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Characterization of head stress proteins in *Borrelia burgdorferi*

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Saxena, Amita, M.A.

San Jose State University, 1989

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**CHARACTERIZATION OF HEAT STRESS PROTEINS
IN *BORRELIA BURGDORFERI***

A Thesis
Presented to
The Faculty of the Department of Biological Sciences
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

By
Amita Saxena
December, 1989

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

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ABSTRACT

CHARACTERIZATION OF HEAT STRESS PROTEINS IN *BORRELIA BURGDORFERI*

by Amita Saxena

We have previously shown that under heat stress *Borrelia burgdorferi* preferentially synthesizes five heat stress proteins (HSPs) HSP 1-5. In the present study this response was further characterized by labeling *B. burgdorferi* proteins with [³⁵S] methionine and analyzing the protein profiles using SDS-polyacrylamide gel electrophoresis and fluorography. In this study we also describe HSP 6 (21.5 kDa) which shows a ten fold increase after shift from 28°C to 40°C. Synthesis of heat stress proteins after a shift from 28°C to 40°C began within 15 minutes and reached maximum levels between 2 and 6 hours. When cells were disrupted using a French press cell and centrifuged at 100,000 × g to obtain membrane and soluble fractions, three of the heat stress proteins (HSP 1, HSP 2, and HSP 3) were found primarily in the soluble fraction. Antigenic cross-reactivity between HSP 1, 75 kDa, and a 31 kDa protein which is not thermoregulated, was demonstrated using Western blots.

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Dr. Boothby's moral support and guidance was also very valuable to me, he helped me not only in my research and in completing this thesis but also advised me how to confidently teach my own laboratory sections in microbiology.

Without Drs. Cluss and Boothby's suggestions, encouragement and most of all belief in me, I could not have become "The Mistress of the Darkfield".

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I will always value the knowledge given to me by all my teachers I have ever taken classes under.

I would also like to thank the faculty staff and my friends in this department who have helped me more than they know. In the future whenever I think of San Jose State University, a smile will come to my face.

DEDICATION

I would like to dedicate my research to my family members who have not only supported me morally but also financially through out my education. I will always be indebted to them.

My ideal researcher and scientist has been my father Dr. K. C. Saxena and the ideal person and best friend in life has been my mother Mrs. Vimla Saxena, I pray to God I continue to learn from them and follow in their footsteps.

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INTRODUCTION

Lyme disease, a complex multisystem disorder caused by the tick-borne spirochete *Borrelia burgdorferi* has been reported in almost every European nation and in 31 states in this country. Both the incidence and the geographic distribution of Lyme disease is increasing with 4,572 new cases reported in 1988 (5, 6, 23). From 1987 to 1988, 94% of the cases reported were from nine states: New York, New Jersey, Wisconsin, Connecticut, Pennsylvania, Rhode Island, California, Massachusetts, and Minnesota and 80% of the cases were from the upper northeast (6). Primary symptoms of Lyme disease may include local skin lesions followed by more systemic secondary and tertiary symptoms which include, lymphocytic meningoradiculitis, or Bannwarth's syndrome, arthritis, myocarditis, and acrodermatitis chronica atrophicans (1, 2, 22).

Borrelia burgdorferi has a biphasic life cycle. Adaptation of the spirochete to either its arthropod vector, the *Ixodes* tick, or its homeothermic host probably involves morphological and/or metabolic changes. One major difference between the two environments, is temperature. As the spirochete is transferred from ambient temperatures found in its poikilothermic arthropod vector to higher temperatures found in homeothermic hosts, a heat stress response occurs (7). This response probably plays a role in the adaptation of the spirochete to life in the mammalian host and may play a role in the spirochete's ability to cause disease.

A variety of cell types respond to thermal stress and other trauma by activating a set of genes that code for heat shock proteins (hsps) (17). Heat shock genes and their regulatory sequences have been highly conserved which suggests that they carry out essential functions (11). The major hsps of most organisms fall into size classes of approximately 80-90, 68-75, and

15-30 kDa (10, 17). The best characterized and most prominent of these classes is the hsp 70 family (68-75 kDa) (11). These proteins play a role in protein assembly and disassembly involving hsp 70 ATPase activity (21).

