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Chlamydia trachomatis Lacks an Adaptive Response to Changes in Carbon Source Availability

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Most bacteria coordinately regulate gene expression as an adaptive response to a variety of environmental changes. One key environmental cue is the carbon source necessary for central metabolism. We used microarray analysis to monitor the global transcriptional response of the obligate intracellular pathogen *Chlamydia trachomatis* to the presence of glycolytic and gluconeogenic carbon sources. In contrast to free-living bacteria, changing the carbon source from glucose to glutamate or α-ketoglutarate had little effect on the global gene transcription of *C. trachomatis*.

Chlamydiae are unusual among bacteria in that they are obligate intracellular organisms with a complex developmental cycle. Chlamydiae develop and grow within an intracellular vacuole, called an inclusion, that is distinct from other intracellular vacuolar compartments (13). The developmental cycle begins with a metabolically inactive infectious form called the elementary body (EB) that, after entry into the target cell, differentiates into a metabolically active form called the reticulate body (RB). After multiple rounds of division, the RB then differentiates back into the EB developmental form. Once the host cell finally lyses, the infectious EBs are released to initiate new rounds of infection (3).

Many organisms have developed sophisticated mechanisms that enable them to sense the nutritional status of their environment and adjust their catabolic capacities by regulatory responses at the transcriptional level (1). Bacteria coordinately regulate genes depending on the carbon source present through a process known as catabolite repression (17, 18). When glucose becomes limiting, bacteria exhibit a transcriptional shift in which genes that are initially repressed by glucose become induced. Such adaptive processes have been intensely investigated in model organisms, such as *Escherichia coli* and *Bacillus subtilis* (12, 18). Recently, with the use of DNA microarray technology, these studies have been expanded to the genomic level where it was found that a conglomeration of gene families are involved in these adaptive processes (9, 15).

When glucose is limiting in the environment, bacteria may utilize a wide variety of substrates, including various sugars, amino acids, and dicarboxylic acids that can serve as gluconeogenic carbon sources (1, 17). Analysis of the chlamydial genome suggested that host-derived glucose-6-phosphate is the primary carbon and energy source used to support chlamydial growth (16). It was also noted that the chlamydial genome contains key gluconeogenic enzymes, suggesting that host-derived glutamate or dicarboxylic acids may support chlamydial growth (16). This hypothesis has been tested experimentally by Iliffe-Lee et al. (4). They demonstrated that gluconeogenic substrates, such as glutamate and α-ketoglutarate, support chlamydial growth and showed that the expression patterns of six genes involved in central metabolism that remained unaltered in response to changes in the type of carbon source available; however, global gene families were not tested.

Microarrays containing 875 validated chlamydial genes and 8 genes of the chlamydial plasmid (8) were used to monitor the global transcriptional response of *Chlamydia trachomatis* to the presence of glucose, glutamate, or α-ketoglutarate as the sole carbon source available in the medium provided to infected L929 cells. To characterize the transcriptional response of *C. trachomatis* grown with these carbon sources, we compared the *Chlamydia*-specific RNA from infected cells cultivated in 20 mM glutamate or 20 mM α-ketoglutarate to *Chlamydia*-specific RNA from cells cultivated in 10 mg of glucose per ml. Previous data have demonstrated that all chlamydial genes are expressed by 24 h postinfection (hpi) (8); therefore, it was decided to culture *C. trachomatis*-infected cells for 24 hpi prior to changing the available carbon source. L929 cells were infected with *C. trachomatis* L2/434/Bu at a multiplicity of infection of 1 and cultured for 24 hpi, at which time the infected cells were washed three times with sterile phosphate-buffered saline and suspended in glucose-free Dulbecco's modified Eagle medium supplemented with 5 mM pyruvate and either 10 mg of glucose per ml, 20 mM glutamate, or 20 mM α-ketoglutarate.

