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Analysis of a *Coxiella burnetii* Gene Product That Activates Capsule Synthesis in *Escherichia coli*: Requirement for the Heat Shock Chaperone DnaK and the Two-Component Regulator RcsC

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A 1.2-kb *Eco*RI genomic DNA fragment of *Coxiella burnetii*, when cloned onto a multicopy plasmid, was found to induce capsule synthesis (mucoidy) in *Escherichia coli*. Nucleotide sequence analysis revealed the presence of an open reading frame that could encode a protein of 270 amino acids. Insertion of a *tet* cassette into a unique *Nru*I restriction site resulted in the loss of induction of mucoidy. Because of its ability to induce mucoidy, we designated this gene *mucZ*. Computer search for homologies to *mucZ* revealed 42% identity to an open reading frame located at 1 min of the *E. coli* chromosome. Interestingly, the C-terminal amino acid residues of MucZ share significant homology with the J domain of the DnaJ protein and its homologs, suggesting potential interactions between MucZ and components of the DnaK-chaperone machinery. Results presented in this paper suggest that *E. coli* requires DnaK-chaperone machinery for Lon-RcsA-mediated induction of capsule synthesis, as noticed first by S. Gottesman (personal communication). The induction caused by MucZ is independent of Lon-RcsA and is mediated through the two-component regulators RcsC and RcsB. DnaK and GrpE but not DnaJ are also required for the RcsB-mediated MucZ induction, and we propose that MucZ is a DnaJ-like chaperone protein that might be required for the formation of an active RcsA-RcsB complex and for the RcsC-dependent phosphorylation of RcsB. Discussions are presented that suggest three different roles for alternative forms of the DnaK-chaperone machinery in capsule production.

Capsule production is an important virulence determinant in some pathogenic bacteria. In *Pseudomonas aeruginosa*, capsule synthesis causes chronic respiratory tract infections in cystic fibrosis patients (11). Other examples include *Neisseria meningitidis*, *Haemophilus influenzae*, and some serotypes of *Escherichia coli* (e.g., K1 and K5), which cause sepsis and meningitis (13), and also group B streptococci, the leading cause of sepsis, meningitis, and pneumonia among neonates in the United States (28). Recently, Cherwonogrodzky et al. (9) have reported that in the case of *Francisella tularensis*, an extremely pathogenic bacterium that causes tularemia, the live vaccine strain becomes extensively encapsulated under certain growth conditions. These investigators have also demonstrated a 1,000-fold increase in virulence of these capsule-producing bacteria. Capsule production has been implicated in protecting *P. aeruginosa* (30) and *S. pneumoniae* (21) from phagocytosis, a host defense mechanism to evade the infecting organism.

Coxiella burnetii is a gram-variable (36) obligate intracellular parasite that causes Q fever in humans (19). It is a moderate acidophile and replicates only in the phagolysosomal compartment of nucleated cells (19). The organism is not known to produce a capsule-like material, which is consistent with its intraphagolysosomal lifestyle of replication. However, we have obtained *C. burnetii* genomic DNA clones that induce capsule synthesis in *E. coli*. This was especially interesting because *C. burnetii* has a small genome, of 1.7×10^6 bp (25), which is approximately one-third the size of the *E. coli* genome (22). In view of its intraphagolysosomal lifestyle of replication and its small genome size, we have speculated that *C. burnetii* probably does not contain much DNA for accessory functions in its genome. This reasoning has prompted us to further analyze the *C. burnetii* genomic DNA clones that induce capsule synthesis in *E. coli* and to characterize the biological activities of the gene product(s).

Colanic acid capsular polysaccharide is produced and secreted by *E. coli*, causing the bacterial colony to be mucoid. The capsule is assembled by proteins encoded by the genes of the *cps* operon. Under normal laboratory conditions, wild-type cells do not form capsule and colonies are not mucoid, because the *cps* operon is not efficiently expressed. The *cps* operon is activated by two positive regulators acting in concert to enhance *cps* transcription. The two positive regulators, RcsA and the phosphorylated form of RcsB, are normally limiting under wild-type conditions and are modulated posttranslationally. Thus, there are two potential pathways by which mucoidy can occur, RcsA and RcsB. RcsA levels are normally limited by degradation caused by the Lon protease, and for this reason, mutations in *lon* that eliminate the protease increase RcsA levels and cause mucoidy. RcsB activity is dependent upon its phosphorylated state. RcsB is part of a two-component regulatory system that also includes the RcsC membrane sensor component. By analogy with other two-component systems, RcsC modulates the phosphorylation state of RcsB in response to a signal(s) received across the membrane. In the case of RcsC, the nature of the signal(s) is not known. However, a special mutation in the *rcsC* gene (*rcsC**) generates a protein (RcsC*) that increases the levels of active (phosphorylated) RcsB. Phosphorylated RcsB activates expression of the *cps* operon in cooperation with limiting RcsA (*lon*$^+$ conditions). Likewise, increased levels of RcsA (in *lon* mutant cells) activate expression of the *cps* operon with limiting levels of phos-

