Transcriptome analysis of chlamydial growth during IFN-γ-mediated persistence and reactivation

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Chlamydia trachomatis **is an obligatory intracellular prokaryotic parasite that causes a spectrum of clinically important chronic inflammatory diseases of humans. Persistent infection may play a role in the pathophysiology of chlamydial disease. Here we describe the chlamydial transcriptome in an *in vitro* model of IFN-γ-mediated persistence and reactivation from persistence. Tryptophan utilization, DNA repair and recombination, phospholipid utilization, protein translation, and general stress genes were up-regulated during persistence. Down-regulated genes included chlamydial late genes and genes involved in proteolysis, peptide transport, and cell division. Persistence was characterized by altered but active biosynthetic processes and continued replication of the chromosome. On removal of IFN-γ, chlamydiae rapidly reentered the normal developmental cycle and reversed transcriptional changes associated with cytokine treatment. The coordinated transcriptional response to IFN-γ implies that a chlamydial response stimulon has evolved to control the transition between acute and persistent growth of the pathogen. In contrast to the paradigm of persistence as a general stress response, our findings suggest that persistence is an alternative life cycle used by chlamydiae to avoid the host immune response.**

microarray analysis | chlamydia | genomics | latency | stimulon

Chlamydia trachomatis is an obligatory intracellular prokaryotic pathogen that exhibits a tropism for conjunctival and urogenital columnar epithelial cells (1). The organism is distinguished from other pathogens by its unique biphasic life cycle that modulates between an infectious elementary body (EB) and a noninfectious, metabolically active reticulate body (RB) (2). Infection of the eye results in blinding trachoma, the world's leading cause of preventable blindness, whereas infection of the female genital tract can produce salpingitis resulting in infertility and ectopic pregnancy (1). Underlying pathophysiological processes that lead to the development of chronic inflammatory disease have not been defined. Persistent infection of mucosal sites may provide a sustained antigenic stimulus that drives the inflammatory response (3, 4).

Chlamydial infection of mucosal surfaces elicits a dominant cellular immune response characterized by antigen-specific IFN-γ-secreting $CD4^+$ and $CD8^+$ T cells (5–9). In human epithelial cells, IFN-γ activates the expression of indoleamine 2,3-dioxygenase, which catabolizes L-tryptophan to *N*-formylkynurenine (4). Chlamydiae are tryptophan auxotrophs that normally acquire this essential amino acid from the host (10). IFN-γ-mediated depletion of tryptophan inhibits chlamydial growth, and continuous exposure results in eradication of infection. Subinhibitory concentrations of IFN-γ are likely to occur *in vivo*, physiological conditions that may favor persistence. During tryptophan-limiting growth, chlamydiae transform into aberrant noninfectious organisms that persist within host cells. These cryptic persistent forms rapidly retransform back to normal RBs and infectious EBs when host tryptophan pools return to normal levels (11).

How chlamydiae modulate between normal and persistent growth is not understood. A depiction of the chlamydial transcriptome profile under these contrasting conditions of growth could yield information about this important host–parasite relationship. Here we describe such studies and report that a chlamydial response stimulon has evolved to direct the conversion between normal and persistent growth.

Materials and Methods

IFN-γ Treatment of Chlamydia-Infected Cells. HeLa 229 cells were grown in DMEM-10 at 37°C in 5% CO_2. Cells were seeded into TC24 culture plates at a density of 4×10^5 cells per ml in DMEM-10 or DMEM-10 containing recombinant human IFN-γ (50 units/ml) and incubated for 24 h at 37°C. Monolayers were infected with *C. trachomatis* serovar D at a multiplicity of infection of 1 (5). Infected cells were incubated in the presence or absence of IFN-γ at 37°C, harvested in sucrose-phosphate-glutamic acid buffer, and titered on monolayers of HeLa 229 cells. Reactivated cultures were prepared from infected cells treated with IFN-γ for 24 h, the IFN-γ was removed, and the cells were pulsed for 24 h with medium containing 10× tryptophan.

Microarray. The spotted cDNA microarray was designed based on the genomic sequence of *C. trachomatis* serovar D (10) (GenBank accession no. AE001273) and constructed as described (12).

RNA Purification, Hybridization, and Microarray Analysis. Total RNA was isolated from 8×10^7 infected cells at 12, 24, and 48 h postinfection (PI) in the presence or absence of IFN-γ. Cells were lysed in 10 ml of TRIzol (Invitrogen) to isolate total RNA. Polyadenylated host mRNA was removed by using oligo(dT) columns (Oligotex, Qiagen, Valencia, CA). Chlamydial mRNA was repurified (RNeasy, Qiagen) and primed for cDNA synthesis (Superscript Choice System, Invitrogen) by using a complete set of complementary 3′ oligonucleotides (12). Chlamydial 16S rRNA levels were determined by using a quantitative RT-PCR (qPCR) procedure (12). Labeled probe samples were prepared from RNA samples containing equivalent amounts of 16S rRNA. Residual RNA was removed by alkaline hydrolysis, and bacterial cDNA was repurified and labeled with Cy3 or Cy5 by using a random priming procedure. Labeled cDNAs from control and IFN-γ-treated cultures at 12 and 24 h PI were compared by cohybridization on the same microarray slides. Comparisons of gene expression profiles during recovery from persistence were done by cohybridizing labeled cDNAs obtained from untreated infected cultures at 48 h PI with cDNAs prepared from IFN-γ-treated reactivated cultures. Fluorescence values were determined by using a ScanArray 5000

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Abbreviations: EB, elementary body; PI, postinfection; PLD, phospholipase D; qPCR, quantitative RT-PCR; RB, reticulate body.

