JOURNAL OF BACTERIOLOGY, May 2010, p. 2569–2574 Vol. 192, No. 10
0021-9193/10/$12.00 doi:10.1128/JB.00068-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Promoters for *Chlamydia* Type III Secretion Genes Show a Differential Response to DNA Supercoiling That Correlates with Temporal Expression Pattern▽

Elizabeth Di Russo Case,[1] Ellena M. Peterson,[2] and Ming Tan[1,3]*

Department of Microbiology and Molecular Genetics,[1] Department of Pathology,[2] and Department of Medicine,[3] University of California, Irvine, California

Received 21 January 2010/Accepted 4 March 2010

Type III secretion (T3S) is important for the establishment and maintenance of a chlamydial infection. The genes encoding T3S components in *Chlamydia* are transcribed as separate temporal classes, but the mechanisms that regulate the timing of their expression are not understood. In this study, we demonstrate that promoters for 10 predicted T3S transcriptional units are each transcribed *in vitro* by the major form of chlamydial RNA polymerase but not by an alternative form of RNA polymerase containing σ^{28}. Since changes in DNA supercoiling during chlamydial development have been proposed as a mechanism for temporal gene regulation, we examined the *in vitro* response of T3S promoters to altered superhelical density. Promoters for three T3S genes that are upregulated at mid times were activated in response to increased DNA supercoiling. In contrast, promoters for three late T3S genes were not sensitive to changes in superhelical density. This differential response to changes in DNA topology is similar to the pattern that has been reported for representative mid and late chlamydial genes that are unrelated to the T3S system. Based on these results, we propose that the temporal expression of T3S genes in *Chlamydia* is controlled by general mechanisms that regulate σ^{66}-dependent gene expression during the developmental cycle. Our results are consistent with a model in which T3S genes that are upregulated in mid cycle are activated together with other mid genes in response to increased DNA supercoiling.

Gram-negative pathogenic bacteria utilize a type III secretion (T3S) system to deliver virulence factors into eukaryotic cells. The components of this specialized secretion machinery include structural proteins that are conserved among different bacteria, as well as specific effector proteins and regulatory chaperones. A wide range of effectors have been described with activities that modulate host cell functions to promote infection. For example, *Salmonella* secretes a T3S effector, SipA, into M cells to modulate actin dynamics and induce bacterial uptake (10). After entry, *Salmonella* secretes a different set of T3S effectors, such as SpiC, which inhibits the fusion of the *Salmonella*-containing vacuole with endosomes (31).

The Gram-negative pathogen *Chlamydia* utilizes a T3S system at different stages of its obligate intracellular infection (11). All *Chlamydia* species encode conserved T3S structural genes (5), and treatment with T3S inhibitors prevents intracellular chlamydial growth (15, 23, 33). Although there is no chlamydial T3S assay, the T3S machinery of other bacteria has been used to provide functional evidence that chlamydial T3S effectors can be secreted (4, 6, 26). An example of a chlamydial T3S effector is the translocated actin-recruiting phosphoprotein (TARP) that is secreted into host cells, where it induces actin recruitment and nucleation (3). These localized cytoskeletal rearrangements are necessary for chlamydial uptake into a membrane-bound cytoplasmic compartment, called the chlamydial inclusion, where chlamydiae replicate. Intracellular chlamydiae secrete T3S effectors to modify the inclusion membrane. For example, IncA is a chlamydial T3S effector that is translocated into the inclusion membrane where it plays an important role in the fusion of chlamydial inclusions (8, 26). These examples demonstrate that the T3S is involved in both the initiation and maintenance of an intracellular chlamydial infection (11).

There are several distinctive features about the organization and regulation of the genes that encode the T3S system in *Chlamydia*. Unlike other Gram-negative bacteria, in which T3S genes are located on a pathogenicity island, the chlamydial T3S genes are present on six loci dispersed throughout the genome (5). This difference has led to the hypothesis that the T3S genes originated in *Chlamydia* and then spread by horizontal gene transfer to other Gram-negative bacteria (13). In *C. trachomatis*, the structural T3S genes have been proposed to be transcribed as 10 operons by the major form of chlamydial RNA polymerase (9) on the basis of their putative promoter sequences, but this prediction has not been experimentally tested (21, 30). Thus, the chlamydial T3S does not appear to be regulated by an alternative form of RNA polymerase, as in the case of *Salmonella enterica* (18). *Chlamydia* spp. also do not encode orthologs of AraC-like transcriptional activators, such as *Yersinia* VirF, that regulate T3S genes in other bacteria (14).

Another unusual feature of chlamydial T3S genes is that they are transcribed at different times during the chlamydial developmental cycle (9, 19, 24). The CT863 operon is transcribed at early time points, soon after entry of the organism into the host cell. In contrast, many T3S genes are transcribed

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, B240 Med Sci. I, School of Medicine, University of California, Irvine, CA 92697-4025. Phone: (949) 824-3397. Fax: (949) 824-8598. E-mail: mingt@uci.edu.

▽ Published ahead of print on 16 March 2010.