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TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Reference or source
Strains		
XL1-Blue	*endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA96 relA1* (*lac*) (F′ *proAB lacI*^{q}Δ*M15* Tn*10*)	Stratagene
TAP56	*hdsR leu thi rpsL supE44* Δ*lacU169 galK*(Am) *bioA* (λN^+ *c*I857)	8
W3110		1
HT115	W3110, *rnc-14*::ΔTn*10*	34
HT120	W3110, *rnc-40*::ΔTn*10*	34
HT210	W3110, *recO*::Kan	34
DC1148	*proC*::Tn*10* Δ*lacU169 lon* (λN^+ *c*I857)	D. Court
BR6010 (=DH5Δ*lac*)	F′ *endA1 recA1 hsdR17 deoR thi-1 supE44 gyrA96 relA1* (Δ*argF-lac*)*U169*	D. Chattoraj
BR4370	BR6010 (Δ*dnaJ*::mini-Kan^r)	32
BR4392	BR6010 (Δ*dnaK-dnaJ*)::mini-Kan^r	32
BL322	F$^-$ *thi-1 argH1 gal-6 lacY1 mtl-2 xyl-7 malA1 ara-13 str-9* λ^r *supE44*	33
BL321	BL322 *rnc*	33
DH5αF′	F′ ϕ80d*lacZ*Δ*M15* Δ*lacU169 deoR recA endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 gyrA96 relA1*	Bethesda Research Laboratories
DH11S	F′ ϕ80d*lacZ*Δ*M15 mcrA* Δ(*mrr hsdRMS mcrBC*) Δ(*lac-proAB*) Δ(*recA1398*) *deoR rpsL srl thi*/F′ *proAB*$^+$ *lacI*^{q}*Z*Δ*M15*	Bethesda Research Laboratories
SG20780	MC4100, F$^-$ Δ*lacU169* Δ*lon-510 cpsB10*::*lac*-Mu-*imm*^{λ}	7
SG20781	*lon*$^+$ *cpsB10*::*lac*-Mu-*imm*^{λ}	7
SG20884	Δ*lon-510 cpsB10*::*lac*-Mu-*imm*^{λ} *dnaJ*::*kan*	S. Gottesman
YJ1127	Δ*lon-510 cpsB10*::*lac*-Mu-*imm*^{λ} *dnaK*	S. Gottesman
MZ51	*cps-lacZ dnaK cat*	YJ1127 + P1 *lon*$^+$. *.cat*
MZ53	*cps-lacZ dnaJ*::*kan cat*	SG20884 + P1 *lon*$^+$. *.cat*
MZ57	*cps-lacZ rcsA*::*kan*	SG20781 + P1 *rscA*::*kan*
MZ60	*cps-lacZ rcsB*::*tet*	SG20781 + P1 *rcsB*::*tet*
MZ63	*cps-lacZ rcsC*::*tet*	SG20781 + P1 *rcsC*::*tet*
MZ70	*lon cps-lacZ rcsB*::*tet*	SC20780 + P1 *rcsB*::*tet*
MZ76	*lon cps-lacZ rcsC*::*tet*	SG20780 + P1 *rcsC*::*tet*
Ω668	N99 *pheA*::Tn*10 grpE280*(λ*c*I$^+$ *ind*)	G. Gaitanaris
MZ81	*cps-lacZ pheA*::Tn*10 grpE280*	SG10781 + P1 Ω668
Plasmids		
pBR322	Ap^r Tet^r general cloning vector	2
pBluescript SK	Ap^r p_{LAC} promoter vector	Stratagene
pMZCb410	p_{LAC}, 0.9- and 1.2-kb *Eco*RI genomic DNA fragments of *C. burnetii*	This work
pMZCb4105	p_{LAC}, *C. burnetii mucZ*	This work
pMZCb4105-11	p_{LAC}, *C. burnetii mucZ*::*tet*	This work
pCbMucZ6	p_{LAC}, *C. burnetii* PCR-amplified *mucZ*	This work
pEcMucZ2	p_{LAC}, *E. coli* PCR-amplified *mucZ*	This work
pWS50	Ap^r λp_L promoter vector	31
pMZGC418	λp_L, *C. burnetii* PCR-amplified *mucZ*	This work

phorylated RcsB, suggesting the possibility of heterodimer formation between RcsA and RcsB. Thus, only one or the other positive regulator has to be increased functionally to activate the *cps* genes and cause a mucoid phenotype.

Results presented in this paper suggest that capsule synthesis can be mediated by a *C. burnetii* gene, *mucZ*, cloned in multiple copy. We find an absolute requirement for the RcsB-RcsC two-component regulatory system (17) for MucZ-dependent induction of capsule synthesis, as well as a requirement for the heat shock DnaK-chaperone system (14).

MATERIALS AND METHODS

Construction of the recombinant bacteriophage lambda *Zap*II library. Construction of the recombinant bacteriophage lambda *Zap*II library with *Eco*RI-digested genomic DNA of *C. burnetii* was described previously (39).

Strains and genetic techniques. The genotypes of *E. coli* strains used in this paper are shown in Table 1. P1*vir* lysates were prepared and transduced by the procedures described by Silhavy et al. (29). L medium (29) was used for general bacterial growth. Ampicillin, chloramphenicol, tetracycline, and kanamycin were used at 50, 10, 12.5, and 50 μg ml^{-1}, respectively. Induction of mucoidy was tested after overnight growth of *E. coli* strains on L agar plates containing appropriate antibiotic(s) at 30°C. MacConkey lactose agar plates were similarly used for scoring the induction of *cps* operon after overnight growth of *cps-lacZ* fusion strains of *E. coli* at 30°C.

P1*vir* lysates of *rcsA*::*kan*, *rcsB*::*tet*, and *rcsC*::*tet* were kindly provided by B. Powell. P1*vir* lysates prepared on a *lon*$^+$ strain with a linked (90%) *cat* gene were kindly provided by S. Gottesman and were used to transduce YJ1127 and SG20884 (Table 1). Chloramphenicol-resistant colonies obtained from these transductions were screened for the Lon$^+$ phenotype by checking for resistance to UV light. The *grpE280* mutation (linked [50%] to the *pheA*::Tn*10* marker) of Ω668 (Table 1) was moved into SG20781 (Table 1) by P1 transduction. Tetracycline-resistant transductants were initially screened for the temperature-sensitive phenotype associated with this *grpE280* mutation; the positive transductants were then subsequently screened for their failure to allow bacteriophage lambda to replicate.

