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Gene Expression Profile of *Campylobacter jejuni* in Response to Growth Temperature Variation

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The foodborne pathogen *Campylobacter jejuni* is the primary causative agent of gastroenteritis in humans. In the present study a whole genome microarray of *C. jejuni* was constructed and validated. These DNA microarrays were used to measure changes in transcription levels over time, as *C. jejuni* cells responded to a temperature increase from 37 to 42°C. Approximately 20% of the *C. jejuni* genes were significantly up- or downregulated over a 50-min period after the temperature increase. The global change in *C. jejuni* transcriptome was found to be essentially transient, with only a small subset of genes still differentially expressed after 50 min. A substantial number of genes with a downregulated coexpression pattern were found to encode for ribosomal proteins. This suggests a short growth arrest upon temperature stress, allowing the bacteria to reshuffle their energy toward survival and adaptation to the new growth temperature. Genes encoding chaperones, chaperonins, and heat shock proteins displayed the most dramatic and rapid upregulation immediately after the temperature change. Interestingly, genes encoding proteins involved in membrane structure modification were differentially expressed, either up- or downregulated, suggesting a different protein membrane makeup at the two different growth temperatures. Overall, these data provide new insights into the primary response of *C. jejuni* to surmount a sudden temperature upshift, allowing the bacterium to survive and adapt its transcriptome to a new steady state.

Since the foundation of the U.S. Foodborne Disease Active Surveillance Network in 1996, *Campylobacter* has been the most frequently diagnosed foodborne pathogen in human, followed by *Salmonella* and *Shigella* spp. (18, 35). *Campylobacter* infection is an acute diarrheal disease that ranges from a day of mild diarrhea to severe abdominal pain (30). Rarely, a *Campylobacter* infection results in the development of the Guillain-Barré syndrome, which is the primary cause of neuromuscular paralysis in the United States (30). Poultry is known to be the main food vehicle for *Campylobacter*, with up to 88% of broiler carcasses being contaminated with this microorganism (2, 26).

Campylobacter jejuni is able to grow at temperatures ranging from 30 to 47°C, with an optimal growth temperature of 42°C. Campylobacters are likely to encounter a wide range of temperatures during a contamination cycle and must therefore be able to sense, adapt, and respond to these temperature fluctuations. Temperature could constitute an important stimulus for *Campylobacter*; the organism may use temperature to sense that it has invaded the chicken reservoir (core temperature of 42°C) or the human host (core temperature of 37°C). Differential gene expression at these two temperatures may allow this organism to colonize its host efficiently, leading to commensalism or pathogenesis. With the complete genomic sequence of *C. jejuni* available (24), it is now possible to identify in silico the presence or absence of a particular gene. The search for chaperone homologues and heat shock proteins in the *C. jejuni* genome reveals up to 17 proteins, several of which have already been characterized, including GroEL, GroES, DnaJ, DnaK, GrpE, HrcA, and Lon (22). Although the function of chaperones and heat shock proteins might be significantly different than bacterial adaptation to host temperature, they should play a crucial role in the first line response. By surmounting a sudden temperature upshift, the bacteria should be able to survive and adapt to the new temperature. The importance of the heat shock response in intestinal tract colonization is supported by two observations: (i) the heat shock proteins GroEL and GroES are immunogenic in experimentally infected rabbits (40), and (ii) a DnaJ mutant is impaired in its ability to colonize chicken and is affected in its growth at 46°C (17). Hence, the heat shock response should play an essential role in intestinal tract colonization and bacterial survival at high temperature. Recently, *C. jejuni* has been shown to upregulate the expression of at least 24 proteins upon temperature upshift (17). Although that study identified DnaJ as being one of the temperature-regulated proteins, the remaining 23 proteins remain to be fully characterized.

DNA microarray has been recently used to compare *Campylobacter* interstrain variations at the genomic level by using a "low-cost" microarray technology (9) that utilized a *C. jejuni* genomic library previously constructed for the genome sequencing project (24). This microarray consists of PCR products amplified from the sequence-defined pUC18 clones by using universal vector primers. One major drawback of this low-cost microarray technology is the presence of more than one gene per element on the array, making this array extremely difficult to analyze and likely ineffective for transcriptome profiling. The construction of *C. jejuni* microarray (carrying a single gene per element) is described here and is used to investigate differential gene expression profile in response to a temperature upshift from 37 to 42°C over a period of 50 min.