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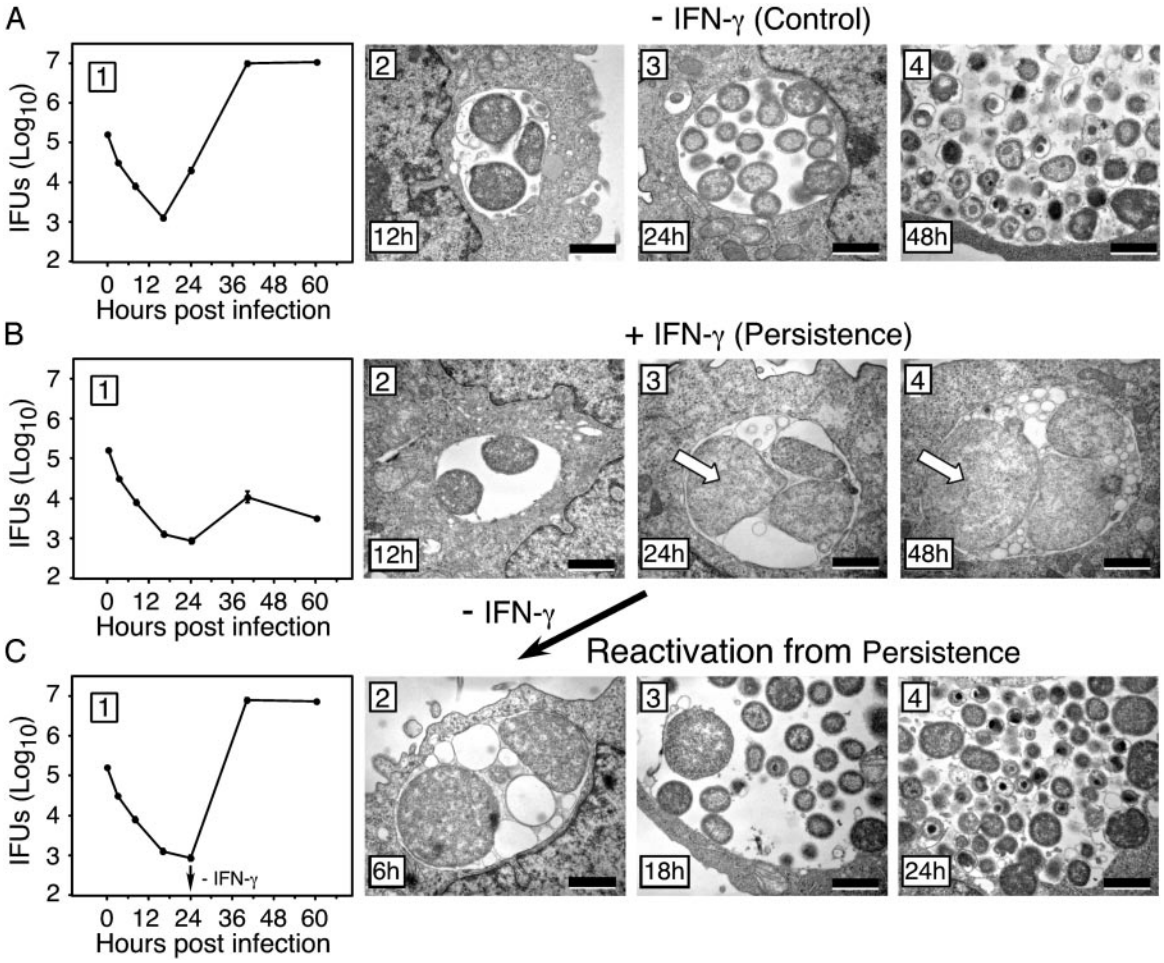