One study reported significant structural similarity between the hsps of some mycobacterial pathogens and hsps produced by host macrophages, implying that the pathogen's stress proteins may not be recognized as foreign by the host's immune system (14). Alternatively, pathogen stress protein synthesis could be boosted in the host environment implying that hsps may serve as major antigens leading to an autoimmune reaction (14). *Borrelia burgdorferi* may undergo similar changes *in vivo* as a result of the temperature shift, leading to autoimmune reactions such as arthritis seen in the third stage of Lyme disease. In this study we characterized *in vitro* protein synthesis by evaluating the kinetics of the heat stress response, and the localization and antigenic characterization of heat stress proteins in *B. burgdorferi*.

MATERIALS AND METHODS

***Borrelia burgdorferi* strains and culture conditions.** *Borrelia burgdorferi* B31 (type strain, ATCC 35210) was provided by Dr. Robert Lane, Department of Entomological Sciences, University of California, Berkeley, CA. Three low passage, pathogenic strains of *B. burgdorferi* (B31, HB19, and N40) were kindly supplied by Dr. Steven Barthold, Section of Comparative Medicine, Yale University, New Haven, CT. Strains B31 and HB19 were isolated from patients with signs of Lyme disease, N40 was isolated from the mid gut of an *Ixodes dammini* tick (3). The cultures were maintained in modified Barbour-Stoenner-Kelly (BSK II) medium as described by Barbour, et. al. (4) except that bovine serum albumin (Fraction V, Sigma Chemical Company, St. Louis MO) was reduced to 1% (w/v) in localization studies.

Cultures of *B. burgdorferi* B31 (type strain) for analysis of heat stress responses and for SDS-PAGE were grown at 28°C to cell densities of 8.5×10^7 or 1.4×10^8 cells per ml, harvested by centrifugation at $17,000 \times g$ for 20 minutes at 6°C, and immediately resuspended to a concentration of 6×10^8 or 1×10^9 cells per ml in BSK II medium containing [^{35}S] methionine (ICN, Irvine, CA) at 100 - 200 μCi per ml. Aliquots of the resuspended spirochetes were incubated at 28° - 42°C for fifteen minutes to 6 hours. These labeled cells were then harvested by centrifugation at $17,000 \times g$ for 20 minutes at 6°C and washed three times in phosphate buffered saline (PBS) before temperature shift studies.

For preparation of whole spirochete and HSP 1 antisera, the type strain was grown at 35°C to a concentration of $1-2 \times 10^8$ cells per ml, and then shifted to 40°C for 18 additional hours. Cells were then harvested and washed as before.

The temporal synthesis and maintenance of the HSPs: *Borrelia burgdorferi*

cells were prepared as above, resuspended in BSK II medium, and divided into three batches. One batch was shifted to 40°C in the presence of [³⁵S] methionine as described. Labeled cells were removed after 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours. A second batch of cells which served as a control, was labeled with [³⁵S] methionine at 28°C for either 15 minutes or 2 hours. In all cases, labeling was terminated by chilling the cells on ice. *Borrelia burgdorferi* was harvested and prepared for SDS-PAGE and fluorography as described above.

HSP synthesis during temperature stress at 40°C and shift to 28°C. To determine patterns in protein synthesis during shifts from 40°C to 28°C, the spirochetes were shifted to 40°C for an additional 2 hours and then harvested, resuspended in BSK II medium containing [³⁵S] methionine as described above. Cells were then split into two batches. One batch was labeled at 28°C and a portion of cells was removed at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours. A second batch of cells was labeled at 40°C, and samples were removed after 15 minutes and 2 hours. Samples were treated for SDS-PAGE and fluorography as described above.