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Array construction and validation. A *C. jejuni* array was constructed by PCR amplification of internal fragments of each open reading frame (ORF) from the annotated genomic sequence of *C. jejuni* NCTC 11168. Of all of the 1,654 predicted ORFs, 1,626 were successfully amplified; these represent 98.3% of the genome. Details of the methods used (microarray construction, RNA extraction, and labeling, as well as data collection and analysis) and a complete list of the genes represented on the microarrays are available online (http://www.cvm.okstate.edu/research/Facilities/CampyLab). The reliability of the data generated by using this microarray was assessed by cohybridization of two identical cDNA samples (prepared from total RNA isolated from two identical but independent bacterial cultures) that were labeled independently with Cy3 or Cy5 dyes. The pattern of hybridization revealed a linear correlation with no more than a 1.5-fold change in the relative gene expression level. This control experiment suggested that genes with an expression ratio beyond this range were either down- or upregulated. Therefore, only genes identified as being up- or downregulated by at least 1.5-fold were chosen for further study. Moreover, a 1.5-fold threshold has recently been reported as being biologically significant (15, 31). In addition, experiments were conducted independently twice (with dye swap) yielding at least four measurements per gene (representing two technical and two biological replicates, since each gene is present twice on each slide) to confirm the reproducibility of the gene expression data. Genes with highly reproducible expression ratios were selected. The Student *t* test was applied to the data, and genes with a *P* value of <0.01 were considered to have significant differential expression. By using a significance level of 0.01, we could anticipate a maximum of 17 false-positive results per time point (with 1,626 variables). However, this number is significantly reduced by the threshold of a 1.5-fold change in gene expression. In summary, genes were selected according to the following algorithm: (i) signal intensity more than three times the standard deviation of the background at least in one channel, (ii) a fold change in relative expression level of >1.5, and (iii) a *P* value of ≤ 0.01. Finally, the data generated were further validated by real-time quantitative reverse transcription-PCR (RT-PCR) by using an ABI Prism 7700 DNA analyzer (Applied Biosystems, Foster City, Calif.) and the QuantiTect SYBR green RT-PCR kit according to the manufacturer's protocol. Ten ORFs, exhibiting high, moderate, and low expression (as identified by microarray analysis), were selected for comparative real-time RT-PCR analysis (Fig. 1). The gene expression levels obtained by real-time quantitative RT-PCR analysis were normalized to that of the *thiC* gene, since its expression was found to be invariant under different temperature changes. The expression level of *thiC* was normalized to that of the *rpoA* gene, the expression of which was also found to be invariant. Quantitative values were obtained by using the comparative threshold cycle ($\Delta\Delta C_T$) method recommended by Applied Biosystems. The C_T value corresponds to the PCR cycle at which occurs the first detectable increase in fluorescence associated with the exponential growth of PCR products. The relative expression of each gene was determined three times in each of the two experimental RNA samples and is expressed as the fold difference in quantity of cDNA molecules present at 42°C relative to that present at 37°C. The resulting gene expression ratio was log transformed and plotted against the average log ratio values obtained by microarray analysis (Fig. 1). A high level of concordance ($r = 0.967$) was observed between microarray and RT-PCR data despite quantitative differences in the level of change. Overall, RT-PCR and DNA microarray data differed by an average of fivefold. Consequently, microarray data could be calibrated to provide a quantitative estimate of differential fold expression. This underestimation of fold changes by DNA microarray analysis has been previously reported (41), suggesting a smaller dynamic range for microarray measurements compared to those of RT-PCR. Nevertheless, this validation study by real-time RT-PCR indicated that our microarray approach produced accurate fold change differences with sufficient sensitivity to identify differentially regulated transcripts.

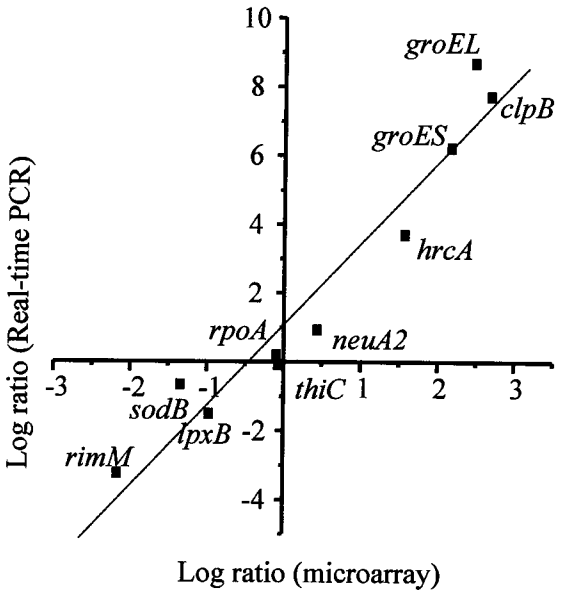

FIG. 1. Comparison of expression measurements by microarray and RT-PCR assays. The fold changes in gene expression in response to temperature upshift from 37 to 42°C were log transformed (in base 2). The real-time RT-PCR $\log_2$ values were plotted against the microarray data $\log_2$ values.