Fig. 1. Model of IFN-γ chlamydial persistence and reactivation from persistence. Shown are one-step growth curves with corresponding ultrastructural images from normal, IFN-γ-treated, and reactivation from IFN-γ treatment cells. (*A*) Untreated chlamydia-infected HeLa 229 cells. (*A1*) One-step growth curve showing a 16-h latency period followed by a rapid burst in recoverable IFU. Typical RB developmental forms are present at 12 (*A2*) and 24 (*A3*) h PI. By 48 h PI (*A4*) the inclusion contains a mixture of characteristic RBs and EBs. (*B*) IFN-γ-treated chlamydia-infected HeLa cells. A similar latency period is observed, but, unlike untreated cells, there is no infectious burst (*B1*). RBs appear similar to untreated cultures at 12 h PI (*B2*); however, at 24 (*B3*) and 48 (*B4*) h PI, the RBs are distinctly different, appearing as large aberrant noninfectious forms. (*C*) Reactivation from persistence. There was a rapid recovery in infectious EB after removal of IFN-γ (*C1*); recoverable IFU was 96% of control (untreated) infections. At 6 h, reactivation chlamydiae remained aberrant (*C2*), but by 18 (*C3*) and 24 (*C4*) h after removal of IFN-γ, inclusions were typical with morphologically normal EBs and RBs. (Scale bar, 1 μm.)

scanner and the QUANTARRAY software package (Perkin–Elmer). Corrected fluorescence intensity values were analyzed by using the GENESPRING software package (Silicon Genetics, Redwood City, CA). The IFN-γ growth experiment was performed twice and the hybridizations were performed in duplicate (i.e., 12 measurements for each gene for a particular time point because each gene was spotted on the microarray in triplicate).

qPCR. Primer and probe sets were designed for selected genes by using PRIMER EXPRESS software (Applied Biosystems). Standard curves were performed by using chromosomal template DNA at concentrations ranging from 10 to 0.001 ng/ml, and qPCR was performed in triplicate as described (12).

Transmission Electron Microscopy (TEM). Chlamydia-infected cells were fixed, and 70- to 80-nm sections were prepared for TEM as described (12).

Results

***In Vitro* Model of Chlamydial Persistent Infection.** One-step growth curves of chlamydiae grown in cells in the absence or presence of IFN-γ and after removal of IFN-γ are shown in Fig. 1 *A1*, *B1*, and *C1*. Morphological characteristics of chlamydiae grown under identical conditions are shown in the accompanying TEM (Fig. 1 *A2–A4*, *B2–B4*, and *C2–C4*). Chlamydiae grown in the absence of IFN-γ exhibit a 16-h latency period followed by a rapid recrudescence of infectious organisms with titers peaking at 40 h PI (Fig. 1*A1*). In contrast, IFN-γ-treated cultures yielded a basal level of infectious organisms at all time points PI (Fig. 1*B1*). After removal of IFN-γ there was a rapid increase in the recovery of infectious progeny yielding titers that equaled control cultures (Fig. 1*C1*). Reactivation from IFN-γ, as determined by the time required to achieve maximal recoverable infectious organisms, was more rapid than that of untreated cultures (Fig. 1 *A1* and *C1*). Morphological characteristics of chlamydiae are shown in Fig. 1. Chlamydial morphology did not differ in control (Fig. 1*A2*) and IFN-γ-treated (Fig. 1*B2*) cultures at 12 h PI; each exhibited small numbers of typical RB forms. In contrast, profound morphological differences were evident at 24 h posttreatment. Inclusions in untreated cells contained dividing, morphologically typical RB forms (Fig. 1*A3*), whereas RBs in IFN-γ-treated cells (Fig. 1*B3*) were fewer, enlarged, and structurally aberrant. Aberrant RBs were not infectious and failed to differentiate into infectious EBs (Fig. 1*B1*) but redifferentiated into typical RBs and EBs after removal of IFN-γ (Fig. 1 *C3*