Fractionation of *B. burgdorferi* B31 (type strain). Spirochetes for fractionation studies were propagated in BSK II medium at 31°C for 5 days to a density of 1-2 X 10⁸ cells per ml. The culture was then divided into two equal portions and incubated further at 31°C and 40°C for an additional 24 hours, before fractionation. To obtain cell lysates washed cells were resuspended in deionized water and disrupted at 15,000 p.s.i. by two passages through a French pressure cell (American Aminco Inst. Co. Inc., Silver Spring, MD) using a Carver Laboratory Press™ (Fred's Carver Inc., Menomonee Falls, WI). The cell lysate was centrifuged at 100,000 x g for two hours. The pellet, designated the crude membrane fraction, was washed in

water. This first supernatant was recentrifuged and the resulting pellet was designated the enriched membrane fraction. The second supernatant contained the enriched soluble fraction.

Preparation of samples for PAGE. All samples were resuspended in 50 μ l-100 μ l of Tris HCl [trihydroxymethyl aminomethane], 0.5M, pH 6.8 [Bio-Rad Laboratories, Richmond, CA]. The protein concentration of each of the samples was determined by the method of Lowry (18), and the samples were stored at -80°C. For SDS-PAGE, the samples were thawed, suspended in sample buffer with 2 mercaptoethanol (5% w/v), and heated for 5 minutes at 95°C.

Analysis of *B. burgdorferi* protein synthesis under various conditions by SDS-PAGE, fluorography, and scanning laser densitometry. SDS-PAGE was performed by the method of Laemmli (15). All reagents were electrophoresis grade and purchased from Bio-Rad Laboratories (Richmond, CA). Molecular weight standards from Bio-Rad included the following; phosphorylase B (97,400), bovine serum albumin (66,200), ovalbumin (42,699), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400). Molecular weight standards from Sigma (St. Louis) included the following; carbonic anhydrase (29,000), egg albumin (45,000), bovine plasma albumin (66,000), phosphorylase B (97,400), β -galactosidase (116,000), and myosin (205,000). Approximately 80-300 μ g of protein was applied on a discontinuous 1.5 mm acrylamide gel containing a 5% stacking and 10% running gel. Electrophoretic separation was done at 12 mAmps overnight under reducing conditions. After electrophoresis the gels were stained with 0.01% Coomassie brilliant blue R-250 and destained in 50% methanol, 10% acetic acid. Gels labeled with [³⁵S]methionine were enhanced for fluorography (EnHance, New England Nuclear, Boston, MA),

and exposed to photographic film (Kodak XAR-5, Rochester, NY) at -80°C. Fluorographed films were developed and quantified by means of a scanning laser densitometer (LKB UltroScan XL, Bromma, Sweden).

Preparation of whole spirochete and HSP 1 specific antisera. Whole *B. burgdorferi* (type strain) cells were prepared as described above. HSP 1 was partially purified by excising sections from preparative SDS-PAGE gels corresponding to a molecular weight of approximately 75 kDa (containing HSP 1) and electroeluting at 100 V overnight in a ElutrapTM electro-separation chamber (Schleicher & Schuell, Inc., Keene, NH) with 2 ml of elution buffer. The final protein concentration of whole spirochete and HSP 1 preparations were determined by using the method of Lowry (18). After obtaining preimmune serum samples rabbits were inoculated with 0.25 mg of antigen (whole *B. burgdorferi* or HSP 1) in Freund's complete adjuvant by intramuscular injection in each hind leg followed three weeks later by similar subcutaneous injections in each prescapular region. Four weeks later each rabbit was injected with 0.10 mg of antigen by intravenous injection in the marginal ear vein. Immune serum was obtained from blood collected one week later from the central artery into a vacutainer serum separator tube (AutoSep, Teruma Medical Elkton, MD) and frozen until used.