Experimental design. We chose to study the *C. jejuni* response to temperature shift over a 50-min period, since the cell densities of the 37 and 42°C cultures were identical at the last time point. This suggests that the observed transcriptional profiles were not influenced by the growth phase or cell density differences of the two temperature conditions. *Campylobacter* sp. was grown microaerobically at 37°C in Mueller-Hinton medium to an optical density at 600 nm of 0.1. The *Campylobacter* broth was centrifuged (5 min, 8,000 × *g*), and the pellet was resuspended in 10 ml of fresh growth medium and then split equally in two flasks containing growth medium (200 ml) preconditioned at 37 or 42°C. Samples of 10 ml were collected at 5, 10, 20, 30, and 50 min after the temperature shift; rapidly mixed with one volume of RNALater solution (Ambion, Austin, Tex.); and placed on ice. Cells were immediately collected by centrifugation at 4°C (3 min, 8,000 × *g*) and resuspended in lysozyme-TE buffer (50 mM Tris-Cl [pH

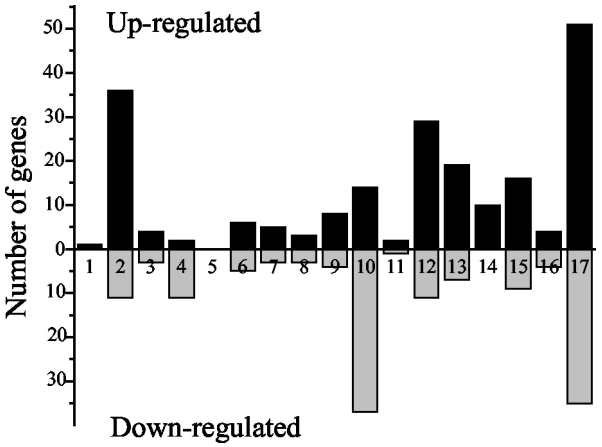

FIG. 2. Differentially expressed genes grouped by functional classification according to the Sanger Center *C. jejuni* genome database. Columns: 1, small molecules degradation; 2, energy metabolism; 3, central intermediary metabolism; 4, amino acid biosynthesis; 5, polyamine synthesis; 6, purines, pyrimidines, nucleosides, and nucleotides; 7, biosynthesis of cofactors, prosthetic groups, and carriers; 8, fatty acid biosynthesis; 9, regulatory functions; 10, synthesis and modification of macromolecules; 11, degradation of macromolecules; 12, cell envelope; 13, cell processes (transport and/or binding proteins); 14, cell processes (heat shock); 15, cell processes (other); 16, other; and 17, miscellaneous (unknown proteins). The solid or shaded bars represent the numbers of genes whose expression increased or decreased, respectively.

8], 1 mM EDTA, 0.5 mg of lysozyme/ml). Total RNA was isolated by using a hot phenol-chloroform protocol (7). Total RNA was reverse transcribed by using random hexamers in the presence of aminoallyl-dUTP, followed by labeling with succinimidyl-ester monoreactive dyes (Cy3 or Cy5). The level of gene expression was monitored by competitive hybridization to the microarray with cDNA obtained from bacteria grown at 42 and 37°C for each time point. Each hybridization experiment was repeated two times by using total RNA isolated from two independent time course experiments. Microarray data were extracted by using GenPix Pro 3 software (Axon) and statistically analyzed as described earlier.

Global gene expression analysis. In total, 336 genes were identified to be differentially regulated by >1.5-fold (with 99% confidence) in at least one of the five time points. This represents ca. 20% of the 1,626 elements on the array. A total of 58% of the differentially transcribed genes were upregulated, and 42% were downregulated. The relative gene expressions in *C. jejuni* grown at 42°C versus *C. jejuni* grown at 37°C varied from 19.7- to 0.15-fold at different times after the temperature shift. Figure 2 provides a summary of the number of differentially expressed genes grouped by functional categories according to the *C. jejuni* NCTC 11168 genome annotation. This functional gene categorization provides information on the molecular mechanisms that allow *C. jejuni* to surmount a sudden temperature upshift. However, many genes whose expression was altered by temperature encode for proteins of unknown function (column 17, Fig. 2), thus displaying the limitations in understanding *C. jejuni* physiology. For genes of known or annotated function, those encoding proteins involved in energy metabolism (column 2, Fig. 2), cell wall and envelope constituents (column 12, Fig. 2), and transport and binding proteins (column 13, Fig. 2) are among the most upregulated genes. However, genes encoding proteins involved in synthesis and modification of macromolecules are among the most downregulated genes (column 10, Fig. 2).