TABLE 1. Plasmids and cloning primers

Plasmid	Promoter	Primer sequence	Region cloned
pMT1441	*cdsU*	5′-CTAGAATTCAAGAAAAAGCAAGATTAGTGCTTCA 5′-AGGAATACTTCGCAAGTTACCG	−153 to +5
pMT1442	*scc2*	5′-CGCGAATTCTTGCAAGGCAACGACACACG 5′-ATTATTAACAATATTAAATTCTAACGATCTGATTTAACAA	−144 to +5
pMT1483	CT863	5′-GGTTCTTTCGCACACCTTTC 5′-AAAGGAGGGAACTTATCTAA	−227 to +5
pMT1511	*cdsJ*	5′-AAAGAATTCGCGGATTCTGTTTTTGAAGC 5′-TTTTTCACTCACCATAGCAAATG	−157 to +5
pMT1512	*lpdA*	5′-ACCGAATTCGGCCAAAGAATCGCTCATAC 5′-TATTTCCCGGGTTGTACAT	−169 to +5
pMT1513	*cdsC*	5′-ACCGAATTCCCCCTAGAGATCCACGAACA 5′-TTGTTTGGCAGAATATATCTATTT	−146 to +5
pMT1514	CT663	5′-CCCGAATTCAGTCGTTCGTGCCCGATT 5′-ATTTTATGCTGAGTTATGCCAATAC	−139 to +5
pMT1515	CT665	5′-ACCGAATTCCGGTATCCAGGCCGTTATC 5′-TAATTTTATATTTCAACCCTTCC	−170 to +5
pMT1516	*fliF*	5′-CCCGAATTCCAGCTCTTTCGAGCTCATGTT 5′-TTTTTGTCTTCTGTAACAAAAAGAGG	−154 to +5
pMT1577	*incA*	5′-GTAGAATTCTTTCGTATCGCCAATGCAACA 5′-TGAAACCAGATCCTATGGCTTTATATG	−150 to +3

at higher levels at mid cycle when chlamydiae are actively growing and replicating by binary fission. Another subset of T3S genes is upregulated late in the developmental cycle, during conversion from the metabolically active intracellular form to an extracellular, infectious form. The mechanisms that regulate this temporal expression of T3S genes in *Chlamydia* have not been defined.

The expression of genes in three temporal classes is a general feature of gene regulation in *Chlamydia* (2, 16, 22). Early genes are transcribed as soon as 1 h after infection, but the majority of chlamydial genes are not transcribed until mid times in the developmental cycle (2). We have proposed that the promoters of mid genes may be activated by the increased DNA supercoiling levels that we have measured during mid cycle (17). Furthermore, we have shown that representative mid-cycle promoters are transcribed at higher levels from more supercoiled templates *in vitro* (17), but we do not know if DNA supercoiling is a general mechanism for the activation of mid genes. Late genes are transcribed only at the end of the developmental cycle, and they appear to be regulated by two mechanisms. A subset is transcribed by the major chlamydial RNA polymerase, σ^{66} RNA polymerase, while another group of late genes is regulated by an alternative form of RNA polymerase containing σ^{28} instead of σ^{66} (34, 36). While the mechanism that regulates the σ^{66}-dependent late genes has not been defined, it does not appear to involve DNA supercoiling since promoters for these genes were insensitive to changes in DNA supercoiling (17).

To understand how the T3S genes are regulated in *Chlamydia*, we examined if T3S promoters belonging to each temporal class are transcribed by different forms of chlamydial RNA polymerase and if transcription is affected by DNA topology. Our results indicate that there are similarities in the regulation of the promoters of T3S genes and non-T3S genes of the same temporal class. These results suggest that the temporal expression of T3S genes in *Chlamydia* may be regulated by general and not T3S-specific mechanisms.

MATERIALS AND METHODS

Construction of *in vitro* transcription plasmids. Each promoter was cloned upstream of a promoterless G-less cassette transcription template as described previously (32). The promoter sequences were amplified by PCR from *Chlamydia trachomatis* serovar D genomic DNA and cloned into pMT1125 (32). All transcription plasmids and the primers used to clone the promoters contained therein are listed in Table 1.

Generation of transcription plasmid topoisomers. For each of the following promoters cloned on a transcription plasmid (CT863, *cdsC*, *fliF*, *incA*, *cdsJ*, *cdsU*, and *scc2*), a series of topoisomers was generated using the method of Rhee et al. (20). For each plasmid, 10 μg of CsCl gradient-purified DNA was incubated for 3 h at 37°C with 5 U of wheat germ topoisomerase I (Promega) in a total of 40 μl containing 50 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM dithiothreitol, 50 mM NaCl, 10% glycerol, and various concentrations of ethidium bromide ranging from 0 to 48 μM. After incubation, the ethidium bromide was removed from the DNA by phenol-chloroform (1:1) extraction, followed by extraction with chloroform. The plasmid DNA was then recovered by ethanol precipitation and suspended in 30 μl of RNase-free TE buffer (10 mM Tris [pH 10], 1 mM EDTA). The purity and concentration of the DNA were assessed using a NanoDrop ND1000 spectrophotometer. The topoisomers were resolved on 1.4% agarose gels in 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA) with concentrations of ethidium bromide ranging from 0.02 to 0.16 μg/ml. Gel electrophoresis was performed at 3.5 V/cm with buffer circulation for 16 to 20 h at room temperature. The average difference in linking-number (ΔLK) for the topoisomers was determined by the band-counting method of Keller (12). The average superhelical density (σ) was calculated using the following formula: $\sigma = -10.5(\Delta LK/N)$, where N is the total number of base pairs in the plasmid.