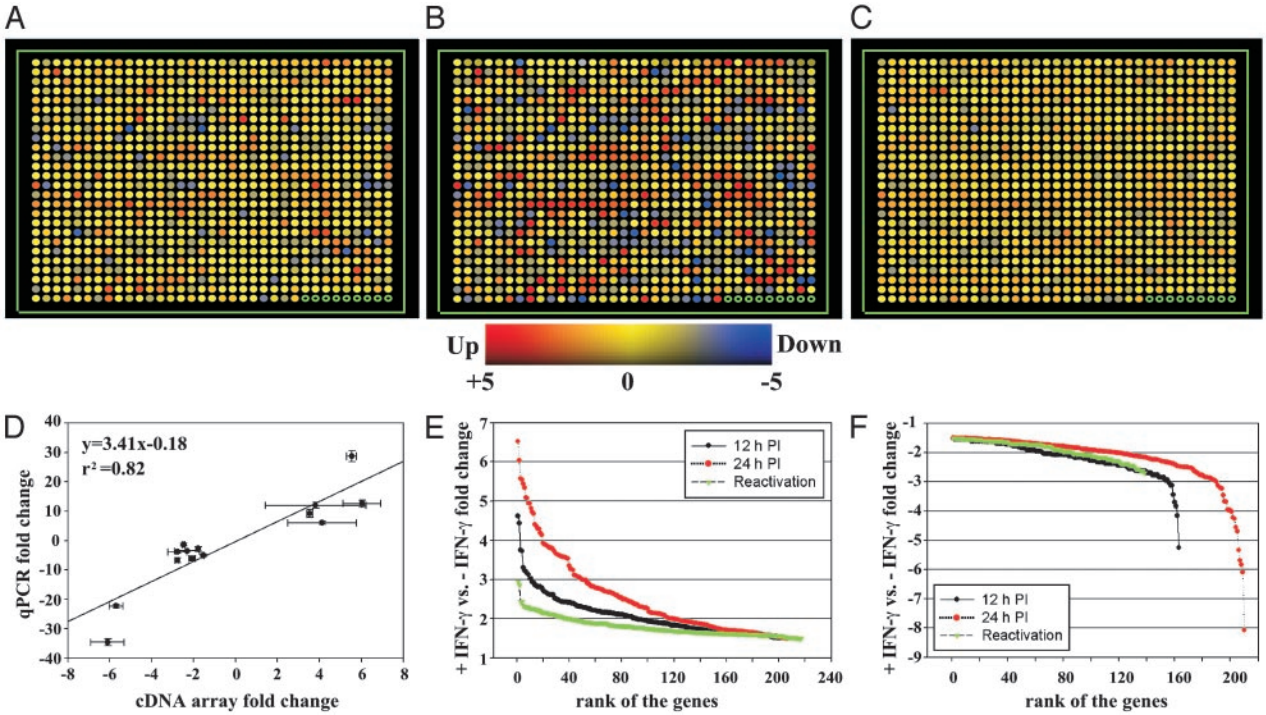

Fig. 2. Microarray analysis of *C. trachomatis* gene expression in infected HeLa 229 cells cultured in the presence and absence of IFN-γ. (*A*) Gene expression differences between infections in the absence and presence of IFN-γ for 12 h. (*B*) Gene expression differences between infections in the absence and presence of IFN-γ for 24 h. (*C*) Gene expression differences between an infection in the absence of IFN-γ for 48 h PI and an infection in the presence of IFN-γ for 24 h, followed by the removal of IFN-γ and tryptophan supplementation for 24 h. Data are presented in an array layout format representing a linear map of the bacterial genome, i.e., starting from the origin of replication in the upper left corner and proceeding from CT001 to CT035 in the top row, CT036 to CT070 in the second row, and proceeding until the final row, which contains the last chromosomal ORF (CT876), the eight plasmid ORFs, and nine negative controls. Fold change in expression is given for each gene according to the colored legend as IFN-γ-treated/control. Correlation between the fold changes (+IFN-γ vs. −IFN-γ at 24 h PI) measured by qPCR and microarray is shown in *D*. Genes exhibiting a ±1.5-fold difference in expression were plotted based on fold change at 12 and 24 h PI, and after reactivation from IFN-γ treatment (*E* and *F*). Up-regulated genes numbered 202, 204, and 217 for the 12-h, 24-h, and reactivation samples, respectively. Fold changes were greatest at 24 h (1.5–6.5) and minimal in reactivation (1.5–3.0). A similar pattern was also observed for the down-regulated genes: 163, 210, and 138 genes for the 12-h, 24-h, and reactivation samples, respectively, with fold changes ranging from 1.5 to 8.1 (24 h), 1.5 to 5.3 (12 h), and 1.5 to 2.7 (reactivation).

and *C4*). Thus, this *in vitro* model provides a controlled biologically relevant system for the transcriptome analysis of chlamydia grown under normal and persistent growth and reactivation from persistence.

Transcriptome of *C. trachomatis* Persistent Infection. The transcriptome of chlamydiae grown in cells treated with IFN-γ and after removal of IFN-γ (reactivation from persistence) is shown in Fig. 2 *A–C*. Transcriptional changes occurred at both 12 and 24 h post-IFN-γ treatment with maximal differences at 24 h. Transcriptional changes at the 12-h period occurred independent of distinguishable morphological changes, indicating that these gene expression differences had not yet been translated into phenotypically observable effects. In contrast, differences in gene expression at 24 h (Fig. 2*B*) posttreatment were associated with marked morphological changes and aberrant noninfectious RB forms (compare *A3* and *B3* of Fig. 1). Chlamydial gene expression normalized after removal of IFN-γ (Fig. 2*C*) and was accompanied by rapid transformation of persistent aberrant forms to typical RBs and infectious EBs (Fig. 1*C1*). A complete list of each of the 901 genes transcribed under these growth conditions is provided in Table 2, which is published as supporting information on the PNAS web site.

We next performed qPCR on a select subset of genes (five up-regulated and nine down-regulated) from the 24-h IFN-γ-treated and untreated samples. The qPCR results exhibited a high correlation coefficient (0.82) with the microarray data (Fig. 2*D*). This correlation was observed even when the fold difference(s) was small (e.g., 1.54-fold down-regulation of *ftsW*). The microarray results tended to underestimate changes in gene expression found by qPCR. These results argue that even a relatively lower fold in up- or down-regulation detected by microarray can be considered valid in consideration for future experimental analysis.