To ensure that a 6-h incubation in the various carbon sources tested would be enough time to produce a measurable biologic effect, the number of infectious EB progeny present at this time was determined by infectivity titration assays. Briefly, *C. trachomatis* EBs isolated from each carbon source were titrated onto fresh, confluent monolayers grown on coverslips. After 24 h, cells were fixed and stained with an antichlamydia monoclonal antibody (Syva; MicroTrak), and the number of inclusions was determined by fluorescence microscopy. After only 6 h of exposure to an alternative carbon source, we found

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TABLE 1. Changes in expression of central carbohydrate metabolism genes of *C. trachomatis*[a]

Gene category, ORF no., and gene name[b]	Putative function[b]	Change in expression (fold change)[c]	
		Glutamate	α-Ketoglutarate
Aerobic			
CT278 *nqr2*	NADH-ubiquinone oxidoreductase	1.48 ± 0.01	−1.33 ± 0.02
CT279 *nqr3*	NADH-ubiquinone oxidoreductase	1.33 ± 0.01	−1.56 ± 0.02
CT280 *nqr4*	NADH-ubiquinone oxidoreductase	1.26 ± 0.02	−1.45 ± 0.02
CT281 *nqr5*	NADH-ubiquinone oxidoreductase	1.05 ± 0.01	−1.70 ± 0.03
CT634 *nqrA*	NADH-ubiquinone oxidoreductase	1.30 ± 0.01	−1.08 ± 0.01
CT714 *gpdA*	Glycerol-3-phosphate dehydrogenase	1.26 ± 0.03	−1.46 ± 0.01
CT740 *nqr6*	NADH-ubiquinone oxidoreductase	1.21 ± 0.01	−1.59 ± 0.02
ATP biogenesis and metabolism			
CT065 *adt*	ADP/ATP translocase	1.59 ± 0.02	−1.04 ± 0.04
CT304 *atpK*	V-type ATP synthase subunit K	1.31 ± 0.02	−1.34 ± 0.04
CT305 *atpI*	V-type ATP synthase subunit I	1.17 ± 0.01	−2.11 ± 0.06
CT306 *atpD*	V-type ATP synthase subunit D	1.37 ± 0.03	−1.43 ± 0.02
CT307 *atpB*	V-type ATP synthase subunit B	1.03 ± 0.05	−1.81 ± 0.03
CT308 *atpA*	V-type ATP synthase subunit A	1.56 ± 0.03	−1.63 ± 0.03
CT310 *atpE*	V-type ATP synthase subunit E	1.27 ± 0.01	−1.63 ± 0.03
CT495 *adt*	ADP/ATP translocase	1.47 ± 0.02	−1.17 ± 0.01
CT719 *fliF*	Flagellar M-ring-like protein	1.18 ± 0.03	−1.48 ± 0.01
Electron transport chain			
CT013 *cydA*	Cytochrome oxidase subunit I	1.18 ± 0.05	−1.30 ± 0.02
CT014 *cydB*	Cytochrome oxidase subunit II	1.17 ± 0.04	−1.33 ± 0.01
CT059 *fer*	Ferredoxin	1.44 ± 0.01	−1.41 ± 0.01
CT312 *fer*	Predicted ferredoxin	1.20 ± 0.05	−1.24 ± 0.04
Glycogen metabolism			
CT042 *glgX*	Glycogen hydrolase	1.54 ± 0.03	−1.35 ± 0.05
CT087 *malQ*	4-α-Glucanotransferase	1.30 ± 0.02	−1.08 ± 0.06
CT248 *glgP*	Glycogen phosphorylase	1.09 ± 0.03	−1.52 ± 0.01
CT489 *glgC*	Glucose-1-phosphate adenylyltransferase	−1.03 ± 0.02	−1.06 ± 0.09
CT710 *pckA*	Phosphoenolpyruvate carboxykinase	1.21 ± 0.03	−1.57 ± 0.01
CT715	UDP glucose pyrophosphorylase	1.18 ± 0.01	−1.38 ± 0.01
CT798 *glgA*	Glycogen synthase	1.04 ± 0.02	−1.22 ± 0.01
CT866 *glgB*	1,4-α-Glucan branching enzyme	1.32 ± 0.03	−1.13 ± 0.02
Glycolysis and gluconeogenesis			
CT205 *pfkA*	Pyrophosphate-fructose-6-phosphate 1-phosphotransferase	1.32 ± 0.05	−1.65 ± 0.18
CT207 *pfkA*	Pyrophosphate-fructose-6-phosphate 1-phosphotransferase	1.49 ± 0.10	−1.36 ± 0.16
CT215 *dhnA*	Predicted aldolase	1.56 ± 0.08	−1.61 ± 0.16
CT295 *yhxB*	Phosphomannomutase	1.19 ± 0.04	−1.77 ± 0.01
CT328 *tpiA*	Triosephosphate isomerase	1.28 ± 0.01	−1.24 ± 0.01
CT332 *pykF*	Pyruvate kinase	1.32 ± 0.02	−1.17 ± 0.01
CT378 *pgi*	Glucose-6-phosphate isomerase	1.13 ± 0.03	−1.26 ± 0.02
CT505 *gapA*	Glyceraldehyde-3-phosphate dehydrogenase	1.25 ± 0.01	−1.73 ± 0.01
CT587 *eno*	Enolase	1.23 ± 0.05	−1.44 ± 0.08
CT693 *pgk*	Phosphoglycerate kinase	−1.06 ± 0.01	−1.63 ± 0.01
CT722 *pgm*	Phosphoglycerate mutase	1.39 ± 0.01	−1.37 ± 0.01
CT815 *mrsA*	Phosphoglucomutase	1.13 ± 0.02	−1.33 ± 0.01
Pentose-phosphate pathway			
CT063 *gnd*	6-Phosphogluconate dehydrogenase	1.21 ± 0.02	−1.45 ± 0.01
CT121 *araA*	Ribulose-3-phosphate epimerase	1.27 ± 0.03	−1.45 ± 0.01
CT185 *zwf*	Glucose-6-phosphate dehydrogenase	1.42 ± 0.01	−1.76 ± 0.03
CT186 *devB*	Glucose-6-phosphate dehydrogenase	1.26 ± 0.01	−1.67 ± 0.02
CT213 *rpiA*	Ribose-5-phosphate isomerase A	1.12 ± 0.05	1.11 ± 0.38
CT313 *tal*	Transaldolase	−1.01 ± 0.01	−1.42 ± 0.01
CT331 *dxs*	1-Deoxy-D-xylulose-5-phosphate synthase	1.65 ± 0.04	−1.70 ± 0.01
CT750 *tktB*	Transketolase	1.25 ± 0.03	−1.61 ± 0.03
Pyruvate dehydrogenase			
CT245 *pdhA*	Pyruvate dehydrogenase E1 alpha	1.40 ± 0.01	−1.24 ± 0.01
CT246 *pdhB*	Pyruvate dehydrogenase E1 beta	1.36 ± 0.03	−1.64 ± 0.01
CT247 *pdhC*	Dihydrolipoamide acetyltransferase	1.32 ± 0.01	−1.54 ± 0.01
CT285 *lplA*	Lipoate protein ligase	1.48 ± 0.01	−1.35 ± 0.04
CT340 *pdhA*	Pyruvate dehydrogenase alpha and beta fusion	1.35 ± 0.01	−1.34 ± 0.01