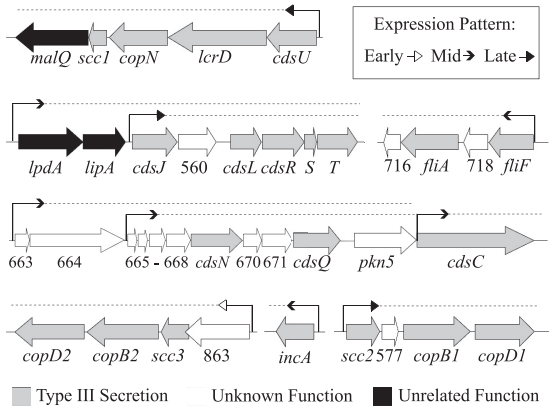

FIG. 1. Transcriptional organization and temporal expression of T3S operons. For each operon, the promoter location is indicated by a bent arrow, followed by a dashed line above each gene in the operon, and the arrowheads indicate the temporal expression patterns of the first gene in each operon, as noted on the figure. Genes or open reading frames are identified by name or number, respectively, below the large filled arrows, which are color coded according to functional class. An unrelated function is one not involved in T3S.

Purification of chlamydial RNA polymerase. L929 cells were infected with *C. trachomatis* serovar L2, and chlamydial σ^{66} RNA polymerase was partially purified at 21 h postinfection by heparin-agarose chromatography as previously described (29). Core enzyme lacking detectable σ^{66} activity was prepared from this partially purified RNA polymerase by gel filtration chromatography using a Superdex S200 10/300 GL column (GE Healthcare) on an AKTA Purifier 10 chromatography system (GE Healthcare). σ^{28} RNA polymerase was reconstituted by adding l μl of 15 nM recombinant chlamydial σ^{28} protein (34) to 4 μl of chlamydial core enzyme. Details of the transcription assays and the quantitation of promoter activity have been previously described (32, 34).

***In vitro* transcription.** Transcription experiments were performed as described previously (29), using 0.5 μl of chlamydial σ^{66} RNA polymerase or chlamydial σ^{28} RNA polymerase and 25 nM CsCl gradient-purified plasmid DNA or an individual plasmid topoisomer. The transcripts were resolved by electrophoresis on an 8 M urea–6% polyacrylamide gel, and the gels were fixed, dried, and exposed to a phosphor screen. The transcripts were visualized with a Personal FX phosphorimager (Bio-Rad). The amount of transcript produced by each promoter topoisomer was quantified using Quantity One software (Bio-Rad). Relative promoter activity was calculated by normalizing the amount of activity obtained from each topoisomer to the maximal promoter activity (defined as 100%) observed over the range of superhelical densities tested. In order to calculate a mean and standard deviation, three independent measurements of relative promoter activity were obtained for each set of promoter topoisomers.

RESULTS AND DISCUSSION

The T3S genes are transcribed by the major form of RNA polymerase in *Chlamydia*. We first tested candidate promoters for 10 T3S operons in the *C. trachomatis* genome (Fig. 1) to determine if they were transcriptionally active. Nine of these promoters were predicted by Hefty and Stephens based on the mapping of *in vivo* transcription start sites (9). In addition we predicted a promoter for the T3S effector, *incA*, immediately upstream of a transcription start site mapped previously (26, 27). Each of these 10 candidate T3S promoters resembles the promoter transcribed by σ^{66} RNA polymerase (Table 2) (21, 28, 30) but not promoter sequences defined for *Chlamydia* σ^{28} RNA polymerase (35) or predicted for *Chlamydia* σ^{54} RNA polymerase (1).

We tested these putative *C. trachomatis* T3S promoters for transcriptional activity with *in vitro* assays (32) since an experimental genetic system to test *in vivo* promoter activity in *Chlamydia* is not yet available. All transcription plasmids and the primers used to clone the promoters contained therein are listed in Table 1. We found that all 10 candidate T3S promoters were transcribed by σ^{66} RNA polymerase, which is the major form of chlamydial RNA polymerase (Fig. 2A). We also examined whether T3S promoters can be transcribed by σ^{28} RNA polymerase since this alternative RNA polymerase regulates a subset of late genes in *Chlamydia* (34, 36). However, σ^{28} RNA polymerase did not transcribe the promoters for three late T3S operons, *cdsU*, *cdsJ*, and *scc2* (Fig. 2B). We were unable to test for σ^{54}-dependent promoter activity because there is no assay for chlamydial σ^{54} RNA polymerase at this time. Taken together, these results indicate that the main T3S genes in *Chlamydia* are transcribed by the major form of RNA polymerase and do not support a role for the late regulator, σ^{28} RNA polymerase, in the expression of T3S genes. Additional mechanisms must exist, however, to explain how the T3S genes are transcribed by σ^{66} RNA polymerase as three temporal groups during the chlamydial developmental cycle (24).