Genes exhibiting a ±1.5-fold change in expression level were rank based according to fold change in expression (Fig. 2 *E* and *F*). A similar subset of genes was up- or down-regulated but with differences in magnitude in each of the experimental conditions. Notably, 70% of the up-regulated and 61% of the down-regulated genes were common between the cultures treated for 12 and 24 h. The greatest change in intensity of gene expression occurred in infected cells treated for 24 h with IFN-γ. Transcriptional differences normalized after reactivation from persistence. These results indicate (*i*) a common subset of genes is differentially regulated in response to IFN-γ-mediated persistent growth and (*ii*) a distinct divergence between the transcriptomes of the persistent and reactivated (normal) growth cycle. Because diverse regulons are differentially regulated, we conclude that a chlamydial response stimulon (13) has evolved that directs the transition between acute and persistent growth in response to IFN-γ.

Chlamydial genes exhibiting greatest differences in expression in the absence or presence of IFN-γ at the 24-h-PI time point are listed in Table 1. Highly up-regulated genes included those required for tryptophan synthesis (*trpR*, *trpB*, and *trpA*), DNA repair and recombination (e.g., *recA* and *yqgF*), phospholipid biosynthesis (e.g., *ct156*, *ct158, ct284*, *lip*A, and *pgsA.2*) and translation (including numerous *rs* and *rl* genes encoding ribosomal proteins). Many early cycle genes were up-regulated at this time, including *incD-G* and

MICROBIOLOGY

Table 1. Expression changes associated with growth in the presence of IFN-γ at 24 h PI

CT no.	Gene	Function	Fold change (+IFN-γ vs. −IFN-γ) cDNA array	Fold change (+IFN-γ vs. −IFN-γ) qPCR
		Up-regulated genes		
Tryptophan utilization				
CT169	*trpR*	Repressor of tryptophan synthase	2.30 ± 0.21	ND
CT170	*trpB*	Tryptophan synthase (beta)	6.52 ± 0.72	458 ± 6.76
CT171	*trpA*	Tryptophan synthase (alpha)	1.80 ± 0.21	ND
DNA repair and recombination				
CT184	*yqgF*	Holliday junction resolvase	2.90 ± 1.86	ND
CT347	*xerC*	Integrase/recombinase	1.57 ± 0.14	ND
CT497	*dnaB*	Replicative DNA helicase	4.81 ± 0.32	ND
CT555	*mot.1*	SNF2 helicase	2.11 ± 0.22	ND
CT643	*topA*	DNA topoisomerase (fused to SWI)	2.08 ± 0.12	ND
CT650	*recA*	Recombination protein A	3.81 ± 2.40	12.0 ± 0.96
Phospholipid biosynthesis				
CT156	*lpd*	Phospholipase D (HKD)	3.59 ± 2.88	ND
CT158	*lpd*	Phospholipase D (HKD)	3.84 ± 0.45	ND
CT284		Phospholipase D-like protein	1.93 ± 0.22	ND
CT402	*lpxK*	Tetraalyldisaccharide 4′ kinase	1.98 ± 0.15	ND
CT558	*lipA*	Lipoic acid synthase	2.54 ± 1.15	ND
CT797	*pgsA.2*	Glycerophosphate synthase	4.13 ± 1.64	6.03 ± 0.52
General stress and early genes				
CT117*	*incF*	Inclusion membrane protein	4.28 ± 0.24	ND
CT228		Inclusion membrane protein	6.03 ± 0.90	12.6 ± 1.21
CT446	*euo*	DNA binding protein	5.54 ± 0.24	28.7 ± 1.80
CT473	*yidD*	Predicted α-hemolysin	4.94 ± 0.58	ND
CT603	*ahpC*	Thiol-specific antioxidant protein	3.62 ± 0.38	ND
CT734		Conserved hypothetical	4.36 ± 0.42	ND
Translation				
CT027	*trmD*	tRNA methyltransferase	2.98 ± 0.17	ND
CT420*	*rl21*	Ribosomal protein L22	5.08 ± 0.19	ND
CT437	*fusA*	Elongation factor G	3.54 ± 0.14	9.21 ± 1.38
CT524*	*rs19*	Ribosomal protein S19	3.93 ± 0.21	ND
CT679	*tsf*	Elongation factor TS	2.49 ± 0.15	ND
CT752	*efp.2*	Elongation factor P	2.54 ± 0.15	ND
CT830	*ytgB.2*	rRNA methyltransferase	5.44 ± 0.33	ND
CT833	*infC*	Translation initiation factor 3	5.07 ± 0.46	ND
General and unknown				
CT038		Conserved hypothetical	3.64 ± 0.59	ND
CT296	*dcrA*	Divalent cation-dependent regulator	3.26 ± 0.23	ND
CT345		Conserved hypothetical	4.29 ± 1.36	ND
CT484		Conserved hypothetical	4.72 ± 0.26	ND
CT505	*gapA*	GAPDH	3.05 ± 0.44	ND
CT591	*sdhB*	Succinate dehydrogenase (Fe/S)	4.68 ± 1.01	ND
CT602		Conserved hypothetical	3.74 ± 0.32	ND