Immunoblots. Transfer of *B. burgdorferi* B31(type strain), low passage B31, HB19, and N40 proteins from SDS-PAGE gels to nitrocellulose membranes (0.45µ, Schleicher and Schuell, Keene, NH) was done according to the procedure described by Towbin et al. (24). Transfer was carried out in a Transblot cell (Hoefer Scientific Instruments, San Francisco, CA.) for 20 hours at 100 mAmps constant current. The transfer buffer consisted of 192 mM glycine, 25 mM Tris base, and 20% methanol. After transfer, the

nitrocellulose membranes were cut and placed in screw cap test tubes containing blocking solution 2% bovine serum albumin (BSA), Fraction V (Sigma Chemical Company, St. Louis, MO) in tris-buffered saline (TBS) (50 mM Tris, 150 mM NaCl, pH 7.4) overnight at 4°C. Subsequent incubation steps were carried out at room temperature with gentle agitation and washing steps (10 minutes each) included 0.1% Triton X100, 0.5% Triton X100, 0.5 M NaCl, TBS, and 0.1% BSA all solutions were diluted in TBS 0.1% BSA. Strips were incubated with preimmune or immune serum (diluted 1:250 in the blocking solution) for 1 hour, followed by several washes as described above. Next the strips were incubated with alkaline phosphatase-conjugated goat anti-rabbit serum (Sigma Chemical Company, St. Louis, MO) (diluted 1:1000 in antibody diluent) for 1 hour, washed as before, and treated with the enzyme substrate [5-bromo-4-chloro-3-indoylphosphate (BCIP) toluidine salt (Sigma Chemical Company, St. Louis, MO) {5% w/v in 100% dimethylformamide stock (Fisher, Pittsburgh, PA)}], 0.33%, nitroblue tetrazolium (NBT) (Sigma Chemical Company, St. Louis, MO) [5% w/v in 70% dimethylformamide stock] 0.66%, in alkaline phosphatase substrate buffer [100mM Tris pH 9.5, 100 mM NaCl, and 5 mM MgCl_2], and stopped with 20 mM EDTA in PBS.

RESULTS

Heat stress response in *B. burgdorferi*. The SDS-PAGE-derived fluorogram of newly synthesized proteins from *B. burgdorferi* grown at 28°C - 43°C indicated that the production of at least four proteins, HSP 1 (75 kDa), HSP 3 (39 kDa), HSP 4 (27 kDa), and HSP 6 (21.5 kDa), designated as heat stress proteins (HSPs) was affected by temperature (Fig. 2). At 40°C, HSP 1 comprised 12.8% of the labeled protein detected by scanning laser densitometry compared to 1.6% at 28°C, an eight fold increase (Fig. 3). Another major heat stress protein, HSP 6, increased ten fold at 40°C compared to 28°C (Fig. 3). However, the syntheses of other *B. burgdorferi* proteins, notably OSP A, OSP B, flagellin, and the 60 kDa Common Antigen (CA) were not thermoregulated. At 43°C, *B. burgdorferi* protein synthesis was negligible (Fig. 1).

The temporal synthesis and maintenance of the HSPs: Fluorograms of *B. burgdorferi* SDS-PAGE profiles showed that HSP 1, 1.4% of the labeled protein at 28°C, increased 4.5 times within 15 minutes after a shift to 40°C, and at two hours reached a maximum of 15.2% of the labeled cell protein; this was 11 times greater than the level at 28°C (Fig. 3). The HSP 1 content then decreased to 10.9% after 4 hours and to 8.4% after 6 hours of incubation at 40°C (Figs. 3 & 4). HSP 3 and HSP 4 reached their respective maximum concentrations at 1 hour and were relatively constant to 6 hours. HSP 6 increased in concentration throughout the labeling period peaking at 6.3 % of the labeled protein at 6 hours (Figs. 3 & 4). The outer surface proteins and CA served as controls during the various labeling periods and were stable at 1.5% and 4%, respectively, at different times.

HSP synthesis during temperature stress at 40°C and shift to 28°C. After 2

hours and 15 minutes at 40°C, HSP 1 represented 13.1% of the labeled protein. After periods of 15 minutes and 6 hours at 28°C, the HSP 1 content decreased to 11.8% and 2.7%, respectively, an approximately five fold decrease in 6 hours (Figs. 5 & 6). HSPs 3 and 4 returned to their normal intracellular concentrations within the first hour at the lower temperature. HSP 6 was stabilized within the first fifteen minutes at 28°C.