T3S promoters show different responses to changes in superhelical density. Since DNA supercoiling has been proposed as a mechanism to regulate mid-cycle genes but not late genes in *Chlamydia* (17), we examined whether T3S promoters are sensitive to alterations in DNA supercoiling. We performed a

TABLE 2. Promoter sequences of chlamydial T3S operons

Operon[a]	Promoter sequence[b]	Temporal class	Discriminator[c] G/C content (%)
cdsU (CT091)	TTGAGAAAAACATTTATATACGGTAACTTGCCAAGTA*	Late	50
lpdA (CT557)	TTGAGATTTTATCCACCCAGATGTACAACCCGGGA*	Mid	83
cdsJ (CT559)	TTGGCACTAATCTCCCCATTTCGTATGGTGAGTGA*	Late	50
scc2 (CT576)	TTGTTAAATCAGATCGTTAGAATTTAATATTGTTAGTA*	Late	25
CT663	TCTTTTTAAAGTAGGTATTGGCATAACTCAGCATAA*	Mid	29
CT665	TTGTATCTTTTTAGAACGGGAAGGGTTGAAATATAA*	Mid	0
cdsC (CT674)	TTGCAAGATAGAGGGCAAATAGATATATTCTGCCAA*	Mid	57
fliF (CT719)	TTGTTTTTAATAGCCTCTTTTTGTTACAGAAGACA*	Mid	33
CT863	TTGCATGAAAAATACTTTTTAGATAAGTTCCCTC*	Early	80
incA (CT119)	TAGAATTTTTATCATATAAAGCCATAGGATCTGGTT*	Mid	50

[a] Each operon is identified by the name and/or the open reading frame identification number of its first gene.
[b] Transcription start sites (*) were mapped by Hefty and Stephens (9), with the exception of that for *incA*, which was mapped by Suchland et al. (27) Promoter elements (−35 and −10 elements, underlined) were predicted by Hefty and Stephens (9), with the exception of the *incA* promoter, which was predicted in this study.

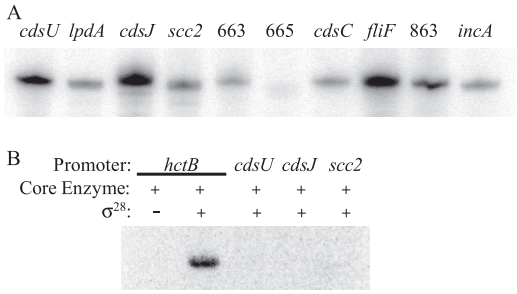

FIG. 2. *In vitro* transcription of candidate *C. trachomatis* T3S promoters. (A) Transcription of 10 candidate promoters with chlamydial σ^{66} RNA polymerase. The promoter for each operon is indicated by the name or the open reading frame number of its first gene. The promoters for the CT663, CT665, and CT863 operons are abbreviated as 663, 665, and 863, respectively. (B) Three late T3S promoters were not transcribed by chlamydial σ^{28} RNA polymerase. The *hctB* promoter is a known σ^{28}-regulated promoter that was used as a positive control (33).

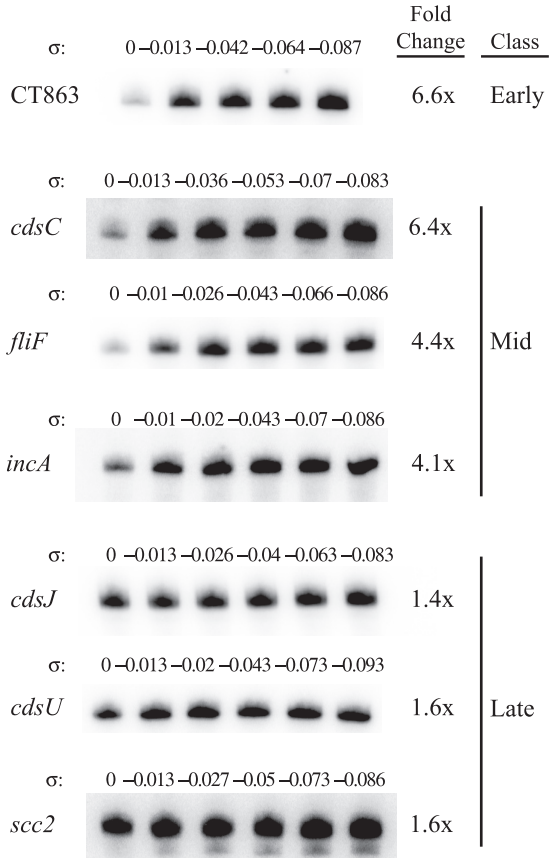

FIG. 3. Supercoiling sensitivity transcription assay. For each T3S promoter cloned on a transcription plasmid, a series of plasmid topoisomers was generated over a range of superhelical densities (σ) from 0 (completely relaxed DNA) to >-0.08 (highly negatively supercoiled). Each topoisomer was transcribed by σ^{66} RNA polymerase in an *in vitro* transcription reaction to measure the promoter activity at different superhelical densities. The greatest difference in promoter activity over this range of superhelical densities was calculated as fold change and is reported as the average of three independent experiments. For each promoter, the temporal expression class (early, mid, or late) is listed.