euo. Up-regulation of the *trpRBA* operon was consistent with the deprivation of host L-tryptophan (4). Increased expression of genes related to phospholipid metabolism during persistence including phospholipase D (PLD) paralogs *ct156* and *ct158* in the plasticity zone (PZ), as well as the extra-PZ PLD paralog *ct284*, is supportive of the hypothesis that the PLDs may have a role in pathogenesis of *C. trachomatis* (14). In agreement with previous reports, we did not observe significant changes in the expression levels of *groEL* during IFN-γ-induced chlamydial growth (4).

Down-regulated genes included genes that function in RB to EB differentiation (*hctA*, *hctB*, *ompB*, and *ompC*), proteolysis and peptide transport (e.g., *clpP.2*, *clpX*, *oppB.2*, and *oppC.2*), cell division (e.g., *dnlJ*, *amiA*, and *ftsW*), TCA cycle (e.g., *sucA*, *sucB.1*, *sucB.2*, and *sucC*), and the major outer membrane protein (*ompA*). Two genes (*ompA* and *ftsW*) were identified that were previously reported to be down-regulated in a monocyte model of persistence (15). In contrast to Gerard *et al.* (15), we did not observe down-regulation of *ftsK*. *In toto*, the transcriptional profiling results are consistent with the biological properties described for aberrant RBs: that they are incapable of normal binary fission and secondary differentiation into infectious EB.

We next performed qPCR temporal analysis of differentially regulated genes in the presence and absence of IFN-γ treatment (12 and 24 h PI) and after reactivation (Fig. 3). Four up-regulated and four down-regulated genes were analyzed between the 12- and 24-h treatment and during reactivation. Expression of each gene normalized during reactivation. For example, *trpB* is ≈500-fold up-regulated in the presence of IFN-γ at 24 h PI but returns to normal levels after the 24-h reactivation. Expression of late cycle genes *hctA* and *omcB*, which encode the chromatin condensing histone-like protein 1 and the cysteine-rich 60-kDa outer membrane cross-linking protein, respectively, resumed rapidly after removal of IFN-γ. Ultrastructural analysis corroborated the expression of this late gene family after reactivation from persistence (Fig. 1 *B3* and *C2–C4*). Chlamydial aberrant forms retransformed into characteristic RBs that further developed into the archetypal smaller EBs exhibiting condensed nucleoid and compact membrane structures.

Euo is a *Chlamydia*-specific protein with DNA-binding activity previously proposed to function as a regulator of late gene expression (16, 17). Augmented expression of *euo* has been associated with glucose deprivation but not specifically persistence (18). Expression of *euo* was 30-fold up-regulated in IFN-γ-treated cells. Elevated expression suggests that additional Euo might be required to ensure complete silencing of late genes during persistent growth.

Table 1. (continued)