Cluster analysis of microarray data. In order to identify and detect genes that were coregulated depending on the stage of temperature stress, a statistical analysis program was used to analyze the 336 genes previously selected. Briefly, the selected genes were subjected to hierarchical clustering by using Genesis software (version 1.0.2; Graz University of Technology [http://genome.tugraz.at]). This cluster analysis rearranges the data into new sets of better-ordered groups of genes sharing similar temporal expression pattern (10, 33). Four clusters were obtained, representing specific patterns of regulation (Fig. 3). Roughly, clusters B and C corresponded to genes that have their expression activated, whereas clusters A and D contained genes that have their expression repressed at 42°C relative to 37°C. One of the remarkable findings observed in this time course microarray analysis was that the global changes in gene expression upon temperature increase were essentially transient (Fig. 3). Immediately after the temperature stress, the bacterial cells responded with large changes in the expression level of 336 genes during the first 10 min, whereas <15% of these genes remained differentially expressed at 50 min. This gene expression pattern suggests that *C. jejuni* is able to adapt its transcript levels to a new steady state at 42°C over time with very few differentially expressed genes between the two growth temperature conditions. These initial global changes in transcript abundance at 5 and 10 min reflect the primary response of *C.jejuni* to surmount the sudden temperature increase, thereby allowing the bacterium to survive and ultimately adapt to the new temperature. This observation is in agreement with findings from Richmond et al. (27), who found very little differences in *E. coli* gene expression after heat shock (27). As exemplified here, time course experiments provide valuable information and are essential for deciphering the mechanism of bacterial response and adaptation to stresses. Temporal gene expression analysis can provide detailed insight into the mechanistic responses to stresses that are otherwise not easy to elucidate or identify using steady-state or single time point studies. Gene expression kinetics allow gene clustering and eventually putative gene function assignment based on a "guilt by association" principle. Steady-state or single time point studies of *C. jejuni* response to temperature changes would have missed ca. 85% of the genes whose expression is affected for the first 30 min.

Cluster A is composed of genes that are significantly downregulated at 20 min and are back near the baseline level at 50 min after the temperature increase. The most notable subgroup of genes with repressed expression includes a number of genes involved in ribosomal protein synthesis and modification (e.g., *rplR*, *rplO*, *rplX*, *rpsH*, *rplL*, *rlpN*, *rpsQ*, *rpsN*, *rplF*, and *rpsI*) and one gene involved in ribosome maturation and modification (*ksgA*, a putative dimethyladenosine transferase). In several gene expression studies with *Saccharomyces cerevisiae*, it has been observed that the expression level of ribosomal genes is similarly affected by various environmental stresses (13, 16). This repression of ribosomal genes suggests a brief growth arrest that allows the cell to reshuffle energy devoted to