supercoiling-sensitivity transcription assay to test promoter activity at different supercoiling levels using plasmid topoisomers that differ only in superhelical density (17). We tested promoters for three T3S operons that are first expressed at mid times in the chlamydial developmental cycle (*cdsC*, *fliF*, and *incA*) and another three T3S operons that are not transcribed until late time points (*cdsJ*, *cdsU*, and *scc2*) (2, 9, 24). We also tested the promoter for the CT863 operon as a putative early T3S promoter based on detection of the CT863 transcript as early as 1.5 h postinfection (24). We cloned each T3S promoter on a transcription plasmid and generated a panel of topoisomers for each plasmid over a range of superhelical densities (σ) from 0 (completely relaxed) to >-0.08 (highly negatively supercoiled) (20) to encompass the physiologic range of supercoiling (-0.028 to -0.077) that we have measured in *Chlamydia* (17).

Four of the seven T3S promoters tested were transcribed in a supercoiling-responsive manner (Fig. 3). The CT863, *cdsC*, *fliF*, and *incA* promoters were upregulated by 4.1- to 6.6-fold in response to increased superhelical density. In contrast, the *cdsJ*, *cdsU*, and *scc2* promoters were insensitive to changes in DNA supercoiling since they showed only 1.4-, 1.6-, and 1.6-fold respective changes in promoter activity across the range of superhelical densities tested. None of these T3S promoters was transcribed at higher levels from completely relaxed DNA.

It has been proposed that the sequence of a chlamydial promoter may determine whether it is responsive to DNA supercoiling and that supercoiling-sensitive promoters contain a high proportion of G or C (G/C) sequence (17). The two most supercoiling-responsive promoters, CT863 and *cdsC*, have a high G/C content (80% and 57%, respectively) in the discriminator region, which is the sequence between the -10 promoter element and the transcription start site (Table 2). However, we found no clear correlation between the supercoiling responsiveness and the G/C content of the other T3S promoters.

The response of T3S promoters to alterations in DNA supercoiling correlates with their temporal expression pattern. We next examined if there is a relationship between this differential response to DNA supercoiling and the temporal expression of T3S genes. For each promoter, we calculated the relative promoter activity by defining the maximal level of transcription at any superhelical density as 100% and normalizing other transcription levels to this value. To compare the promoters, we graphed the effects of superhelical density on the relative activity of each promoter. The promoters for the three mid-cycle T3S operons were transcribed at low levels from a relaxed template and at much higher levels from more supercoiled templates. Transcription of the *cdsC*, *fliF*, and *incA* promoters was highest at a superhelical density (σ) of approximately -0.06 and leveled off or decreased slightly for a σ of >-0.06 (Fig. 4A). These superhelical density optima are close to the *in vivo* superhelicity of -0.063 to -0.077 that we measured for the chlamydial plasmid in the reticulate body (RB) stage of the developmental cycle (17). In addition, this response to DNA supercoiling closely mirrors the supercoiling response pattern for promoters of two other non-T3S chlamydial mid genes, *ompA* (Fig. 4D) and *pgk* (17). *ompA* encodes the major outer membrane protein (7), and *pgk* is the gene for