CT no.	Gene	Function	Fold change (+IFN-γ vs. −IFN-γ) cDNA array	qPCR
		Down-regulated genes		
Late genes				
CT046	*hctB*	Histone-like protein 2	1.92 ± 0.37	ND
CT080	*ltuB*	Late-transcription unit protein 2	2.07 ± 0.16	6.10 ± 0.85
CT443	*omcB*	Cysteine-rich OMP (60 kDa)	6.09 ± 0.80	34.6 ± 1.16
CT444	*omcA*	Cysteine-richOMP (9 kDa)	2.82 ± 1.03	ND
CT546		Predicted OMP	2.31 ± 0.59	3.50 ± 0.09
CT565		Conserved hypothetical	8.08 ± 1.76	ND
CT659		Conserved hypothetical	5.84 ± 2.25	ND
CT694		Hypothetical	3.00 ± 0.39	ND
CT743	*hctA*	Histone-like protein 1	5.69 ± 0.33	22.3 ± 0.65
CT780		Protein disulfide isomerase	2.78 ± 0.09	6.75 ± 0.83
CT783		Protein disulfide isomerase	3.70 ± 0.12	ND
CT868	*mtpB*	Membrane thiol protease	2.13 ± 0.33	ND
Proteolysis and peptide transport				
CT113	*clpB*	Clp protease (chaperonin)	2.12 ± 0.23	ND
CT286	*clpC*	Clp protease (chaperonin)	2.71 ± 0.52	ND
CT478	*oppC.2*	Oligopeptide transport (permease)	4.02 ± 0.47	ND
CT479	*oppB.2*	Oligopeptide transport (permease)	2.56 ± 0.36	ND
CT480	*oppA.4*	Oligopeptide transport (lipoprotein)	2.48 ± 0.06	1.34 ± 0.48
CT705	*clpX*	Clp protease (ATP binding subunit)	2.43 ± 0.35	ND
CT706	*clpP.2*	Clp protease (proteolytic subunit)	2.29 ± 0.21	ND
CT806	*ptr*	Protease III	2.38 ± 0.30	ND
CT823	*htrA*	Periplasmic serine protease	2.48 ± 0.21	ND
CT858		Multidomain protease	2.24 ± 0.47	ND
Cell division				
CT146	*dnaJ*	DNA ligase	2.89 ± 1.10	ND
CT267	*ihfA*	DNA binding protein (IHF-like)	2.77 ± 0.46	3.82 ± 0.45
CT268	*amiA*	N-acetylmuramoyl alanine amidase	3.97 ± 2.67	ND
CT275	*dnaA.2*	Replication initiation factor 2	3.46 ± 0.13	ND
CT760	*ftsW*	Cell division membrane protein	1.54 ± 0.06	5.04 ± 0.31
TCA cycle				
CT054	*sucA*	α-Ketoglutarate dehydrogenase	2.77 ± 0.80	ND
CT055	*sucB.1*	Lipoamide succinyltransferase 1	2.35 ± 0.30	ND
CT400	*sucB.2*	Lipoamide succinyltransferase 2	2.47 ± 0.32	ND
CT821	*sucC*	Succinyl CoA synthase (beta)	4.27 ± 0.12	ND
CT822	*sucD*	Succinyl CoA synthase (alpha)	4.23 ± 1.24	ND
General and unknown				
CT223		Conserved hypothetical	5.34 ± 0.29	ND
CT259		Protein phosphatase (Ser/Thr)	3.98 ± 0.49	ND
CT610	*cadd*	Death domain receptor protein	2.85 ± 0.60	ND
CT681	*ompA*	Major OMP	1.78 ± 0.05	2.80 ± 0.72
CT713	*ompB*	OMP	4.70 ± 0.98	ND
CT812	*pmpD*	Polymorphic OMP	4.56 ± 0.58	ND
CT869	*pmpE*	Polymorphic OMP	3.68 ± 0.13	ND

ND, not determined.

*Data are representative of other genes in the same operon (i.e., *inc* F for the *inc* D,E,F,G operon) or the same functional groups (*rl*22 for 14 *rl* genes and *rs*19 for 12 *rs* genes).

Consistent with this interpretation, *euo* expression decreases 20-fold in the 12-h period after the removal of IFN-γ, and this decrease is accompanied by concomitant up-regulation of the late gene family and differentiation of aberrant RBs into infectious EBs.

Chlamydial Growth and Cell Division During Persistence and Reactivation. Transcription of peptidoglycan-related biosynthetic and cell division-associated genes did not significantly decrease during persistence with two notable exceptions, *ftsW* and *amiA*. In *E. coli*, FtsW and AmiA are involved in the final stages of cell division, as a chaperone to PBP 3 and a septal amidase, respectively (19, 20). Transcription of the chlamydial homologues of *ftsW* and *amiA* in *C. trachomatis* was decreased during persistence but rapidly returned to normal after removal of IFN-γ (Table 1). In contrast, expression of the *C. trachomatis ftsK* homologue was similar in persistent and normally grown *C. trachomatis*. In *E. coli*, FtsK is recruited to the FtsZ ring before final septal peptidoglycan synthesis by PBP 3 and separation of daughter cells by amidase action. These findings suggest that all but the terminal phases of septation are complete in the aberrant persistent RB forms.

Discussion

The transcriptome results described here demonstrate that an IFN-γ-inducible stimulon mediates *C. trachomatis* persistence. This stimulon likely evolved in response to host cellular immunity as a consequence of the indoleamine 2,3-dioxygenase-mediated tryptophan deprivation effector function of IFN-γ (4). Although we show that some stress response regulons are components of and may be mediated by the persistence stimulon, the persistence stimulon regulates more than a simple stress response. It is in fact, a more global response that permits chlamydiae to survive and multiply in opposition to host immunological defenses.

Correlating the transcriptional profiles of persistence stimulon genes, putative functions of these genes in other bacteria, and

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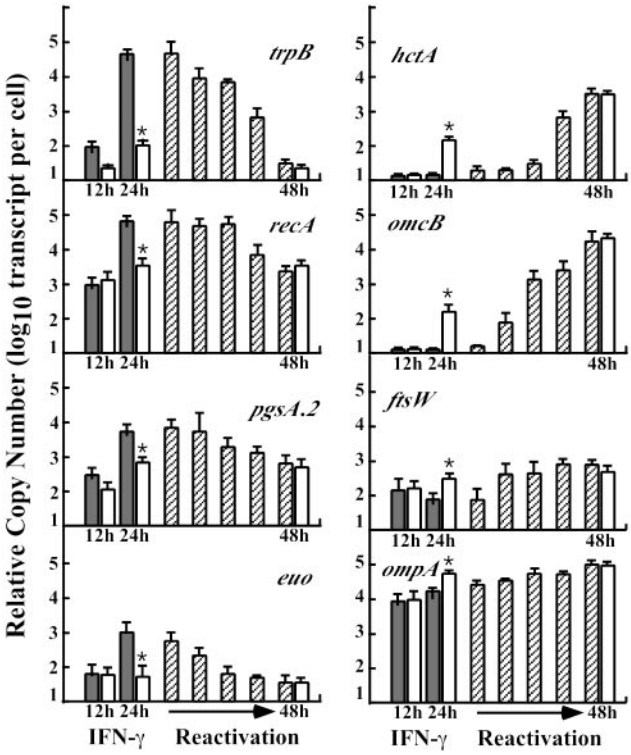