Bacteriophage lambda G3 (λ*imm*^{434}), obtained from the National Institutes of Health collection, was routinely used for checking for λ growth in appropriate *E. coli* hosts.

Plasmids. Plasmids used in this study are listed in Table 1. A Tet^r cassette contained on the *Eco*RI-*Ava*I restriction fragment of plasmid pBR322 (2) was used, after Klenow filling in, to clone into the unique *Nru*I restriction site of the *mucZ* open reading frame (ORF) and to generate pMZCb4105-11. PCR-amplified DNA fragments containing the *mucZ* genes of *C. burnetii* and *E. coli* were cloned into the *Eco*RV site of pBluescript SK$^+$ (Stratagene, La Jolla, Calif.). The resulting plasmids, pCbMucZ6 and pEcMucZ2, respectively, were transformed into strain BR6010 (Table 1) for further study, and their DNA was verified by restriction analysis. The PCR-amplified *mucZ* gene of *C. burnetii* was also cloned into the *Nru*I site of pWS50 (31), and the resulting plasmid, pMZGC418, was subsequently used for high-level expression in *E. coli* TAP56 (Table 1). Recombinant DNA procedures were carried out as described previously (23, 29).

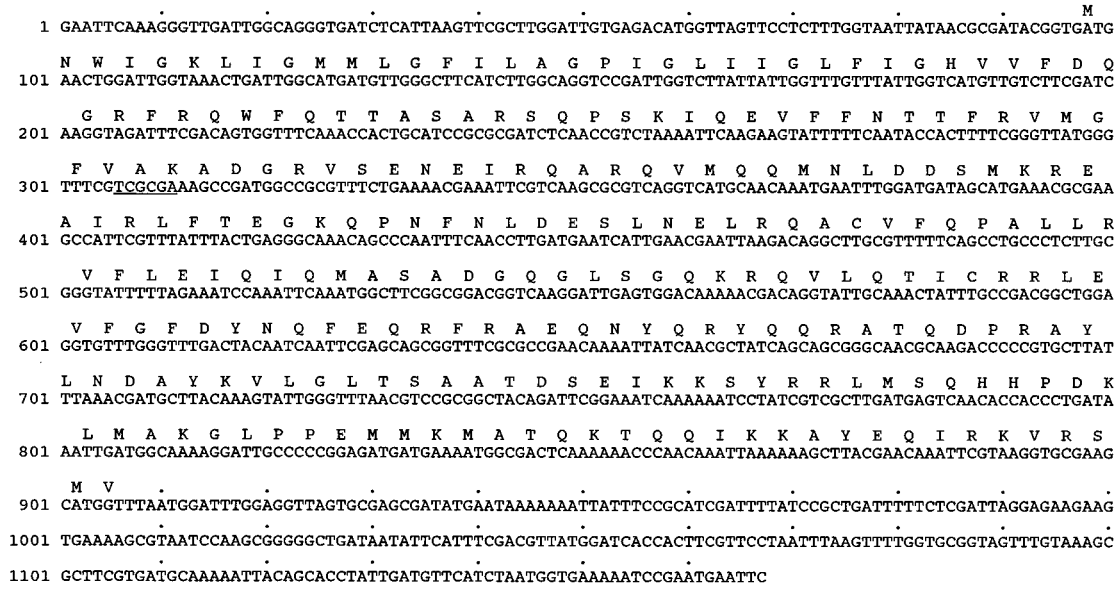

FIG. 1. Nucleotide sequence of the 1.2-kb *Eco*RI genomic DNA fragment of *C. burnetii* contained in plasmid pMZCb4105 (Table 1). An ORF that could potentially encode a protein of 270 amino acids is shown. The underlined sequence refers to the unique *Nru*I restriction site located in this ORF.

DNA sequencing. The nucleotide sequence of the 1.2-kb *C. burnetii* DNA insert in pMZCb4105 was obtained by automated DNA sequencing procedures on an Applied Biosystems model 370A DNA sequencer by procedures described previously (39). Oligonucleotides were synthesized on an Applied Biosystems model 391 PCR-Mate DNA synthesizer. The DNA sequence was analyzed for ORFs, database homologies, and other features with an IBM computer equipped with the PCGene software package (IntelliGenetics, Inc.) and programs in the University of Wisconsin Genetics Computer Group package (12).

β-Galactosidase assays. Cultures were grown in minimal (M9) glucose or tryptone broth medium containing the appropriate antibiotic(s) at 30°C for determining the *cps-lacZ* fusion expression (16). β-Galactosidase activity was assayed by the method of Miller (24). β-Galactosidase values reported in this paper are the averages of three experiments.

Nucleotide sequence accession number. The DNA sequence data reported here have been assigned GenBank accession number 42518.

RESULTS

Isolation of *C. burnetii* genomic DNA clones that induce capsule synthesis in *E. coli*. During our efforts to clone the *dnaJ* gene homolog from a *C. burnetii* (38) recombinant bacteriophage lambda *Zap*II *Eco*RI library, one lambda clone that hybridized to a *dnaJ* probe (38) yielded ampicillin-resistant (Ap^r) mucoid colonies at 37°C following in vivo excision of the plasmid DNA from the phage. Restriction analysis demonstrated that the plasmid DNAs prepared from mucoid colonies contained two *Eco*RI DNA inserts of 1.2 and 0.9 kb in the vector pBluescript SK^-, and one such plasmid was designated pMZCb410 (Table 1). The two *Eco*RI fragments from pMZCb 410 were cloned individually in the vector, and only the 1.2-kb fragment was able to confer the mucoid property to cells (Table 1). pMZCb4105 induced mucoidy at 30 and 37°C in several other *E. coli* strains tested: DH11S, DH5αF′, DC1148, BL321, W3110, HT115, HT120, HT210, and BR6010 (Table 1). Southern blot analysis clearly demonstrated hybridization of the 1.2-kb *Eco*RI DNA insert of pMZCb4105 to the genomic DNA of *C. burnetii* (data not shown).