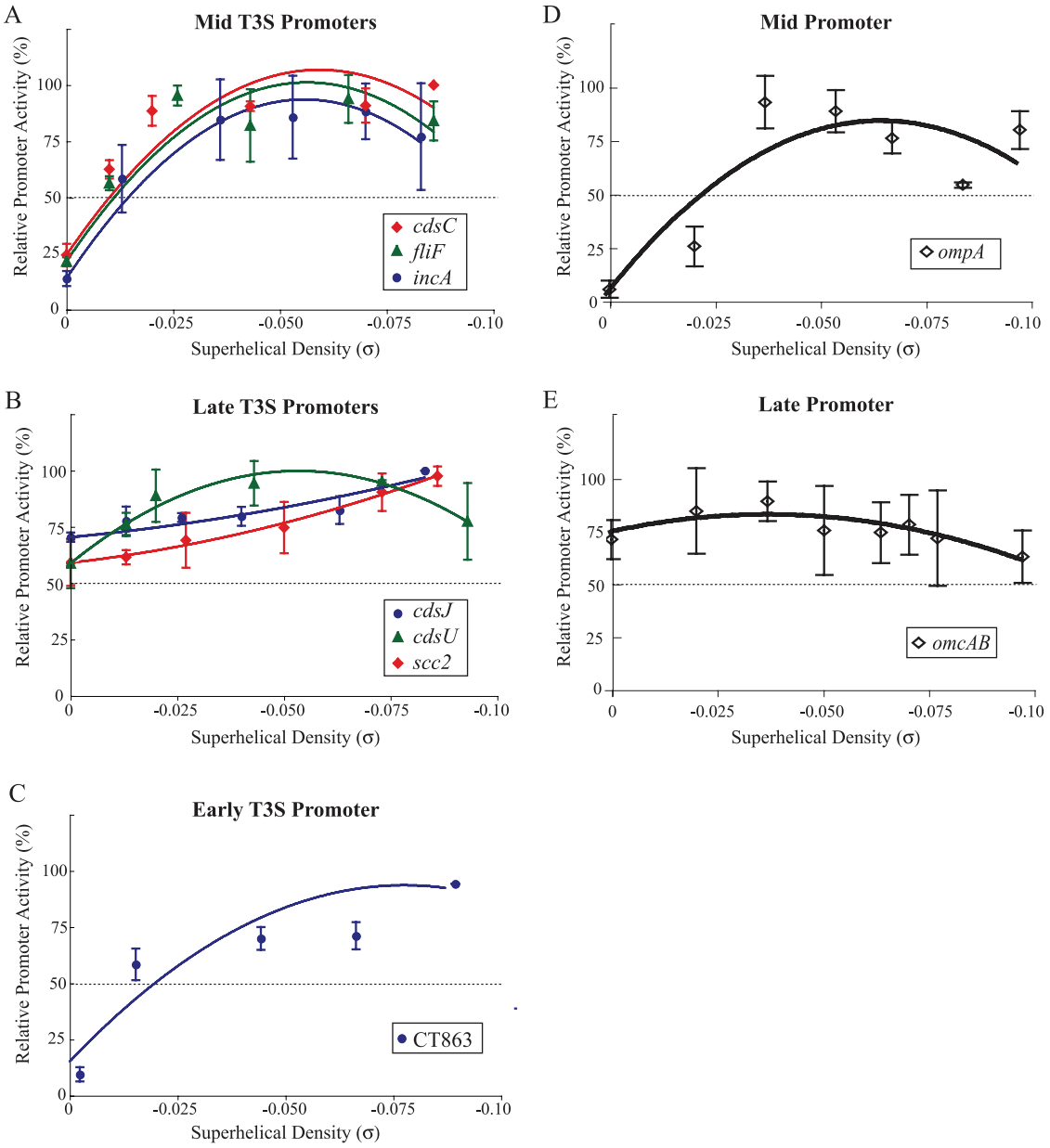

FIG. 4. Graphs showing the relationship between promoter activity and superhelical density for promoters of T3S genes belonging to mid (A), late (B), and early (C) temporal classes. For comparison, the graphs for mid (D) and late (E) promoters of non-T3S genes are shown (adapted from reference 17). Relative promoter activity was calculated as a percentage of the maximal promoter activity over the range of superhelical densities (σ) tested. A relative promoter activity of 50% is indicated by a dashed horizontal line on each graph. All reactions were performed as three independent experiments, and error bars represent standard deviations.

phosphoglycerol kinase (25); neither is known to be associated with the T3S system in *Chlamydia*.

In contrast, the promoters for three late T3S operons, *cdsJ*, *cdsU*, and *scc2*, were not affected by alterations in DNA supercoiling. Transcript levels from each late promoter did not change over the range of superhelical densities, and the relative promoter activity was above 50% at all superhelical densities tested (Fig. 4B). Thus, the promoters for late T3S genes show a lack of response to DNA supercoiling that is similar to promoters for the late genes *omcAB* (Fig. 4E), *hctA*, and *ltuB* (17), which have no known connection to the T3S system.

The promoter for the single early T3S operon showed a supercoiling-responsive transcription pattern. The CT863 promoter was transcribed at low levels from a relaxed template and at higher levels with increasing superhelical density (Fig. 4C). The maximal activity was at the highest superhelical density tested (σ of −0.087), but unlike the three mid T3S promoters, the CT863 promoter did not reach a supercoiling optimum prior to this supraphysiologic level of DNA supercoiling. The CT863 promoter is the first early chlamydial promoter whose supercoiling response has been characterized, and thus it remains to be seen whether its supercoiling depen-

dence is typical of early promoters. In summary, promoters of T3S genes show differential responses to changes in DNA supercoiling that correlate with their temporal patterns of gene expression. We found that three mid T3S promoters were transcribed in a supercoiling-dependent manner that is similar to the pattern described for two other mid chlamydial promoters (17). These *in vitro* findings are consistent with a model in which the T3S mid genes, together with other supercoiling-responsive mid genes, are activated by increased DNA supercoiling during mid cycle. In contrast, we demonstrated that three late T3S promoters were transcribed in a supercoiling-insensitive manner by σ^{66} RNA polymerase but were not transcribed by σ^{28} RNA polymerase. Thus, the late T3S genes appear to belong to the subset of supercoiling-independent, σ^{66}-regulated late genes that includes *omcAB*, which encode two outer membrane proteins that are specific for elementary bodies, and *hctA*, the gene for the histone-like protein, Hc1, that condenses chlamydial DNA. The ability of late promoters to be well transcribed from relaxed templates is consistent with the low DNA supercoiling levels that we have measured at late time points in the chlamydial developmental cycle (17). Our results indicate that the temporal expression of T3S genes in *Chlamydia* appears to be controlled by general mechanisms that regulate developmental gene expression in this pathogenic bacterium.