Fig. 3. Quantitative RT-PCR analysis of gene expression. (*Left*) Up-regulated genes. (*Right*) Down-regulated genes. Copy numbers of gene transcripts per bacterial cell are given for control cultures (open bars), IFN-γ-treated cultures (filled bars), and reactivated cultures at 3, 6, 12, 18, and 24 h post-IFN-γ removal (hatched bars). Copy number differences at 24 h PI for control and IFN-γ-treated cultures are indicated by an asterisk if their differences are considered significant at a level of $P < 0.005$.

described phenotypes of persistent *C. trachomatis* in cell culture, we can make certain predictions as to how the stimulon may mediate persistence *in vivo*. Four biological functions must be achieved by this stimulon for *C. trachomatis* to survive in a persistent state: the bacteria must (*i*) sense and respond to tryptophan depletion, (*ii*) arrest secondary differentiation from RB to EB by silencing the expression of late cycle genes, (*iii*) block cell division but not chromosome replication, and (*iv*) maintain metabolic pathways that prepare the bacteria for rapid reactivation and differentiation.

Host cell tryptophan depletion, mediated by IFN-γ, is a primary means of defense against *C. trachomatis* (8). Our work reinforces this hypothesis and suggests that this phenomenon is relevant to infection *in vivo*. Tryptophan synthase (*trpBA*) is massively up-regulated during persistence, highlighting the importance of restoring tryptophan availability to bacterial survival. Interestingly, neither the host nor chlamydiae produce the indolic substrates necessary for formation of tryptophan by this enzyme. It has been suggested that the source of indole is other microflora of the female genital tract (21). Collectively, these results suggest that the *C. trachomatis* persistence stimulon evolved as an adaptation to the host immune response, and, conceivably, it appears that the pathogen has similarly evolved a strategy of reactivation from persistence by interaction with the polymicrobial flora present in the genital tract.

Persistent chlamydiae decrease expression of late gene products and avert the process of differentiation from RBs to EBs. Expression of *euo*, whose protein product has been suggested to repress late transcription (17), is dramatically up-regulated in persistent *C. trachomatis* but markedly drops after removal of IFN-γ. Thus, strict silencing of late gene products during persistence may be a function of this protein.

During persistent growth, aberrant RBs continue chromosome replication but fail to divide (15). Transcript levels for DNA replication and for cell wall biosynthetic genes, are similar between normal and persistent forms. However, important exceptions are the *ftsW* and *amiA* genes, which are significantly down-regulated during persistence. How can these seemingly contradictory observations help us understand the biological basis for persistence and reactivation? First, one must infer functional aspects of FtsW and AmiA from *E. coli*, which are as a chaperone of PBP 3 and as an amidase that separates the biosynthetically complete cell wall into distinct sacculi with the resultant formation of daughter cells, respectively. Thus, in chlamydiae these functions can be inferred as functional blocks in the very late stages of cell division during persistence. Similar *ftsK* transcript levels in persistent and normal chlamydiae suggest that cell division in persistent forms is blocked before resolution of daughter RBs. This unique block in cell division explains why equal numbers of infectious EBs are rapidly recovered from cultures after removal of IFN-γ (Fig. 1*C1*). Thus, during persistent growth chlamydiae continue most metabolic processes but block the last stages of cell division. The trigger to reactivation is sensitive and provides a mechanism for quick reactivation in a permissive host environment.

The paralogous family of PLD genes localized within the chlamydial plasticity zone (PZ) were up-regulated during persistence. Multiple PLD genes reside in the PZ, which is the location of the large chlamydial cytotoxin gene and the *trpRBA* operon (21). The function of chlamydial PLDs is not known; however; homologues are virulence factors of viral (22) and bacterial (23) pathogens. Mammalian PLDs serve diverse functions in signal transduction cytoskeletal dynamics, membrane vesicle trafficking, and blebbing (24). Chlamydial PLDs might function in vesicular budding in persistent growth as a mechanism to control the mass of aberrant persistent forms.

In summary, our analysis of the chlamydial transcriptome during normal and persistent growth provides insight into the pathobiology of this unique host–parasite interaction. The findings suggest a stimulon response to IFN-γ that involves synchronized changes in gene expression during persistent infection. Moreover, the findings of potentially novel gene functions in persistence might have application to the development of new antiinfectives effective in managing persistent chlamydial infections.

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