Nucleotide sequence of the 1.2-kb *Eco*RI genomic DNA fragment of *C. burnetii*. Figure 1 shows the nucleotide sequence of the 1.2-kb *Eco*RI genomic DNA fragment of *C. burnetii*. At 90 bp downstream from one of the *Eco*RI sites, there is a methionine initiation codon for an ORF that could potentially encode a protein of 270 amino acids. There is a single-nucleotide overlap between the TAA translational stop of this ORF and an ATG initiation codon of a truncated ORF located downstream (the second ORF is not indicated in Fig. 1). Long ORFs were not found in the opposite direction. To further characterize the larger ORF, we used oligonucleotide primers specific to the flanking sequences of the gene encoding this ORF (Fig. 1) to PCR amplify a minimal 1-kb fragment containing only that gene from the *C. burnetii* genomic DNA and cloned it into pBluescript SK^+ (pCbMucZ6) and into pWS50 (31) under the *lac* and λ p_L promoter controls, respectively. The PCR-amplified gene on plasmid pCbMucZ6 (Table 1) does induce mucoidy in *E. coli* BR6010 (Table 1) and, from the λ p_L promoter on the plasmid pMZGC418, directs the synthesis in *E. coli* of a protein product of about 27,000 Da (data not shown). This overexpressed product fails to cross-react on Western immunoblots with human serum from a patient with Q fever, suggesting that the protein is not an immunologically important

TABLE 2. *C. burnetii* MucZ-mediated induction of mucoidy in *E. coli*

Plasmid[a]	Colony morphology[b] of:		
	BR6010 (*dnaK*$^+$*J*$^+$)	BR4370 (Δ*dnaJ*)	BR4392 (Δ*dnaKJ*)
pBluescript SK	Normal	Normal	Normal
C. burnetii mucZ::*tet*	Normal	Normal	Normal
C. burnetii mucZ$^+$	Mucoid	Mucoid[c]	Normal
E. coli mucZ$^+$	Mucoid	NC[d]	Normal

[a] *C. burnetii mucZ*::*tet* refers to plasmid pMZCb4015-11, where a *tet* cassette was inserted into the unique *Nru*I site of *C. burnetii mucZ*. *C. burnetii mucZ*$^+$ refers to plasmid pMZCb4105 containing *C. burnetii mucZ*. *E. coli mucZ*$^+$ refers to plasmid pEcMucZ2 containing *E. coli mucZ*.

[b] Colony morphologies were determined following transformation of the indicated plasmids in the three strains listed on L agar plates at 30°C after overnight growth.

[c] Very tiny colonies that were seen after overnight growth at 30°C were mucoid upon further incubation.

[d] NC, no colonies were obtained after transformation.

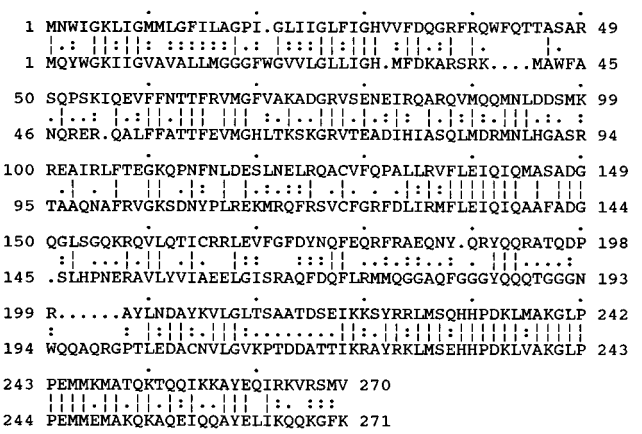

FIG. 2. Comparison of the deduced amino acid sequences of the MucZ proteins of *C. burnetii* (top line) and *E. coli* (bottom line).

antigen. It is interesting that there are two additional methionines at amino acid positions 10 and 11 after the first methionine identified in the gene. We have not unambiguously determined which of the three AUG codons is the correct initiation codon, since no sequence with similarities to the consensus ribosome-binding site was seen upstream of the three AUG codons of *mucZ* (Fig. 1). Because of its ability to induce mucoidy in *E. coli* (Table 2), we would like to designate this *C. burnetii* ORF *mucZ*. No *E. coli* consensus promoter-like sequence or Rho-independent transcription terminator-like sequence (27) is seen in the noncoding sequence located 5′ to *mucZ*.

Computer analysis for homologies in protein databases. Computer analysis of *C. burnetii mucZ* (Fig. 1) revealed significant homology to an uncharacterized *E. coli* ORF that had been identified by systematic sequencing of the 0- to 2.4-min region of the genome (37). Comparison of the deduced amino acid sequences of *C. burnetii mucZ* and its *E. coli* homolog is presented in Fig. 2. The *mucZ* ORFs of *C. burnetii* (270 amino acids) and *E. coli* (271 amino acids) encode proteins of similar size. Sequence comparison showed 41.8% identity and 63% similarity between these two proteins, with the N-terminal 32-amino-acid segment of the two MucZ proteins containing characteristics of a membrane-spanning domain. The calculated molecular mass of *C. burnetii* MucZ is 31,291 Da, with a pI value of 10.53. *E. coli* MucZ has a calculated molecular mass of 30,579 Da, with a pI value of 10.32.