Continued on following page

TABLE 1—*Continued*

Gene category, ORF no., and gene name[b]	Putative function[b]	Change in expression (fold change)[c]	
		Glutamate	α-Ketoglutarate
CT499 *lplA*	Lipoate protein ligase	1.24 ± 0.05	−1.54 ± 0.02
CT557 *lpdA*	Lipoamide dehydrogenase	1.47 ± 0.01	−1.59 ± 0.04
Tricarboxylic acid cycle			
CT054 *sucA*	2-Oxoglutarate dehydrogenase E1	1.20 ± 0.01	−1.53 ± 0.01
CT055 *sucB*	Dihydrolipoamide succinyltransferase E2	−1.16 ± 0.02	−1.83 ± 0.02
CT376 *mdh*	Malate dehydrogenase	1.16 ± 0.02	−1.20 ± 0.01
CT390 *aspC*	Aspartate aminotransferase	1.31 ± 0.02	−1.45 ± 0.03
CT400 *sucB*	Dihydrolipoamide succinyltransferase E2	1.24 ± 0.04	−1.39 ± 0.01
CT591 *sdhB*	Succinate dehydrogenase iron-sulfur protein	−1.19 ± 0.08	−1.62 ± 0.05
CT592 *sdhA*	Succinate dehydrogenase flavoprotein	1.19 ± 0.02	−1.36 ± 0.04
CT593 *sdhC*	Succinate dehydrogenase cytochrome subunit	1.21 ± 0.03	−1.40 ± 0.09
CT821 *sucC*	Succinyl-CoA[d] synthetase beta chain	1.13 ± 0.01	−1.46 ± 0.03
CT822 *sucD*	Succinyl-CoA synthetase alpha chain	1.11 ± 0.01	−1.50 ± 0.02
CT855 *fumC*	Fumarate dehydratase class II	1.14 ± 0.01	−1.50 ± 0.04

