoculation, which affected the modulation of the host's immune response. Evidence for the spirochaetes altering their phenotypes between *in vitro* growth and infection in ticks compared with that of mammals was still lacking.

In 1995, work in our laboratory demonstrated that *B. burgdorferi* altered its Osps in ticks during feeding, and also with changes in temperature during *in vitro* growth [30]. Spirochaetes in unfed *I. scapularis* nymphs had OspA, but no OspC; yet, immediately after the ticks had engorged on mice, a significant number of spirochaetes stained positive for OspC whereas some were negative for OspA [30]. Shifting the *in vitro* growth temperature from 24°C to 37°C also stimulated the spirochaetes to synthesize OspC (Figure 1), whereas decreasing the temperature back to 24°C caused OspC to be diminished to below detectable levels. Serological examination of mice infected with *B. burgdorferi* by tick bite showed strong antibody responses to OspC, but not to OspA, suggesting that OspC, but not OspA, was present during infection in mammals [30]. These results were the first to demonstrate that Lyme-disease spirochaetes changed phenotypically during tick feeding, that temperature was one environmental cue that controlled the change, and that proteins that were expressed by the spirochaetes in ticks may vary from those expressed in mammals.

Later in 1995, Barthold et al. [32] demonstrated that mice seroconverted to OspA only if they were inoculated with $>10^4$ live spirochaetes that had been grown *in vitro* (most *B. burgdorferi* produce OspA *in vitro*). Mice inoculated with fewer spirochaetes became infected, but they did not seroconvert to OspA [32]. This observation suggested that the smaller number of spirochaetes did not provide sufficient amounts of OspA to stimulate an antibody response, and that OspA may be down-regulated after the spirochaetes were inoculated into mice. These investigators went on to show that mice that were immunized with recombinant OspA were protected from infection when challenged with

**Figure 2 | *Borrelia burgdorferi* grown *in vitro* and allowed to adhere to Vero cells**

A Lyme-disease spirochaete adhering to a mammalian cell is visualized by scanning electron microscopy. OspA and OspB, which are produced by spirochaetes that are grown *in vitro* are not produced *in vivo*. Thus such attachment experiments with eukaryotic cells and spirochaetes grown in culture may not reflect interactions with mammalian cells during natural infection. The lower panel shows a magnification of the region indicated on the upper panel.



spirochaetes grown *in vitro* that were expressing OspA. However, when OspA-immunized mice were challenged with skin samples from mice infected with *B. burgdorferi*, the challenged mice became infected. These results demonstrated that after culture-grown spirochaetes were inoculated into mice, they became 'host-adapted' by no longer producing OspA. Our study with ticks [30] and Barthold's study with mice [32] demonstrated that Lyme-disease spirochaetes had a more complex life cycle than had been appreciated previously and many studies that examined the differential regulation of proteins by these bacteria have followed.

The first reports that *B. burgdorferi* changed phenotypically during its life cycle did not quantify the number of spirochaetes with OspA or OspC, or elucidate the changes in these proteins temporally. We now know OspA is a tick-associated protein that is down-regulated by spirochaetes while nymphal ticks feed [33,34]. Most spirochaetes in the midgut of unfed ticks produce OspA, but after 3–4 days of tick attachment and feeding, only 30–40% of the spirochaetes contain this protein. The prevalence of OspA-positive spirochaetes in the tick's midgut increases to pre-feeding levels after the ticks have completed their blood meal and have detached from their host [34]. Hence the down-regulation of OspA by spirochaetes is closely associated with their transmission during tick feeding. OspA-positive and OspA-negative spirochaetes can be found in tick salivary glands approx. 2 days after the initiation of tick feeding [35], but only OspA-negative spirochaetes are likely to persist in mammals once infection is established.