Interestingly, we have also found significant amino acid homology (Fig. 3) between the C terminus of MucZ and the N termini of the DnaJ chaperone and the recently described DnaJ homolog of *E. coli*, referred to as CbpA (35). The N-terminal 70 amino acid residues of *E. coli* DnaJ constitute the most highly conserved domain of the protein (referred to as the J domain) and are important for the biological function of DnaJ. This domain of DnaJ protein is specifically used to interact with DnaK chaperone (14), another heat shock protein whose gene is located immediately upstream of *dnaJ* in *E. coli* (3, 5). Despite the J domain similarities between MucZ and DnaJ proteins, multicopy *mucZ* clones (from *E. coli* or *C. burnetii*) do not complement a *dnaJ* genetic defect in *E. coli* for λ growth (14); also, multicopy *dnaJ* clones (from *E. coli* or *C. burnetii*) do not cause a mucoid phenotype (6, 38).

No homolog to the truncated ORF located downstream of *mucZ* in *C. burnetii* is found downstream of *mucZ* in *E. coli*. However, this ORF does share a >50% identity with two *E. coli* ORFs of unknown function located at 92.5 min (GenBank accession no. P32719) and 74 min (GenBank accession no. P32661) in the *E. coli* genetic map. All of these ORFs share strong homologies with a pentose-5-phosphate-3-epimerase Calvin cycle enzyme from *Alcaligenes eutrophus* (GenBank accession no. M64173).

Induction of capsule synthesis in *E. coli* requires the DnaK heat shock system. In view of the significant similarities between the C termini of MucZ and the J domains of the DnaJ homologs (Fig. 3) and the involvement of the J domain with DnaK (14), we were interested in examining potential interactions between multicopy *mucZ* and the DnaK-DnaJ-GrpE heat shock system of *E. coli*. Results of this analysis are presented in Table 2.

As discussed above, pMZCb4105 induces mucoidy in several *E. coli* strains tested at 30 and 37°C. Insertion of the *tet* cassette into the *Nru*I site of *mucZ* resulted in loss of induction of mucoidy (Table 2). Also, deletions extending into *mucZ* prevented induction (data not shown). We also PCR amplified and cloned the wild-type *mucZ* coding region from the *E. coli* chromosome onto pBluescript to generate pEcMucZ2; this *E. coli mucZ* clone also caused mucoidy in wild-type cells (Table 2). Because all the strains used in this study carry wild-type *mucZ* on the chromosome and because only multicopy *mucZ* causes the mucoid phenotype, high gene dosage is apparently required for MucZ-mediated mucoidy in *E. coli.*

Multicopy *mucZ* may be toxic in the absence of *dnaJ*, since pEcMucZ2 transformants could not be obtained in the *dnaJ* mutant background and pMZCb4105 transformants grew very poorly (Table 2). Interestingly, multicopy MucZ-mediated toxicity was not observed in *dnaJ* mutants in which the *lacZ* reporter gene is fused to the *cpsB* gene within the *cps* operon (Table 3) (7). Since the *cps-lacZ* fusion prevents *cps* expression and capsule synthesis, toxicity in the *dnaJ* mutant may be caused by high-level expression of one or more *cps* genes or by capsule production itself.

Whereas DnaJ was not required for MucZ-induced mucoidy or stimulation of *cps-lacZ* expression, both DnaK and GrpE were required (Tables 2 and 3). The *dnaKJ* double defect did not allow MucZ-induced mucoidy (Table 2), presumably because of the *dnaK* requirement for mucoidy (Table 3). We also note that the toxicity observed in *dnaJ* mutant hosts does not occur in the double mutant *dnaK dnaJ cps*$^+$ strain (Table 2), presumably because activation of *cps* (mucoidy) is blocked by the *dnaK* defect.

Lon independence of MucZ-induced capsule synthesis: requirements for the DnaK chaperone machinery. Capsule syn-