Localization of heat stress proteins using whole spirochetes. The electrophoretic pattern of various *B. burgdorferi* fractions obtained by lysing cells in a French press resemble those described previously (17, 18). Of note are the 31 and 34 (OSP A and OSP B), 41 (flagellin), and 66 kDa proteins that fractionate in the pellet (crude membrane fraction). HSP 1 represented 2.0 to 4.6% of the total protein in the supernatant (enriched soluble fraction) vs 0.0 to 0.7% in the crude membrane fraction (Fig. 7). HSP 2 accounted for 4.9 to 3.5% of the enriched soluble fraction protein and 0.0 to 1.2% in the crude membrane fraction. Similarly, HSP 3 was enriched in the soluble fraction (7.5 to 6.3% vs 0.0 to 0.7%). HSP 4 fractionated in both the membrane and soluble fractions (3.3 to 2.0% and 1.7 to 1.4%, respectively) (Fig. 8). It was also seen that the fractions obtained by this fractionation scheme were not completely clean as the outer surface proteins OSP B and OSP A were present in both fractions.

Immunoblots. Preimmune serum from the rabbit injected with HSP 1 (75 kDa) reacted with a protein in the 60 kDa range (Fig. 9, Lane 4). As expected, immune serum from the same rabbit reacted nonspecifically with a protein in the 60 kDa range and specifically with the 75 kDa protein (HSP 1); however, a 31 kDa protein from the type strain and the pathogenic strains also reacted (Fig. 9). Furthermore, this lower molecular weight protein reacted more strongly than the higher molecular weight protein (Fig. 9).

Immunoblots from lanes 1 to 6 were obtained from the same gel. Lanes 1 to 5 contained whole cell protein from the B31 type strain, and lane 6 contained whole cell protein of the low passage, pathogenic strain B31. Immunoblots in lanes 7 and 8 were obtained from a separate gel which showed slightly lower mobilities for the 75 kDa, 60 kDa and 31 kDa proteins. Lane 7 contained whole cell proteins from the pathogenic strain N40 and lane 8 contained whole cell proteins from the pathogenic strain HB19. The reactivity between the 75 kDa protein and the 31 kDa protein is common among each strain tested.

DISCUSSION

During thermal stress in *B. burgdorferi* normal protein synthesis is inhibited and synthesis of stress proteins is induced, similar to responses in a number of other organisms. Stress proteins are abundant and well characterized; however, their functions remain poorly described (11). Analysis of the heat stress response in *B. burgdorferi* is of interest because of the potential role of HSPs in the adaptation of the spirochete to the mammalian host. The major heat stress protein, HSP 1 (75 kDa) increased from 1.6% of the total cell protein at 28°C to 12% at 40°C, an eight fold increase. HSP 6 (21.5 kDa) increased ten fold. Other *B. burgdorferi* HSPs, HSP 2 (42 kDa) and HSP 5 (36 kDa) reported elsewhere were not strongly thermoregulated in this study (7). The 60 kDa antigen of *B. burgdorferi* cross reacts with the *E. coli* 60 kDa CA heat shock protein known as GroEL protein (13). Synthesis of this protein was not thermoregulated in *B. burgdorferi*. The apparent molecular weights of *B. burgdorferi* HSPs are typical of stress proteins found in many organisms (7).

Experiments with a number of organisms showed a correlation between induction of HSP synthesis and resistance of cells to a severe heat shock. Cells adapted to an initial, mild non-lethal heat treatment before shifts to lethal temperatures survive better (11). In our experiments with *B. burgdorferi*, no increase in thermotolerance was generated with the various thermal pretreatments (data not shown). Further experiments on thermotolerance need to be carried out to confirm these results.