ACKNOWLEDGMENTS

We thank Christopher Rosario for providing σ^{28} RNA polymerase and Christine Sütterlin, Johnny Akers, Allan Chen, Eric Cheng, Kirsten Johnson, and Karen Kelley for critical reading of the manuscript and helpful advice.

This work was supported by a grant from the NIH (AI 71104 to E.M.P.). M.T. was supported by an NIH Independent Scientist Award (AI 057563).

REFERENCES

1. **Barrios, H., B. Valderrama, and E. Morett.** 1999. Compilation and analysis of σ^{54}-dependent promoter sequences. Nucleic Acids Res. **27:**4305–4313.
2. **Belland, R. J., G. Zhong, D. D. Crane, D. Hogan, D. Sturdevant, J. Sharma, W. L. Beatty, and H. D. Caldwell.** 2003. Genomic transcriptional profiling of the developmental cycle of *Chlamydia trachomatis*. Proc. Natl. Acad. Sci. U. S. A. **100:**8478–8483.
3. **Clifton, D. R., K. A. Fields, S. S. Grieshaber, C. A. Dooley, E. R. Fischer, D. J. Mead, R. A. Carabeo, and T. Hackstadt.** 2004. A chlamydial type III translocated protein is tyrosine-phosphorylated at the site of entry and associated with recruitment of actin. Proc. Natl. Acad. Sci. U. S. A. **101:**10166–10171.
4. **Fields, K. A., and T. Hackstadt.** 2000. Evidence for the secretion of *Chlamydia trachomatis* CopN by a type III secretion mechanism. Mol. Microbiol. **38:**1048–1060.
5. **Fields, K. A., and T. Hackstadt.** 2006. The *Chlamydia* type III secretion system: structure and implications for pathogenesis, p. 219–234. *In* P. Bavoil and P. Wyrick (ed.), *Chlamydia*: genomics and pathogenesis. Horizon Bioscience, Wymondham, United Kingdom.
6. **Fields, K. A., D. J. Mead, C. A. Dooley, and T. Hackstadt.** 2003. *Chlamydia trachomatis* type III secretion: evidence for a functional apparatus during early-cycle development. Mol. Microbiol. **48:**671–683.
7. **Frost, E. H., S. Deslandes, S. Veilleux, and D. Bourgaux-Ramoisy.** 1991. Typing *Chlamydia trachomatis* by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein. J. Infect. Dis. **163:**1103–1107.
8. **Hackstadt, T., M. A. Scidmore-Carlson, E. I. Shaw, and E. R. Fischer.** 1999. The *Chlamydia trachomatis* IncA protein is required for homotypic vesicle fusion. Cell Microbiol. **1:**119–130.
9. **Hefty, P. S., and R. S. Stephens.** 2007. Chlamydial type III secretion system is encoded on ten operons preceded by σ^{70}-like promoter elements. J. Bacteriol. **189:**198–206.
10. **Higashide, W., S. Dai, V. P. Hombs, and D. Zhou.** 2002. Involvement of SipA in modulating actin dynamics during *Salmonella* invasion into cultured epithelial cells. Cell Microbiol. **4:**357–365.
11. **Jamison, W. P., and T. Hackstadt.** 2008. Induction of type III secretion by cell-free *Chlamydia trachomatis* elementary bodies. Microb. Pathog. **45:**435–440.
12. **Keller, W.** 1975. Determination of the number of superhelical turns in simian virus 40 DNA by gel electrophoresis. Proc. Natl. Acad. Sci. U. S. A. **72:**4876–4880.
13. **Kim, J. F.** 2001. Revisiting the chlamydial type III protein secretion system: clues to the origin of type III protein secretion. Trends Genet. **17:**65–69.
14. **Lambert de Rouvroit, C., C. Sluiters, and G. R. Cornelis.** 1992. Role of the transcriptional activator, VirF, and temperature in the expression of the pYV plasmid genes of *Yersinia enterocolitica*. Mol. Microbiol. **6:**395–409.
15. **Muschiol, S., S. Normark, B. Henriques-Normark, and A. Subtil.** 2009. Small molecule inhibitors of the *Yersinia* type III secretion system impair the development of *Chlamydia* after entry into host cells. BMC Microbiol. **9:**75.
16. **Nicholson, T. L., L. Olinger, K. Chong, G. Schoolnik, and R. S. Stephens.** 2003. Global stage-specific gene regulation during the developmental cycle of *Chlamydia trachomatis*. J. Bacteriol. **185:**3179–3189.
17. **Niehus, E., E. Cheng, and M. Tan.** 2008. DNA supercoiling-dependent gene regulation in *Chlamydia*. J. Bacteriol. **190:**6419–6427.