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CbmucZ 202 LnDaYkVLGl tsaAtdsEIK KsYRRLmsqh HPDklmakGL PPEMMKmAtq KtqqIKkAYE qirkvrsmv 270
EcmucZ 203 LeDacnVLGV KPTddattIK rAYRkLmseh HPDklvakGL PPEMMemAkq KaqEIqqAYE likqgkgfk 271
CbdnaJ   3 krDYYEVLGV nlnAtEaEvK KAfRRLAmKY HPDrnpgd.. .....KdAEv KFKEarEAYE VLcDSrKRA  64
EcdnaJ   3 kqDYYEiLGV skTAeErEIr KAYkRLAmKY HPDrnqgd.. .....KeAEa KFKEIKEAYE VLtDSqKRA  64
EccbpA   3 LkDYYaimGV KPTddlktIK tAYRRLArKY HPDvske... .....pdAEa rFKEvaEAwE VLsDeqrRA  63
Consensus    L-DYYEVLGV KPTA-E-EIK KAYRRLA-KY HPD-----GL PPEMMK-AE- KFKEIKEAYE VL-DS-KRA
```

FIG. 3. Sequence homologies between the C termini of MucZ and the J domains of the DnaJ protein homologs. The abbreviations Cbmucz, Ecmucz, CbdnaJ, EcdnaJ, and EccbpA refer to the deduced amino sequences encoded by the genes *C. burnetii mucZ*, *E. coli mucZ* (GenBank accession no. D10483), *C. burnetii dnaJ* (GenBank accession no. L36455), *E. coli dnaJ* (GenBank accession no. M12544), and *E. coli cbpA* (GenBank accession no. D10483), respectively.

TABLE 3. MucZ-mediated induction of capsule synthesis in *E. coli* requires DnaK and GrpE

Plasmid[a]	Lac phenotype and *cps-lacZ* expression[b] of:			
	SG20781 (*lon*$^+$)	MZ51 (*lon*$^+$ *dnaK*)	MZ53 (*lon*$^+$ *dnaJ*::*kan*)	MZ81[c] (*lon*$^+$ *grpE*)
mucZ::*tet*	White (28 ± 1)	White (26 ± 2)	White (26 ± 1)	White
C. burnetii mucZ$^+$	Red (321 ± 33)	White (30 ± 5)	Red (259 ± 8)	White

[a] See Table 2, footnote *a*, for definitions.
[b] *cps-lacZ* expression was determined from cells grown in minimal M9 medium by measuring β-galactosidase activity as described previously. Values are given in parentheses (24). The Lac phenotype is on MacConkey lactose plates at 30°C, with the color of the colony indicated. Red means high expression of *cps-lacZ*.
[c] β-Galactosidase activity was not determined.

thesis in *E. coli* is normally regulated at the level of transcription activation by RcsA and RcsB. The RcsA transcriptional activator is a natural substrate for the *E. coli* Lon protease; consequently, RcsA protein is limiting under *lon*$^+$ conditions. It is clear, however, from the results presented in Tables 2 and 3 that MucZ induces capsule synthesis under *lon*$^+$ conditions, when the level of RcsA protein is normally limiting. It was of interest, therefore, to examine the multicopy *mucZ* effects under *lon* mutant conditions, when the RcsA level is high.

Despite the ability of MucZ to induce mucoidy at 37°C, a temperature at which *lon* mutants are not normally mucoid (18), it seemed possible that excess MucZ protein itself titrates the Lon protease and thus causes mucoidy. If such a scenario were true, multicopy *mucZ* should not stimulate capsule expression in *lon* mutant *E. coli* strains. However, pMZCb4105 caused the *lon* mutant *E. coli* DC1148 to be more mucoid. In addition, twofold-higher β-galactosidase activities were seen in *lon cps-lacZ* strain SG20780 harboring multicopy *mucZ* than in the strain harboring multicopy *mucZ*::*tet*. Thus, MucZ induces capsule synthesis in *E. coli* irrespective of its *lon*$^+$/*lon* genetic background.

In *lon* mutant cells (Table 4), MucZ-induced capsule synthesis requires DnaK but not DnaJ, as observed under *lon*$^+$ conditions (Table 3). Unexpectedly, in *lon* cells, in the absence of multicopy *mucZ*, both DnaK and DnaJ appeared to be required for capsule gene expression (Table 4, line 1). Thus, the DnaK chaperone system appears to be involved in Lon-RcsA-mediated as well as MucZ-induced capsule synthesis.

Requirements for RcsA, RcsB, and RcsC for MucZ-induced capsule synthesis. As discussed above, MucZ induces capsule synthesis in *lon*$^+$ cells, in which RcsA protein is limiting. We tested the requirement for RcsA with an *rcsA*::*kan* mutant. Interestingly, a low level of capsule expression was induced by the presence of multicopy *mucZ* (Table 5). This low-level expression of *cps-lacZ* in the absence of RcsA further supports the independence of the MucZ and Lon pathways for capsule expression. In fact, this type of low-level RcsA-independent expression is observed when the RcsB-RcsC two-component system is constitutively activated, for example by a special *rcsC** mutant or by multicopy *rcsB* (7).

To test if RcsB and RcsC are also required for MucZ-induced expression, we tested null mutants for each. As expected for *rcsB* mutants, no capsular synthesis was induced by MucZ, since RcsB is an essential activator of the *cps* genes (7, 18). Importantly, for the *rcsC* mutant, no capsule synthesis was induced by MucZ (Table 5). This result is different from that found for a *lon* mutant, in which capsule synthesis is independent of RcsC (Table 6) (7, 18), reaffirming the distinct pathways of Lon and MucZ. This result indicates that MucZ activates the RcsB-RcsC two-component pathway.

TABLE 4. Effects of *E. coli* Lon protease on the MucZ-mediated induction of capsule synthesis[a]

Plasmid	Lac phenotype of:		
	SG20780 (*lon*)	YJ1127 (*lon dnaK*)	SG20884 (*lon dnaJ*::*kan*)
mucZ::*tet*	Red (266 ± 5)	White (26 ± 1)	White (35 ± 1)
C. burnetii mucZ$^+$	Red (259 ± 10)	White (29 ± 2)	Red (145 ± 11)

[a] See footnotes to Tables 2 and 3 for details.

DISCUSSION

In this study, we have identified a *C. burnetii* gene and its *E. coli* homolog, which we call *mucZ*, by virtue of its ability in multicopy to induce mucoidy in *E. coli*. This effect of MucZ is caused by the 10-fold-increased synthesis of *cps* gene products, leading to an accumulation of colanic acid capsular polysaccharide. In *E. coli*, two cooperating transcriptional activators, RcsA and RcsB, control capsule synthesis. RcsA itself is controlled by proteolysis via the Lon protease, and RcsB is the activator component of the two-component regulators RcsC and RcsB. In *lon* mutants, RcsA is overproduced and, with RcsB, activates the *cps* genes. Under *lon* mutant conditions, RcsB need not be activated by RcsC. Under *lon*$^+$ conditions, however, where RcsA levels are low, RcsB must be activated by RcsC phosphorylation to cause capsule synthesis. RcsC is the sensor membrane protein; however, no sensory signal that stimulates its activation is known. Special RcsC* mutants that increase phosphorylation of RcsB activate *cps* genes constitutively (17).