[a] At least three independent infections in which chlamydiae were harvested from infected cells from each specific carbon source were performed. Data for duplicate readings and each hybridization experiment were normalized based on the total percent intensity to eliminate slide-to-slide variation. Gene expression data were then normalized 16S rRNA. The statistical significance of the gene expression changes observed during the presence of glutamate versus glucose and α-ketoglutarate versus glucose were assessed by using the significant analysis of microarrays as previously described (19).

[b] Gene category, open reading frame (ORF) number, gene name, and putative function were assigned by Stephens et al. (16).

[c] Change in expression of cells grown with glutamate or α-ketoglutarate as the sole carbon source compared to expression in cells grown with glucose as the sole carbon source. Change in expression is shown as the mean fold change ± standard deviation.

[d] CoA, coenzyme A.

a 48% reduction in the number of inclusion-forming units (IFUs) recovered from the cells cultured in glutamate and a 33% reduction in the number of IFUs recovered from cells cultured in α-ketoglutarate compared to the IFUs from cells cultured in the presence of glucose. These results suggest that exposure to the carbon sources tested should have an effect on gene expression by 6 h; therefore, infected L929 cells were cultured in either 10 mg of glucose per ml, 20 mM glutamate, or 20 mM α-ketoglutarate for 6 h at which time chlamydia-specific RNA was isolated as previously described (8). For each hybridization, equal quantities of cDNA generated from cells grown with glutamate or α-ketoglutarate were compared to equal quantities of cDNA generated from cells grown with glucose. At least three independent infections in which chlamydiae were harvested from infected cells grown with each specific carbon source were performed. Gene expression data were then normalized to 16S rRNA.

The expression profiles for all the genes involved in carbon metabolism and transport are listed in Table 1. Minimal or no changes in central metabolism gene expression levels were found when cells were cultivated in the presence of glutamate as the sole carbon source versus glucose (Table 1). Additionally, minimal transcriptional changes were also found on a global scale (data not shown). Statistical significance was assessed using the significance analysis of microarray program by Tusher et al. (19). Of the entire genome, only four genes were found to be significantly upregulated (*q* value of 0.04) (19): CT084 (+1.94-fold), CT198 *oppA* (+1.91-fold), CT451 *cdsA* (+1.84-fold), and CT057 *gcpE* (+1.74-fold). Although these genes were reproducibly and reliably upregulated, each gene displayed low fold changes (i.e., less than twofold) in gene expression. Conversely, no genes were significantly downregulated (*q* value of 0.04) (19). *C. trachomatis* has a conserved 7.5-kb plasmid (2, 11, 14), which has been proposed to play a role in glycogen accumulation, since strains of *C. trachomatis* lacking the plasmid no longer accumulate glycogen (6). Plasmid-specific genes had no changes in gene transcription when cells were cultivated in the presence of glucose compared to glutamate as the sole carbon source (data not shown).

When the expression profiles from cells cultured in the presence of 20 mM α-ketoglutarate as the sole carbon source were tested, very little change in global gene transcription was observed, including genes involved in central carbohydrate metabolism (Table 1). Additionally, the dicarboxylate-specific porin gene *porB*, which has been shown to transport α-ketoglutarate in vitro (5), remained unaffected. Four genes were found to be significantly upregulated (*q* value of 0.39) (19): CT576 *lcrH* (+2.36-fold), CT288 (+1.80-fold), CT181 (+1.79-fold), and CT814 (+1.69-fold). In contrast to the global transcriptional response seen in the presence of glutamate, the transcriptional response when cells were grown in the presence of α-ketoglutarate as the sole carbon source involved a high number of genes having a low negative fold change (data not shown), suggesting an overall lower chlamydial vitality. This conclusion is consistent with previous findings (4) and the results from the infectivity titration assays, showing a lower number of infectious EBs produced when chlamydia-infected cells were cultured in α-ketoglutarate as the sole carbon source than cells grown in glucose.