The accumulation, rate of synthesis, and stability of HSPs are important when considering the function of these proteins. In response to heat stress, HSP 1 accumulates rapidly and reaches its maximum within 2 hours after shift to a high temperature. Thereafter, HSP 1 levels decreased relative to total cell protein, although they remained substantial even after 6 hours.

Further incubation at high temperature will be necessary to determine the maintenance level of HSP 1 in *B. burgdorferi* under heat stress conditions.

During recovery from heat stress, HSP 1 levels decreased with time after low temperature shift, and by 6 hours were close to pre-stress amounts. Some of the smaller HSPs returned to pre-stress levels quite rapidly, within 15 minutes of recovery.

The stability of proteins in the hsp 70 family studied in different organisms varies extensively. In *Drosophila* larvae and adults hsp 70 peaks within 1 hour and decreases significantly within 4 hours of recovery. After 12 hours, hsp 70 returns to pre-shock levels, indicating a half-life of 1 to 2 hrs (11). The first part of this eukaryotic response compares closely to the regulation of HSP 1 in *B. burgdorferi*. In rats, hsp 70 accumulates rapidly but the decline in concentration to the basal levels varied between 4 days in the brain and 8 to 16 days in other organs. In rats hsp 70 is stable, with a half-life of greater than 10 hrs (11).

The fractions obtained from *B. burgdorferi* indicate that three of the four HSPs are associated with the soluble fraction (HSP 1, HSP 2, and HSP 3), while HSP 4 was found mostly in the membrane fraction. However, the soluble fractions were contaminated with significant amounts of the outer surface proteins OSP A and OSP B. Other attempts to localize proteins of *B. burgdorferi* have also resulted in outer surface protein contamination of the fraction containing the protoplasmic cylinder (20). Some eukaryotic hsps have been localized to the nucleus. This has prompted the speculation that these proteins may be involved in transcriptional regulation (11).

Immunoblots of *B. burgdorferi* using preimmune rabbit serum showed reactivity to a protein in the 60 kDa range. Hansen et al. (13) have shown that a 60 kDa protein in *B. burgdorferi* is a common antigen (CA) that is cross reactive with an equivalent antigen from approximately 20 distinct bacteria. Since this antigen is homologous and commonplace, exposure to a

number of bacteria containing CA explains the presence of its reactivity in preimmune serum.

Antiserum directed against HSP 1 reacted not only to the 75 kDa protein but also to a 31 kDa protein which is not thermoregulated. This antiserum also reacted with greater intensity to the 31 kDa band than to the 75 kDa protein. Two possible explanations are that these proteins are antigenically related or that the 31 kDa protein is a subunit of HSP 1. The production of monospecific antibody to HSP 1 and its reactivity with the 31 kDa protein would clearly establish the relatedness of these 2 proteins. It has been shown in *Escherichia coli* that the DnaK (70 kDa) protein is a heat shock protein which under normal temperature conditions serves as the prominent σ factor regulating transcription. Upon heat shock in *E. coli*, the synthesis of another transcriptional activator, σ 32, is enhanced and is responsible for increased transcription at heat shock promoters (12). Landic et al. have also shown that the amino acid sequence of these two sigma subunits are homologous (16). If HSP 1 and the 31 kDa protein of *B. burgdorferi* are homologous with the *E. coli* σ factors then a similar model of transcription activation could be proposed for *B. burgdorferi*.