When the RcsB protein is highly activated in the RcsC* mutant, activation of the *cps* genes occurs, albeit weakly, even without RcsA, i.e., in an *rcsA* null mutant. The RcsB regulator is essential for *cps* expression (7, 18). Normally, RcsA and RcsB interact to form a complex and permit promoter activation. This interaction also stabilizes RcsA from Lon protease. When RcsB is not activated by RcsC, it fails to interact efficiently with low levels of RcsA in *lon*$^+$ cells; however, in *lon* cells, the increased level of RcsA forms complexes with RcsB to generate the strong RcsA-RcsB activator. For this reason, the *rcsC* gene and its product are dispensable under *lon* conditions for the mucoid phenotype (Table 6, line 2).

MucZ induces mucoidy in *lon*$^+$ cells at 37°C and enhances mucoidy in *lon* cells, suggesting that MucZ induction is different from the Lon pathway to mucoidy. The alternate pathway to Lon-RcsA involves RcsC and RcsB. Null mutants of both RcsC and RcsB eliminate the expression of *cps* genes and production of colanic acid induced by MucZ (Table 5). Since *rcsC* null mutants have no effect on Lon-RcsA-dependent expression of colanic acid (Table 6) (17) but do affect MucZ-mediated production, a role for MucZ activating RcsB through RcsC is suggested. The fact that MucZ causes *cps-lacZ* expression, albeit weak, even in an *rcsA* null mutant (Table 5, pink

TABLE 5. Effects of RcsA, RcsB, and RcsC on the MucZ-mediated induction of capsule synthesis in *E. coli*[a]

Plasmid	Lac phenotype[b] of:			
	SG20781 (*lon*$^+$)	MZ57 (*lon*$^+$ *rcsA*::*kan*)	MZ60 (*lon*$^+$ *rcsB*::*tet*)	MZ63 (*lon*$^+$ *rcsC*::*tet*)
mucZ::*tet*	White (9 ± 0.7)	White (9 ± 0.3)	White (9 ± 0.4)	White (9 ± 0.3)
C. burnetii mucZ$^+$	Red (744 ± 32)	Light pink (56 ± 4.5)	White (9 ± 0.2)	White (9 ± 0.4)

[a] See footnotes to Tables 2 and 3 for details.
[b] *cps-lacZ* expression was determined from cells grown in tryptone broth medium by measuring β-galactosidase activity as described previously. Values are given in parentheses (24).

colonies), further supports a role for MucZ in the RcsC-RcsB two-component activation system rather than in the Lon-RcsA pathway.

MucZ has a C-terminal domain that is similar to the J domain of the DnaJ protein. In DnaJ, this domain interacts with DnaK to form the DnaK molecular chaperone complex that includes DnaK, DnaJ, and GrpE. Because MucZ contains this domain, we asked whether the three components of this chaperone were also involved in capsule synthesis. To our surprise, under *lon* mutant conditions, capsular expression and the mucoid phenotype are dependent upon DnaK-chaperone components (Table 4). Jubete et al. (20a) have made similar observations for the Lon-RcsA pathway to capsule production and suggest that the DnaK-chaperone system is maintaining RcsA in a soluble form that is able to productively interact with limiting RcsB function. When the MucZ system was tested for its dependence on the DnaK-chaperone system, a similar but qualitatively different result was found. DnaK and GrpE were both required for MucZ-induced capsule expression; however, DnaJ was not. Our interpretation of this result is that MucZ is required in place of DnaJ for activation of the RcsC-RcsB pathway. We suggest that the RcsC activation (phosphorylation) of RcsB requires the interaction of the DnaK-chaperone machine. In this instance, MucZ protein, instead of DnaJ, is required to target RcsC (and RcsB) for chaperone assistance.

Another role of the DnaK-chaperone machinery is evident during capsule expression induced by MucZ. In wild-type cells with MucZ, colonies express excess capsule and grow well (Table 1). In *dnaJ* mutants with MucZ, colonies express capsule but grow very poorly. This toxicity caused by MucZ may be dependent upon capsule synthesis, since *dnaK* mutants that fail to produce capsule form normal colonies, even when combined with a *dnaJ* mutant. In support of this argument, when the *cps* operon is disrupted by a *lacZ* fusion, *dnaJ* mutants that are *dnaK*$^+$ form normal nonmucoid colonies in the presence of MucZ (Table 3). Thus, induction of the mucoid phenotype under *dnaJ* mutant conditions appears to be the cause of toxicity. We speculate that the normal DnaK-chaperone machinery (DnaK, DnaJ, and GrpE) may be required to control this toxicity problem and that MucZ cannot substitute for this function of DnaJ.

The toxicity discussed above could be caused by overexpression of intermediates of the *cps* pathway or by excess capsule production itself. In an example of the latter in the mucoid alginate-producing *Pseudomonas aeruginosa*, Hassett et al. (20) have reported considerable increase in the levels of manganese superoxide dismutase to counterbalance the potentially harmful effects of elevated levels of superoxide radical caused by capsule production. This scenario, although yet to be demonstrated for other capsule-producing bacteria, may be true for *E. coli*. Assuming that it is true for *E. coli*, it is tempting to hypothesize, from the results presented here, that DnaK and DnaJ proteins are required in some way for manganese superoxide dismutase expression to overcome the toxic effects of superoxide radicals in capsule-producing strains of *E. coli.*

TABLE 6. Effects of RcsB and RcsC on the induction of capsule synthesis in *lon E. coli* hosts[a]

Plasmid	Lac phenotype of:		
	SG20780 (*lon rcs*$^+$)	MZ70 (*lon rcsB*::*tet*)	MZ76 (*lon rcsC*::*tet*)
mucZ::*tet*	Red	White	Red
C. burnetii mucZ$^+$	Red	White	Red

[a] See footnotes to Tables 2 and 3 for details.