To confirm the array results, quantitative reverse transcriptase PCR (qRT-PCR) was used to assess key central metabolism genes as well as CT576 *lcrH* and CT084, which were upregulated in the microarray assays. CT084 is believed to be involved in pathogenesis with homology to a phospholipase D-like HKD superfamily-secreted protein, and CT576 *lcrH* is thought to be involved in pathogenesis by serving a role in type III secretion (16). The metabolic genes CT815 *mrsA*, CT489 *glgC*, CT798 *glgA*, and CT866 *glgB* are involved in glycogen synthesis, while CT042 *glgX* and CT248 *glgP* are glycogen-degrading enzymes. The key carbohydrate transporters ana-

TABLE 2. qRT-PCR analysis of total RNA extracted from *C. trachomatis* L2-infected L929 cells[a]

ORF no. and gene name[b]	Change in expression (fold change)[c]	
	Glutamate	α-Ketoglutarate
CT042 *glgX*	1.40 ± 0.13	−1.06 ± 0.04
CT084	2.13 ± 0.23	1.31 ± 0.14
CT129 *glnP*	1.22 ± 0.17	−1.63 ± 0.08
CT204 *sodTi*	1.76 ± 0.15	−1.46 ± 0.05
CT216 *xasA*	1.97 ± 0.13	−2.28 ± 0.06
CT230	2.00 ± 0.09	1.85 ± 0.04
CT290 *ptsN*	1.89 ± 0.33	−1.61 ± 0.11
CT401 *gltT*	1.59 ± 0.17	−1.34 ± 0.10
CT544 *uhpC*	1.19 ± 0.08	−1.55 ± 0.03
CT576 *lcrH*	2.06 ± 0.29	2.18 ± 0.28
CT613 *folP*	1.87 ± 0.24	−2.69 ± 0.05
CT798 *glgA*	1.73 ± 0.10	−1.67 ± 0.04

[a] At least three independent infections in which total RNA was harvested from infected cells from each specific carbon source were performed. Total RNA (1 μg) was used to generate cDNA using random hexamers. qRT-PCR was performed in an ABI 7700 sequence detection system using SYBR green master mix. Gene expression data were normalized to 16S rRNA.

[b] Open reading frame (ORF) number and gene names are based on the naming system of Stephens et al. (16).

[c] Change in expression of cells grown with glutamate or α-ketoglutarate as the sole carbon source compared to expression in cells grown with glucose as the sole carbon source. Change in expression is shown as the mean fold change ± standard deviation.

lyzed by qRT-PCR include the following: CT544 *uhpC*, a glucose phosphate transporter; CT401 *gltT*, a glutamate transporter; CT204 *sodTi*, a dicarboxylate translocator which takes up oxaloacetate or α-ketoglutarate in return for malate; and two genes that have homology to glutamate transporters, CT216 *xasA* and CT230. CT290 *ptsN* has been proposed to encode a potential regulator of the glycolytic/gluconeogenic flux. We found a high correlation coefficient (0.93) between the qRT-PCR results and the microarray data. For example, both CT576 *lcrH* and CT230 were slightly upregulated in the presence of glutamate or α-ketoglutarate as the sole carbon source (Table 2). There was little to no change in gene transcription of the other genes assayed as measured by qRT-PCR, regardless of the type of carbon source available (Table 2). In summary, global gene expression in *C. trachomatis* was largely unaffected by changes in the availability of the carbon sources tested. These results are consistent with the report by Iliffe-Lee et al. (4), which demonstrated that six genes key to central metabolism were unaffected by carbon source availability.

Chlamydiae, like other obligate pathogenic bacteria living within an eukaryotic host (7), were transcriptionally unresponsive or inflexible to nutrient-based environmental changes, such as carbon source availability. This is in striking contrast to the expression profiles for free-living bacteria, where the growth of *E. coli* in media rich in glucose compared to growth in media containing only gluconeogenic substrates leads to a massive switch in global gene expression affecting more than 700 genes (9, 10). The eukaryotic host contains a dynamic assortment of energy sources, such as ATP, amino acids, and various carbon sources. As chlamydiae bacteria grow and develop within their eukaryotic host, these energy sources are consumed and are ultimately depleted. An attractive hypothesis is that depletion of such energy stores could serve as the molecular switch behind the morphological changes that occur during the developmental cycle; however, carbon source depletion does not appear to serve as a signal for developmental regulation of chlamydiae.

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