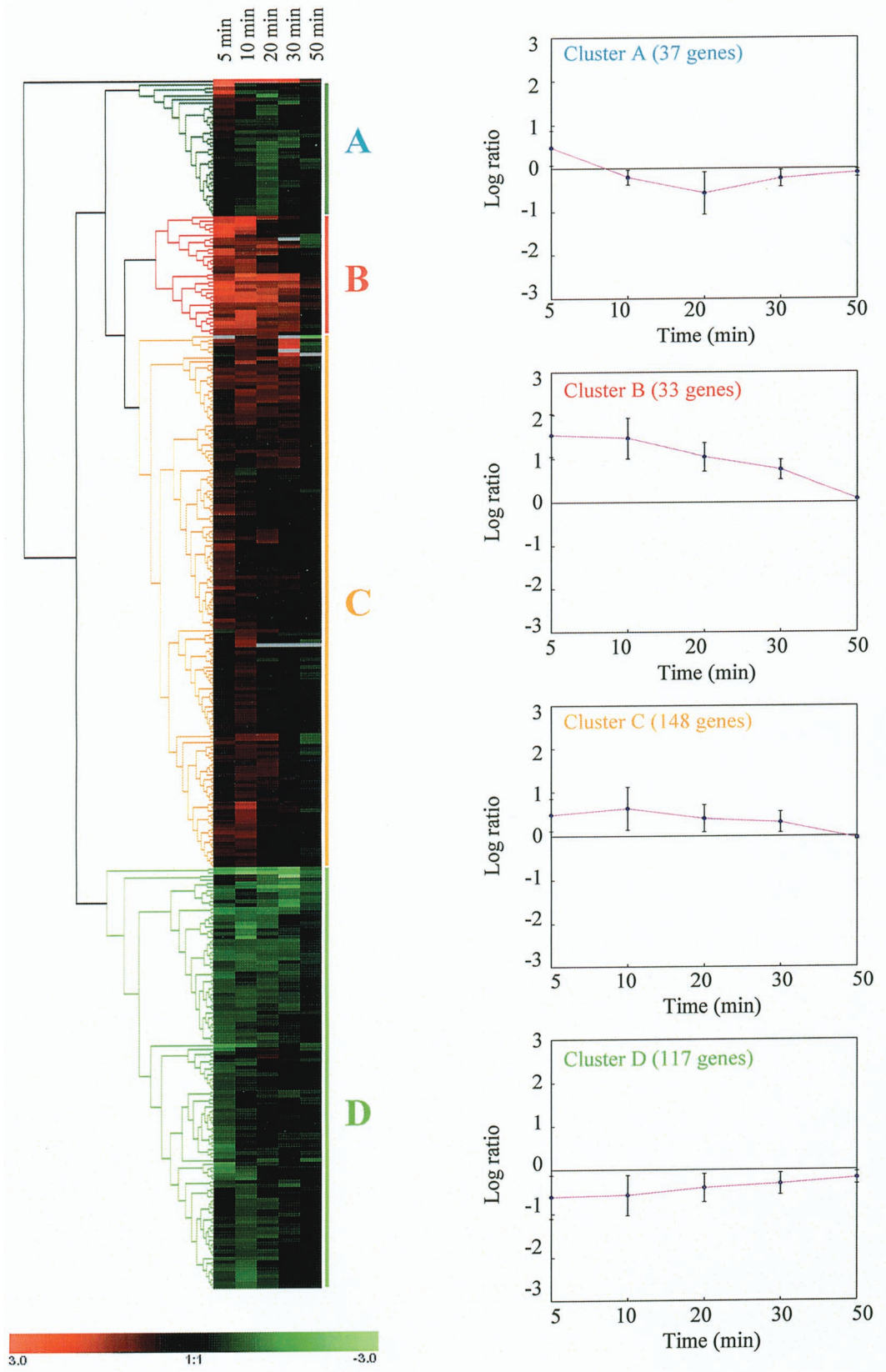

FIG. 3. Hierarchical clustering of 336 genes that varied significantly ($P < 0.01$ and a fold change > 1.5) in their expression profiles in response to a temperature change from 37 to 42°C. Each row represents a single gene expression, and each column represents an individual time point after the temperature upshift. An increasing green intensity indicates genes whose expression decreased, whereas an increasing red intensity indicates genes whose expression increased in response to the temperature upshift. The gray color indicates missing data. The mean of the gene expression patterns of each cluster is represented by plotting the $\log_2$ value of the expression ratio versus time. The error bars represent the standard deviation of the gene expression ratio.

TABLE 1. Fold change in the expression level of *C.jejuni* heat shock genes in response to a temperature upshift from 37 to 42°C

Gene	Gene product or function	Induction ratio[a]
groEL	60-kDa chaperonin	5.5
groES	10-kDa chaperonin	3.8
grpE	Heat shock protein	19.7
dnaK	Heat shock protein	3.5
dnaJ	Chaperone DnaJ	1.4
hspR	Putative heat shock transcriptional regulator	2.6
cbpA	Putative curved DNA-binding protein	5.1
hrcA	Putative heat shock regulator	3.7
lon	ATP-dependent protease	1.4
clpB	Protease	5.1
hslU	Putative heat shock protein	1.4
Cj1034c	Possible DnaJ-like protein	1.6

[a] The induction ratio is the maximum value obtained over five time points.

an increased expression of genes involved in protective response and adaptation to the new growth condition. This reduction in ribosomal gene expression, together with the upregulation of genes encoding proteins involved in energy metabolism, reflects the energy-starved condition of the cell and the necessity for saving and reshuffling energy for the increased expression of proteins involved in repairing damages caused by the temperature upshift. In addition, this transient decrease in ribosomal proteins probably resulted in a modest repression of de novo protein synthesis, allowing the bacterium to surmount heat shock damage and adapt its transcriptome to the new growth temperature. At 50 min after the temperature shift, the expression of the ribosomal genes returned to a basal level, indicating that *C. jejuni* maintains a constant level of ribosomal protein and RNA between 37 and 42°C. Since 42°C is the optimal growth temperature for *C. jejuni*, this bacterium probably responds, like *Escherichia coli*, to temperature augmentation by increasing the rate of peptide chain elongation.

