Supporting information for Revel *et al*. (2002) *Proc. Natl. Acad. Sci. USA* **99** (3), 1562–1567. (10.1073/pnas.032667699)

Materials and Methods

SDS/PAGE and Immunoblotting. SDS/PAGE and immunoblotting were carried out as described (1); for Coomassie blue staining, 2.5×10^7 B31 were loaded per gel lane, whereas 5.0×10^7 B31 per lane were used for immunoblotting. Rat polyclonal antiserum for OspC (2) and monoclonal antibody 8H3-33 for FlaB (2) have been described. A glutathione *S*-transferase fusion protein (3) was used to prepare rat polyclonal antiserum against OspA (4).

Microarray Construction. ORF-specific primer pairs were purchased from Sigma-Genosys and used to PCR-amplify in 96-well plates 1,754 (95.4%) of the 1,838 predicted ORFs from *Borrelia burgdorferi* B31 DNA according to a general protocol provided by the manufacturer. Parameters for PCR amplification were as follows: 95°C for 3 min; 30 cycles of 95°C for 30 s; 50°C for 30 s; 72°C for 2.5 min; and a final extension cycle of 72°C for 10 min. PCR products >150 bp were observed via 1.5% agarose gel electrophoresis. PCR fragments <150 bp were subjected to 10% polyacrylamide gel electrophoresis. Fragments were stained with ethidium bromide and imaged digitally by using a Kodak Image Station. Amplification was scored on a visual scale of 1+ (low) to 4+ (high); amplification was considered acceptable if a single PCR product within 10% of the predicted ORF size was 3+ or better (5). Unsuccessful reactions were characterized as either (*i*) 0 or 1+, (*ii*) DNA smears, (*iii*) multiple bands, or (*iv*) single bands of unexpected size. Primers from unsuccessful PCR reactions were rearrayed into new 96-well plates and reamplified with altered $MgCl_2$ concentration and annealing temperatures to favor amplification of the desired single products. All PCR products were precipitated with cold isopropanol, dried, dissolved in 7% DMSO, and rearrayed into 384-well plates for glass-slide printing. The PCR products then were deposited in duplicate on poly-L-lysine-coated glass slides utilizing a MagnaPrinter designed and manufactured by University of Texas Southwestern Medical Center (microarraycore.swmed.edu). Negative control samples (n = 720) containing vehicle buffer only (7% DMSO) also were deposited on the array for determination of threshold values for both channels. Post-processing of the slides was performed as reported (6). Briefly, to ensure uniformity of DNA features, the slides spotted with DNA were rehydrated by suspension over boiling water for 1 min and then flash-dried on a heating block (100°C). UV crosslinking was performed by using a UV Stratalinker (Stratagene). Removal of unreacted lysine residues on the glass slides was accomplished by submersion in blocking solution (20 mM boric acid (pH 8.0)/55.2 mM succinic anhydride/90% 1-methyl-2-pyrrolidinone) for 30 min with rotation followed by submersion in distilled water (95°C) for 2 min. Slides then were dipped briefly in 95% ethanol and spun dry. Slides were stored, protected from dust and light, and kept at room temperature.

Twenty-six of the amplicons also were sequenced to validate their identities; 21 of these amplicons corresponded to genes from 10 paralogous families. For 8 of the 10 families, 2–3 genes were tested and for two families, one representative gene was sequenced. The remaining five amplicons corresponded to nonparalogous genes.

Fluorescent Probe Labeling and Hybridization. Ten µg of total *B. burgdorferi* RNA was used to generate Cy3-labeled first-strand cDNA via the SuperScript II reverse transcription kit (GIBCO); in lieu of standard hexamer random priming, 40 genome-directed primers (GDPs; ref. 7) designed for strain B31 (Table 1) were used to generate Cy3-labeled cDNA. Each GDP primer was supplied in the reaction to a final concentration of 250 ng/µl. The 40 GDPs were designed as octamers that anneal within the distal 40% of each predicted ORF. On average, each ORF was complementary to three GDPs. To guard against potential bias from spurious enzyme activity, each slide was subjected to hybridization with Cy3-labeled cDNA generated in separate reverse transcription reactions. After cDNA synthesis, the remaining RNA was hydrolyzed by the addition of a 1:10 volume of 10 N NaOH followed by heating at 65°C for 10 min. The solution then was brought to neutral pH by the addition of a 1:10 volume of 10 N HCl. For normalization and data analysis, 1 µg of Cy5-labeled genomic DNA was synthesized by nick translation (Promega; ref. 7). Microcon YM-30 cellulose centrifugal filters (Millipore) were used to remove unincorporated Cy dyes from both Cy5-labeled genomic DNA and Cy3-labeled cDNA. Fifty-microliter hybridization solutions were comprised of 20 ng/µl Cy5-labeled genomic DNA (1.0 µg), 200 ng/µl Cy3-labeled cDNA (10 µg), 0.7 µg/µl yeast tRNA, 0.4% SDS, and 4× SSC buffer (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7). The hybridization solution was heat-denatured for 2 min, allowed to cool to room temperature, and deposited onto the microarray slides. Slides were covered with cover slips (12-544G; Fisher Scientific) and placed into a hybridization chamber (ArrayIt). Hybridization with both probes was performed at 62.5°C with rotation overnight in a hybridization oven. The slides then were washed twice in buffer A (0.2% SDS + 0.1× SSC; 62.5°C), twice in buffer B (0.1× SSC; 62.5°C), and once in buffer B at 23°C and then spin-dried.

Data Acquisition and Analysis. Data acquisition was performed on a GenePix 4000B scanner using GENEPIX 3 software (Axon Instruments, Foster City, CA). The fluorescence intensity of each feature was measured, and spots with intensity values below threshold (2 SDs above the mean of the negative control features) in the Cy5 (normalizing) channel were omitted from further analysis. Log ratios (Cy3/Cy5) were compared between slides of the same condition, and only slides with a correlation (r) of >0.75 were included in subsequent analyses. To achieve statistical reliability, analyses were performed on a minimum of three slides per condition (8), yielding a possible six data points per condition. For *B. burgdorferi* cultivated *in vitro*, at least two independent cultures were represented in the data sets, whereas spirochetes cultivated in 20 DMCs were pooled to obtain sufficient RNA for microarray analysis. Each feature with Cy3/Cy5 ratios >3 SDs from the mean log ratios for each gene on replicate slides was considered an outlier and omitted. The resultant data set for each slide then was transformed to a mean log ratio of zero, thus allowing the calculation of average log ratios, SD, and uncertainty values for each gene within replicates. Expression changes with 95% confidence intervals greater than a conservative 1.8-fold threshold were designated as differentially expressed genes. The fold-change threshold was defined as twice the mean of all feature uncertainties (SD) above background, where the average uncertainty was calculated to be 0.4-fold, and background was defined as no change between conditions (i.e., a ratio of 1.0).

Hierarchial Cluster Analysis. Cluster analysis utilizing the CLUSTER and TREEVIEW programs (Stanford University; ref. 9) was performed for visual representation and profiling of data. A filter cut-off of 0.4 on log-transformed ratios resulted in 709 genes being selected for inclusion in average

linkage clustering analysis.

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