LITERATURE CITED

1. **Asbrink, E., B. Hederstedt, and A. Hovmark.** 1984. The spirochetal etiology of erythema chronicum migrans Afzelius. *Acta Derm Venereol.* 64:291-295.
2. **Asbrink, E., A. Hovmark, and B. Hederstedt.** 1984. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. *Acta Derm Venereol (Stockh).* 64:506-511.
3. **Barthold, S. W., K. D. Moody, G. A. Terwillger, P.H. Duray, R. O. Jacoby, and A. C. Steere.** 1988. Experimental Lyme arthritis in rats infected with *Borrelia burgdorferi*. *J. Infect. Dis.* 157:842-846.
4. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.* 57:512-525.
5. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis,** 1982. Lyme disease - a tick borne spirochetosis? *Science.* 216:1317-1319.
6. **Centers for Disease Control.** October 6, 1989. Current Trends. Lyme Disease - United States, 1987 and 1988. *Morbidity And Mortality Weekly Report.* 38:668-672.
7. **Cluss, R. G., and J. T. Boothby.** 1990. Thermoregulation of protein synthesis in *Borrelia burgdorferi*. *Infection and Immunity.* In Press.
8. **Cohen, R. S. and M. Meselson.** 1988. Periodic interactions of heat shock transcriptional elements. *Nature.* 332:856-858.
9. **Coleman, J. L., J. L. Benach, G. Beck, and G. S. Habicht.** 1986. Isolation of the outer envelope from *Borrelia burgdorferi*. *Zbl. Bakt. Hyg.* 263:123-126.
10. **Craig, E. A.** 1985. The heat shock response. *Crit. Rev. Biochem.* 18:239-280.
11. **Craig, E. A., T. Ingolia, M. Slater, L. Manseau, and J. Bardwell.** 1982. *Drosophila*, Yeast, and *Escherichia coli* genes related to the *Drosophila* heat-shock genes. In: *Heat Shock from Bacteria to Man* (Schlesinger, M. J., Ashburner, M. and Tissieres, A., eds), pp. 11-18, Cold Spring Harbour, New York.
12. **Grossman, A. D., J. W. Erikson and C. A. Gross.** 1984. The htpR gene product of *E. coli* is a sigma factor for heat-shock promoters. *Cell.* 38:175-182.
13. **Hansen K., J. M. Bangsberg, H. Fjordvang, N. S. Pedersen, and P. Hindersson** 1988. Immunochemical characterization of and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60-kilodalton antigen common to a wide range of bacteria. *Infect. Immun.* 56:2047-2053.
14. **Koga, T., A. Wand-Wurttenberger, J. DeBruyn, M. E. Munk, B. Schoel, S. H. E. Kaufmann.** 1989. T. cells against a bacterial heat shock protein recognize stressed macrophages. *Science.* 245:1112-115.
15. **Laemmli, U. K., and Favre M.** 1973. Maturation of the head of the

- bacteriophage T4. J. Mol. Biol. 80:575-580.
16. Landic, R., V. Vaughn, E. T. Lau, R. A. VanBogelen, Erikson J. W., and F. C. Neidhardt. 1984. Nucleotide sequence of the heat shock regulatory gene of *E. coli* suggests its protein product may be a transcriptional factor. Cell. 38:175-182.
 17. Lindquist, S. 1986. The Heat-Shock Response. Ann. Rev. Biochem. 55:1151-91.
 18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.
 19. Luft, B. J., W. Jiang, P. Munoz, R. J. Dattwyler, and P. D. Gorevic. 1989. Biochemical and immunological characterization of the surface proteins of *Borrelia burgdorferi*. Infect. and Immun. 57:3637-3645.
 20. Magnarelli, L. A., J. F. Anderson, and A. G. Barbour. 1989. Enzyme-linked immunosorbent assays for Lyme disease: reactivity of subunits of *Borrelia burgdorferi*. J. Infect. Dis. 159:43-49.
 21. Pelham, H. 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell. 46:959-961.
 22. Pfister, H. W., K. M. Einhaupl, P. Franz, and C. Garner. 1988. Corticosteroids for radicular pain in Bannwarth's syndrome: A double-blind, randomized, placebo-controlled trial. p. 485-487. In J. L. Benach and E. M. Bosler (ed.), Lyme Disease and Related Disorders, vol. 539. Annals of the NY Academy of Sciences.
 23. Steere, A. C., R. L. Grodzicki, A. N. Kornblat, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista. 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. 308:733-740.
 24. Towbin H. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Proc. Natl. Acad. Sci., USA. 76:4350-4355.