18. **Osborne, S. E., and B. K. Coombes.** 2009. RpoE fine tunes expression of a subset of SsrB-regulated virulence factors in *Salmonella enterica* serovar Typhimurium. BMC Microbiol. **9:**45.
19. **Ouellette, S. P., Y. M. Abdelrahman, R. J. Belland, and G. I. Byrne.** 2005. The *Chlamydia pneumoniae* type III secretion-related *lcrH* gene clusters are developmentally expressed operons. J. Bacteriol. **187:**7853–7856.
20. **Rhee, K. Y., M. Opel, E. Ito, S. Hung, S. M. Arfin, and G. W. Hatfield.** 1999. Transcriptional coupling between the divergent promoters of a prototypic LysR-type regulatory system, the *ilvYC* operon of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. **96:**14294–14299.
21. **Schaumburg, C. S., and M. Tan.** 2003. Mutational analysis of the *Chlamydia trachomatis dnaK* promoter defines the optimal −35 promoter element. Nucleic Acids Res. **31:**551–555.
22. **Shaw, E. I., C. A. Dooley, E. R. Fischer, M. A. Scidmore, K. A. Fields, and T. Hackstadt.** 2000. Three temporal classes of gene expression during the *Chlamydia trachomatis* developmental cycle. Mol. Microbiol. **37:**913–925.
23. **Slepenkin, A., P. A. Enquist, U. Hagglund, L. M. de la Maza, M. Elofsson, and E. M. Peterson.** 2007. Reversal of the antichlamydial activity of putative type III secretion inhibitors by iron. Infect. Immun. **75:**3478–3489.
24. **Slepenkin, A., V. Motin, L. M. de la Maza, and E. M. Peterson.** 2003. Temporal expression of type III secretion genes of *Chlamydia pneumoniae*. Infect. Immun. **71:**2555–2562.
25. **Stephens, R. S., S. Kalman, C. Lammel, J. Fan, R. Marathe, L. Aravind, W. Mitchell, L. Olinger, R. L. Tatusov, Q. Zhao, E. V. Koonin, and R. W. Davis.** 1998. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science **282:**754–759.
26. **Subtil, A., C. Parsot, and A. Dautry-Varsat.** 2001. Secretion of predicted Inc proteins of *Chlamydia pneumoniae* by a heterologous type III machinery. Mol. Microbiol. **39:**792–800.
27. **Suchland, R. J., B. M. Jeffrey, M. Xia, A. Bhatia, H. G. Chu, D. D. Rockey, and W. E. Stamm.** 2008. Identification of concomitant infection with *Chlamydia trachomatis* IncA-negative mutant and wild-type strains by genomic, transcriptional, and biological characterizations. Infect. Immun. **76:**5438–5446.
28. **Tan, M.** 2006. Regulation of gene expression, p. 103–132. *In* P. Bavoil and P. Wyrick (ed.), *Chlamydia*: genomics and pathogenesis. Horizon Bioscience, Wymondham, United Kingdom.
29. **Tan, M., and J. N. Engel.** 1996. Identification of sequences necessary for transcription *in vitro* from the *Chlamydia trachomatis* rRNA P1 promoter. J. Bacteriol. **178:**6975–6982.
30. **Tan, M., T. Gaal, R. L. Gourse, and J. N. Engel.** 1998. Mutational analysis of the *Chlamydia trachomatis* rRNA P1 promoter defines four regions important for transcription *in vitro*. J. Bacteriol. **180:**2359–2366.
31. **Uchiya, K., M. A. Barbieri, K. Funato, A. H. Shah, P. D. Stahl, and E. A. Groisman.** 1999. A *Salmonella* virulence protein that inhibits cellular trafficking. EMBO J. **18:**3924–3933.
32. **Wilson, A. C., and M. Tan.** 2002. Functional analysis of the heat shock regulator HrcA of *Chlamydia trachomatis*. J. Bacteriol. **184:**6566–6571.
33. **Wolf, K., H. J. Betts, B. Chellas-Gery, S. Hower, C. N. Linton, and K. A. Fields.** 2006. Treatment of *Chlamydia trachomatis* with a small molecule inhibitor of the *Yersinia* type III secretion system disrupts progression of the chlamydial developmental cycle. Mol. Microbiol. **61:**1543–1555.
34. **Yu, H., and M. Tan.** 2003. σ^{28} RNA polymerase regulates *hctB*, a late developmental gene in *Chlamydia*. Mol. Micro. **50:**577–584.
35. **Yu, H. H. Y., E. G. Di Russo, M. A. Rounds, and M. Tan.** 2006. Mutational analysis of the promoter recognized by *Chlamydia* and *Escherichia coli* σ^{28} RNA polymerase. J. Bacteriol. **188:**5524–5531.
36. **Yu, H. H. Y., D. Kibler, and M. Tan.** 2006. *In silico* prediction and functional validation of σ^{28}-regulated genes in *Chlamydia* and *Escherichia coli*. J. Bacteriol. **188:**8206–8212.