Cluster B contains 33 genes and displays the most highly and rapidly upregulated genes. The genes in this cluster were immediately upregulated after the increase in temperature. After 10 min of exposure at 42°C, their expression gradually decreased with time to reach a baseline level at 50 min. Cluster B is dominated by genes encoding chaperones, chaperonins, and heat shock proteins. Specifically, *groEL*, *groES*, *dnaK*, *dnaJ*, *hspR*, *cbpA*, *hrcA*, *lon*, *clpB* (protease), *hslU*, and Cj1034c transcripts all increased (Table 1). Proteins belonging to the heat shock family have been intensively studied and have been shown to be induced in response to stress, in particular as a result of a sudden temperature upshift (42). These proteins act by repairing and preventing damages caused by an accumulation of unfolded proteins. Several of the heat shock proteins (e.g., DnaK and GroEL) also play a crucial role under normal physiological conditions by assisting in the proper folding of newly synthesized proteins. Recently, *C. jejuni* has been shown to mount a heat shock response, with the preferential synthesis of 24 proteins upon temperature increase (17). Of these 24 proteins, only DnaJ has been further characterized. Our study confirms the upregulation of *dnaJ* upon temperature stress and demonstrates the temperature-responsive regulation of many other heat shock proteins (Table 1). The molecular mechanism of heat shock regulation in *C. jejuni* is poorly understood. Many studies have demonstrated that heat shock regulation differs among bacterial species. In *E. coli*, the alternative sigma activator σ^{32} (encoded by *rpoH*) mediates the expression of most heat shock proteins (42). Analysis of the *C. jejuni* genome reveals the absence of a σ^{32} homologue, suggesting a regulation mechanism of the heat shock response different than those in other gram-negative bacteria such as *E. coli*. In *Bacillus subtilis* and other gram-positive bacteria, the heat shock response is regulated by diverse regulatory strategies depending on the specific heat shock genes (42). In *B. subtilis*, class I heat shock genes are negatively regulated by HrcA, class II genes are positively regulated by the transcriptional activator σ^{B}, and class III genes are negatively regulated by CtsR. The HrcA repressor acts at the DNA level by binding to conserved palindromic sequence, named CIRCE (for controlling inverted repeat of chaperone expression) (43). Upon heat shock, HrcA dissociates from its operators, leading to the transcriptional expression of class I (*groE* and *dnaK*) heat shock genes. Interestingly, the *C. jejuni* genome contains a homologue of the *hrcA* gene arranged in a cluster with two heat shock genes, *grpE* and *dnaK*. Consequently, HrcA might serve as a negative regulator of *grpE* and *dnaK* in *C. jejuni* just as it does in *B. subtilis*. This hypothesis is supported by the identification of a *cis*-acting regulatory element (CIRCE) upstream of the *hrcA* gene (36). The *C. jejuni* CIRCE sequence exhibits a putative stem-loop structure with some similarities to the consensus CIRCE sequence. The *C. jejuni* CIRCE sequence is 5′-CTAG CAATC-N_8-GAGTGCTAG-3′, with unmatching bases underlined (the loop of the putative hairpin structure is composed of eight nucleotides instead of nine in the consensus sequence). This campylobacter CIRCE sequence was also identified upstream of the *groESL* operon translation start site (37). However, this CIRCE sequence could not be identified in front of the other genes from cluster B, suggesting that these other genes might be regulated differently than the *groESL* and *hrcA-grpE-dnaK* operons. The *C. jejuni* genome lacks homologues of the other two *B. subtilis* heat shock regulators, σ^{B} and CtsR. Interestingly, the *C. jejuni* genome contains a homologue of *Streptomyces albus* HspR regulator (4). HspR is organized in an operon with the *cbpA* gene, which belongs to the heat shock protein DnaJ family. HspR is contained within cluster B, and thus HspR might likely regulate several genes from cluster B. Indeed, recent investigations have shown that HspR regulates the expression of *dnaK*, *groESL*, and *cbpA* genes in *C. jejuni*. Therefore, *C. jejuni* may use more than one strategy to simultaneously regulate distinct sets of heat shock genes. This complex regulatory network may allow the bacterium to fine tune heat shock gene expression in response to a temperature upshift or other stimuli (e.g., oxidative shock, acid shock, and osmotic shock).

Another set of genes in cluster B contains *napABGH*, Cj1358c and Cj1357c genes, which encode a nitrate reductase of the periplasmic nap type, a NapC/NirT/NrfH homologue, and a putative periplasmic cytochrome *c* (homologous to the nitrate reductase NrfA of *E. coli*), respectively. This set of genes could be predicted to allow the bacterium to carry out respiration with nitrate and nitrite as electron acceptors. In fact, it has recently been reported that nitrate sustains *C. jejuni* growth under oxygen-limited conditions, indicating its role in

energy conservation (28). Thus, the upregulation of these genes may be caused by a decreased pO_2 of the culture medium at 42°C compared to that which occurs at 37°C (since gas solubility decreases with increasing temperature) allowing *C. jejuni* to respire on nitrate. This hypothesis was supported by the downregulation of genes encoding for proteins involved in aerobic metabolism (constituting the cluster D) upon temperature increase. Analysis of the *C. jejuni* genome revealed the presence of two other reductases that are predicted to allow the use of fumarate (FrdCAB homologues) and N- or O-oxide reductase (DorA/TorA homologue) as alternative electron acceptors to oxygen. In contrast to the nitrate reductase, these two other reductases were not differentially expressed at 42°C relative to 37°C. One explanation for their absence of upregulation could be the lack of sufficient electron acceptors (fumarate and N- or O-oxide compounds) in Mueller-Hinton broth. Likewise, Mueller-Hinton broth should contain only traces of nitrate, and yet genes involved in nitrate reduction were differentially expressed. The homologue *E. coli* Nap enzymes have been shown to exhibit a high affinity for nitrate, making it ideal for scavenging a low nitrate concentration as encountered in vivo (32). Thus, the trace amount of nitrate within the broth medium might be sufficient to induce the *nap* genes expression at a low oxygen concentration.