Fig. 1. Fluorogram of newly synthesized proteins from *B. burgdorferi* grown at 28°C prior to 2 hour labeling periods at 28°C, 31°C, 34°C, 37°C, 40°C and 43°C. Approximately 100 µg of protein was loaded per lane and the film was exposed to the gel for 36 hours. The synthesis of at least four major proteins we designate as heat stress proteins (HSPs) was increased by shifts to higher temperatures; HSP 1 (75 kDa), HSP 3 (39 kDa), HSP 4 (27 kDa), and HSP 6 (21.5 kDa). Other proteins are as indicated; Common Antigen (CA) (60 kDa), FLAGELLIN (41 kDa), and outer surface proteins A and B, OSP A (31 kDa) and OSP B (34 kDa). Numbers on the left indicate molecular weights (in kilodaltons).

Fig. 2. Histograms of the various HSPs as percent of total protein at indicated temperatures as determined by scanning laser densitometry.

Fig. 3. Fluorogram of *B. burgdorferi* proteins synthesized at 28°C and labeled at 40°C for 15, 30, 60, 120, 240, or 360 min. Lanes 1 and 2 represent control cells left at 28°C and labeled for 15 and 120 min. Approximately 60 µg of protein was loaded per lane and the film was exposed to the gel for 7 days. HSPs are indicated on the right and molecular weight markers (in kilodaltons) on the left.

Fig. 4. Synthesis of the various *B. burgdorferi* proteins as percent total protein at 40°C at 0.25, 0.5, 1, 2, 4, and 6 hours.

Fig. 5. Fluorogram of proteins synthesized from *B. burgdorferi* grown at 28°C stressed for 2 hours at 40°C, and then labeled at 28°C for 15, 30, 60, 120, 240, or 360 min. Lanes 1 and 2 represent control cells left at 40°C and labeled for 15 and 120 min. Approximately 70 µg of protein was loaded per lane and the film was exposed to the gel for 9 days. HSPs are indicated on the right and molecular weight markers (in kilodaltons) on the left.

Fig. 6. Synthesis of the various *B. burgdorferi* proteins at 28°C after heat stress at 40°C at 0.25, 0.5, 1, 2, 4, and 6 hours.

Fig. 7. Coomassie-stained gel of *B. burgdorferi* proteins in whole cell lysates and cell fractions at 28°C (lanes 1, 2, 3, and 7) and 40°C (lanes 4, 5, 6, and 8). Lanes 1 and 4, whole cell lysates; lanes 2 and 5, crude membrane fractions; and lanes 3 and 6, are enriched soluble fractions. Each lane contains 150 µg of protein. Lanes 7 and 8, are enriched membrane fractions and contain less than 50 µg protein. Spirochetes were cultured and fractionated as given in the materials and methods. Osp A (31 kDa) and Osp B (34 kDa) are outer surface proteins, Flagellin (41 kDa) is associated with the inner membrane, and a 70 kDa protein is associated with the soluble fraction. Molecular weight standards (in kilodaltons) are indicated on the right.

Fig. 8. Histogram of various *B. burgdorferi* proteins as determined by scanning laser densitometry of Coomassie-stained SDS-PAGE prepared from whole spirochetes, crude. Cells were grown at 28°C and shifted to 40°C for 18 hours.

Fig. 9. Immunoblots of *Borrelia burgdorferi* (type strain, B31) and the low passage, pathogenic strains (B31, HB19, & N40) reacted with anti-whole *B. burgdorferi* or anti-HSP 1 (75 kDa). Lane 1 molecular weight standards, lanes 2 & 3 preimmune and immune sera respectively from immunized with whole *B. burgdorferi* cells, lanes 4 & 5 preimmune and immune sera respectively from a rabbit immunized with gel-purified HSP 1, lane 6 whole *B. burgdorferi* (low passage, pathogenic, strain B31) reacted with anti-HSP 1, lane 7 whole *B. burgdorferi* (N40) reacted with anti-HSP 1, and lane 8 whole *B. burgdorferi* (HB19) reacted with anti-HSP 1.