The high prevalence of OspA-positive spirochaetes in the midgut of unfed ticks, and the down-regulation of this protein by spirochaetes during tick feeding, stimulated the hypothesis

that OspA is a tick midgut adhesin [34]. The synthesis of this protein with adhesive properties as the spirochaetes are acquired by ticks would prevent the spirochaetes from being eliminated in tick faeces, and from being disseminated from the midgut to other tick tissues when transmission is not possible. Two recent studies provide support for this hypothesis: purified recombinant OspA of *B. burgdorferi* binds to extracts of tick midgut cells from *I. scapularis* [36], and adherence by OspA-positive spirochaetes to the midgut epithelium in ticks is greatly reduced when non-borreliacidal, anti-OspA antibody is present in the tick's blood meal [37]. Intuitively, the primary role of OspA to bind spirochaetes to the tick midgut is appealing, but conclusive evidence to support this hypothesis is still lacking.

Spirochaetes in feeding ticks down-regulate OspA and up-regulate OspC [30,33,34,38]. Spirochaetes lack OspC in unfed ticks; however, 75% of spirochaetes in the tick midgut become OspC-positive after only 48 h of tick attachment to a mammalian host, and then cease making the protein rapidly after the cessation of tick feeding [34]. Variability in the pattern of OspC and OspA synthesis has been reported for different isolates of spirochaetes [39,40] and a variety of OspA and OspC phenotypes can exist after infected ticks have fed [35]. Yet most investigations have shown that spirochaetes in ticks up-regulate OspC when ticks feed. This suggests a function for OspC associated with spirochaete transmission from ticks to vertebrates that includes any or all of the following: (i) dissemination from the tick midgut, (ii) immunoevasion while traversing the tick haemolymph, (iii) penetration of the salivary glands, or (iv) the initial colonization of the vertebrate host after delivery via saliva into the feeding lesion into the host's dermis.

The migration of *B. burgdorferi* from the tick midgut to salivary glands is inhibited when infected ticks feed on OspC-immunized mice [41]. This observation could support a role for OspC to assist spirochaetes in penetration of the midgut wall for dissemination, or the inhibition may simply result from OspC-positive spirochaetes being killed in the midgut lumen by the immune serum before their escape, mediated by other proteins. However, OspC-negative spirochaetes have been detected in salivary glands of recently fed ticks, and in mouse skin associated with the mouthparts of ticks pulled from their hosts [35]. The up-regulation of OspC during tick feeding may also facilitate spirochaete dispersal in ticks by being a receptor for host proteases ingested in the blood meal [38]. Spirochaetes exposed to tick haemolymph *in vitro* also up-regulate OspC [42], which could protect *B. burgdorferi* from antibacterial defences in the tick haemocoel, or prepare the bacteria for entry into the salivary glands. I believe that spirochaetes probably require OspC for initial infection in mammals, but definitive proof for this hypothesis does not yet exist. Spirochaetes in the midgut of unfed ticks that lack OspC are not infectious when inoculated into mice, whereas spirochaetes expressing OspC in recently fed ticks are infectious [43]. The relapsing fever spirochaete, *Borrelia hermsii*, persists in the salivary glands of its tick vector while expressing a protein homologous with OspC, Vsp33, that is down-regulated after transmission to mammals [44]. The phenotypic changes of *B. burgdorferi* and *B. hermsii* in their respective tick vectors points strongly to a shared function of OspC and Vsp33 that is related to the early colonization of the vertebrate host [45].

## Concluding remarks

The reciprocal synthesis of OspA and OspC by Lyme-disease spirochaetes during tick feeding are only two of an expanding list of differentially expressed proteins [46]. Several environmental stimuli affect their regulation, including temperature [30], pH [47], cell density [48], cultivation with tick cells [49] and exposure to tick haemolymph [42]. Several of these stimuli may work in concert to accentuate Osp production or suppression [50]. The biological roles for these surface-exposed lipoproteins in the infection and transmission cycle of these pathogenic spirochaetes are, as yet, undetermined, but are of keen interest. The suggested roles of OspA and OspC as a tick midgut adhesin and an early vertebrate colonization factor respectively, will require additional work with infectious mutants lacking these proteins for use in transmission experiments to test whether these hypotheses are correct.

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