Cluster C contains 148 genes. The gene expression in cluster C resembled that of cluster B. The level of gene expression in this cluster was upregulated between 1.5- to 2-fold at 5 and 10 min after a temperature increase from 37 to 42°C. As with genes from clusters A and B, cluster C gene expression gradually decreased over time until it reached basal level at 50 min after the temperature change. Interestingly, many genes encoding proteins known or presumed to be involved in chemotaxis, flagellum biosynthesis, and flagellar motility are part of this cluster. A similar effect on the expression of flagellar and chemotaxis genes in response to heat shock has also been recently demonstrated in *E. coli* (27). This set of genes includes *fliI* (flagellum-specific ATP synthase), *fliQ* (flagellar biosynthetic protein), *fliD* (flagellar hock-associated protein), *flgH* (flagellar L-ring protein precursor), *flgD* (flagellar hock assembly protein), *flgE2* (flagellar hock subunit protein), *flgI* (flagellar P-ring protein precursor), *flaA* (flagellin), *flaC* (flagellin), Cj1312 (possible flagellum protein), *cheV* (chemotaxis protein), *cheW* (chemotaxis protein), Cj0262c (putative methyl-accepting chemotaxis signal transduction protein), and *neuB3* (*N*-acetylneuraminic acid synthetase, known to be involved in flagellin glycosylation). This observation is in agreement with the findings of Alm et al. (1) that the *flaB* gene expression is induced 2.5-fold at a growth temperature of 42°C compared to induction at 37°C, suggesting that flagellar gene expression is temperature regulated. Although *flaB* was not selected as differentially expressed by using the gene selection algorithms described above, the microarray data show that the *flaB* gene expression is in fact upregulated by 1.4-fold ($P < 0.01$) at 5, 10, 20, and 30 min and reaches a basal expression level at 50 min. This microarray data definitely demonstrates that a growth temperature of 42°C induces flagellar biosynthesis and consequently motility. The molecular mechanism of *Campylobacter* chemotaxis and flagellar gene regulation are ill defined. The present study reveals that these genes are upregulated upon a temperature increase. Undoubtedly, flagellum and chemotaxis proteins should play an essential role in *Campylobacter* gut colonization. Indeed, nonmotile mutants are unable to colonize intestinal tracts (19, 20, 25, 38). Therefore, the upregulation of chemotaxis and flagellar genes at 42°C, which is the core temperature of the chicken reservoir, might condition *C. jejuni* in order to spread rapidly through large broiler flocks and to colonize efficiently the human intestinal tract. This hypothesis is supported by the observation that flagellin proteins are overexpressed when *Campylobacter* is maintained within chicken implants compared to in vitro growth observed at 37°C (6) and that the chick colonization potential of *C. jejuni* could be increased 10,000-fold after a single passage in chickens (5).

Interestingly, several genes encoding for restriction and modification enzymes are included in cluster C. These genes are Cj0031 (putative type IIS restriction-modification enzyme, N-terminal half), Cj0032 (putative type IIS restriction-modification enzyme, C-terminal half), Cj1549c (putative type I restriction enzyme R protein), and Cj1551c (putative type I restriction enzyme S protein). The role of the DNA restriction and modification systems in *C. jejuni* is unclear at present. These restriction-modification enzymes might be involved in the breakdown of foreign DNA. These enzymes might also be necessary for stimulating the formation of DNA fragmentation and recombination resulting in antigenic diversity and variation, such as the homologous recombination observed for the virulence-associated flagellin locus of *C. jejuni* (14, 21, 39). Interestingly, the presence of restriction-modification systems in *Helicobacter pylori* has been recently associated with the bacterial ability to infect its host (3), implying that these enzymes might affect the virulence gene expression. Based on this observation, it is tempting to suggest a similar function in *C. jejuni*, where these enzymes would control the expression of genes involved in chicken gut colonization.

Another group of genes from cluster C encodes for proteins involved in energy metabolism. Representative genes from this functional group encode for Ni/Fe hydrogenase (*hydAB*), Ni/Fe hydrogenase B-type cytochrome (*hydC*), formate dehydrogenase (*fdhABC*), and cb-type cytochrome *c* oxidase (*ccoO*, *ccoN*, and *ccoQ*). The upregulation of these genes at 42°C clearly correlates with the upregulation of the nitrate reductase *napABGH*, Cj1358c and Cj1357c from cluster B. Undoubtedly, *C. jejuni* shifted from aerobic metabolism to anaerobic metabolism upon temperature increase; this was probably caused by a decrease in the oxygen concentration in the broth medium at the higher temperature.

Finally, another worthwhile functional set of genes from cluster C encodes proteins involved in surface structure biosynthesis and modification, UDP-glucose 4-epimerase (*galE*), phosphoheptose isomerase (*gmhA2*), 3-deoxy-D-manno-octulosonic acid transferase (*kdtB*), putative transferase (Cj1321), acylneuraminate cytidylyltransferase (*neuA2*), *N*-acetylneuraminic acid synthetase (*neuB3*), putative ADP-heptose synthase (*waaE*), and a putative glycosyltransferase (*wlaE*). It has been previously reported that the protein encoded by *galE* plays an essential role in the synthesis of *C. jejuni* lipopolysaccharide (LPS), probably by catalyzing the interconversion of UDP-glucose into UDP-galactose (11). A *galE* mutant expresses a truncated lipid A core molecule and is affected in its ability to invade and adhere to human intestinal cells, thus demonstrat-

ing the involvement of GalE in LPS biosynthesis and the significance of LPS as a virulence factor (11). *GalE* gene is part of a 16-kb region, named *pgl*/*wla* locus (12), which contains 13 genes encoding proteins involved in LPS and lipooligosaccharide biosynthesis. Genes from this locus (*galE* and *wlaBCDEFGHIKLM*) have also been shown to be involved in a mechanism of general protein glycosylation in *C. jejuni* (34). Surprisingly, the microarray data show that several genes from this cluster are expressed differently. Whereas *wlaE* and *galE* are upregulated upon temperature increase, *wlaK* is downregulated (and is part of cluster D). These results suggest that the genes from the *pgl*/*wla* locus are transcribed as multiple operons. In support of this conclusion is the absence of a polar mutation by insertion of a chloramphenicol or kanamycin cassette in either orientation within several genes from the *wlaB-M* region (12). From these microarray data, it is apparent that the expression of several genes from the protein glycosylation locus is temperature regulated in *C. jejuni*. This differential regulation of glycosylases genes should lead to differential glycosylation patterns of surface structures between 37 and 42°C. This complex mechanism of protein glycosylation may allow the bacterium to efficiently colonize different ecological niches, such as the intestinal tract of birds (core temperature of 42°C) and mammals (core temperature of 37°C), and/or to modify the protein membrane "make-up" in order to evade the host immune system.

Cluster D contains 117 genes. The expression level of the genes in this cluster is decreased by 1.5- to 3-fold at 5 and 10 min after the temperature upshift. A subgroup of noteworthy genes encode for the succinate dehydrogenase (*sdhABC*). This enzyme complex is a membrane component of the tricarboxylic acid cycle. It catalyzes the oxidation of succinate to fumarate and participates in the aerobic respiratory chain by reducing the ubiquinone pool in the membrane. The gene expression of *E. coli* succinate dehydrogenase has been studied and appears to be under the control of both oxygen concentration and carbon sources (23, 29). The *E. coli* enzyme is subjected to anaerobic repression via the two regulators, ArcA and Fnr (23, 29). Whereas the *C. jejuni* genome lacks homologues of both ArcA and Fnr, *Campylobacter sdhABC* genes seem to be similarly repressed under a low oxygen concentration as a result of a temperature increase to 42°C. The downregulation of this set of genes is in agreement with the upregulation of genes encoding proteins involved in the anaerobic metabolism (genes from cluster B and C). Given that ArcA and Fnr homologues are absent in the *Campylobacter* genome, this microorganism should regulate gene expression in response to oxygen deprivation via a novel and uncharacterized mechanism. *E. coli* ArcA has also been shown to repress other enzymes involved in aerobic metabolism, including manganese superoxide dismutase (encoded by the *sodA* gene) (8). Interestingly, *C. jejuni* iron superoxide dismutase gene (named *sodB*) is part of cluster D, suggesting that *sodB* and *sdhABC* might be coregulated via an anaerobic responsive repressor.

One other major functional group of genes in cluster D encodes proteins involved in surface structure biosynthesis and modification, including WlaK (putative aminotransferase), LpxB (lipid A-disaccharide synthase), and Fcl (putative fucose synthase). The downregulation of these proteins, together with the upregulation of other proteins belonging to the same functional group, clearly suggests a differential surface structure pattern between 42 and 37°C.

Conclusion. The present study provides a genome-wide expression profile of *C. jejuni* in response to a temperature change from 37 to 42°C. A complete repertoire of genes are either induced or repressed at 42°C relative to 37°C. This time course study of gene expression reveals that a large number of genes are differentially expressed during the first 10 min, whereas only a few of them remain differentially expressed 50 min after temperature upshift. This report identifies up to 336 genes that have their expression affected by the temperature change. Whereas clustering analysis was used as a first step to identify coregulated genes, a genetic analysis of the regulatory pathways needs to be undertaken in order to fully characterize the major regulators.

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