Results presented in this paper describing our analysis of *mucZ* have unravelled the following DnaK chaperone effects. (i) *E. coli* requires the DnaK and DnaJ molecular chaperones during induction of capsule synthesis mediated by the Lon-RcsA pathway; it may also require GrpE, but this could not be tested, since the *grpE* mutation could not be placed in the *lon* mutant strain. (ii) DnaK and GrpE, but not the DnaJ chaperones, are required for the MucZ-mediated activation of the RcsB-RcsC two-component system and subsequent capsule synthesis; the requirement for MucZ in this pathway might reflect its direct involvement with the DnaK-GrpE chaperone complex. (iii) The DnaK and DnaJ chaperones may also be required to ward off toxic effects of high expression of intermediates of the *cps* system or of excess capsule production itself.

The *mucZ* gene has the same effect as another gene, *rcsF*, because of its ability to cause mucoidy in multicopy at 37°C; however, unlike multicopy *mucZ*, which induces mucoidy via RcsB and RcsC, the multicopy *rcsF* gene has its effect through RcsA (15). Interestingly, defects in the Lps (lipopolysaccharide synthesis) or DsbA (periplasmic disulfide bond formation) genes (4, 16a, 26) also cause increased capsule production dependent upon the RcsB-RcsC pathway. In this respect, we have not determined whether multicopy *mucZ* causes its phenotype by reducing Lps or DsbA levels in the cell.

We have reported previously that multicopy expression of *C. burnetii rnc* results in suppression of capsule synthesis in *E. coli* (39). Although *C. burnetii* is not known to produce a capsule-like material, it is conceivable that *rnc* plays a global regulatory role in the biology of this organism. In the case of bacteriophage λ and *E. coli*, *rnc* has been shown to mediate both positive and negative regulatory effects on the expression of many genes (10). The chaperone-like gene product of *mucZ* might also prove to be an important controlling element for several metabolic processes in *C. burnetii*, an organism adapted to replication in the acidic environment of the phagolysosomes of eukaryotic cells. The activation of the RcsB-RcsC two-component system and the induction of capsular polysaccharide synthesis by MucZ may be explained in two ways. MucZ overproduction may reduce the levels of Lps and DsbA (or some other factor), thereby having an indirect effect on the mem-

brane sensor RcsC. Alternatively, MucZ might act more directly, perhaps as a cosensor with RcsC. We have noticed that the N-terminal 32 amino acid residues of MucZ have significant similarity to membrane-spanning protein domains, supporting the notion of a MucZ-RcsC interaction. We believe that future work directed at the isolation of *E. coli mucZ* mutations and investigation of their effects on the regulation of capsule expression is crucial for a better understanding of the precise biological role of this interesting function.

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REFERENCES

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli*. Bacteriol. Rev. **36:**525–557.
2. **Balbas, P., X. Soberon, E. Merino, M. Zurita, H. Lomeli, F. Valle, N. Flores, and F. Bolivar.** 1986. Plasmid vector pBR322 and its special-purpose derivatives—a review. Gene **50:**3–40.
3. **Bardwell, J. C., and E. A. Craig.** 1984. Major heat shock gene of *Drosophila* and *Escherichia coli* heat inducible *dnaK* gene are homologous. Proc. Natl. Acad. Sci. USA **81:**848–852.
4. **Bardwell, J. C. A., K. McGovern, and J. Beckwith.** 1991. Identification of a protein required for disulfide bond formation in vivo. Cell **67:**581–589.
5. **Bardwell, J. C. A., K. Tilly, E. Craig, J. King, M. Zylicz, and C. Georgopoulos.** 1986. The nucleotide sequence of the *Escherichia coli* K12 *dnaJ*$^+$ gene. A gene that encodes a heat shock protein. J. Biol. Chem. **261:**1782–1785.
6. **Blum, P., J. Ory, J. Bauernfeind, and J. Krska.** 1992. Physiological consequences of DnaK and DnaJ overproduction in *Escherichia coli*. J. Bacteriol. **174:**7436–7444.
7. **Brill, J. A., C. Quinlan-Walshe, and S. Gottesman.** 1988. Fine-structure mapping and identification of two regulators of capsule synthesis in *Escherichia coli* K-12. J. Bacteriol. **170:**2599–2611.
8. **Chen, S. M., H. E. Takiff, A. M. Barber, G. C. Dubois, J. C. Bardwell, and D. L. Court.** 1990. Expression and characterization of RNase III and Era proteins. Products of the rnc operon of *Escherichia coli*. J. Biol. Chem. **265:**2888–2895.
9. **Cherwonogrodzky, J. W., M. H. Knodel, and M. R. Spence.** 1994. Increased encapsulation and virulence of *Francisella tularensis* live vaccine strain (LVS) by subculturing on synthetic medium. Vaccine **12:**773–775.
10. **Court, D.** 1993. RNA processing and degradation by RNaseIII, p. 71–116. *In* G. Brawerman and J. Belasco (ed.), Control of messenger RNA stability. Academic Press, Inc., San Diego, Calif.
11. **Deretic, V., C. Mohr, and D. W. Martin.** 1991. Mucoid *Pseudomonas aeruginosa* in cystic fibrosis: signal transduction and histone-like elements in the regulation of bacterial virulence. Mol. Microbiol. **5:**1577–1583.
12. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
13. **Frosch, M., and A. Müller.** 1993. Phospholipid substitution of capsular polysaccharides and mechanisms of capsule formation in *Neisseria meningitidis*. Mol. Microbiol. **8:**483–493.
14. **Georgopoulos, C., K. Liberek, M. Zylicz, and D. Ang.** 1994. Properties of the heat shock proteins of *Escherichia coli* and the autoregulation of the heat shock response, p. 209–249. *In* R. I. Morimoto, A. Tissières, and C. Georgopoulos (ed.), The biology of heat shock proteins and molecular chaperones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
15. **Gervais, F. G., and G. R. Drapeau.** 1992. Identification, cloning and characterization of *rcsF*, a new regulator gene for exopolysaccharide synthesis that suppresses the division mutation *ftsZ84* in *Escherichia coli* K-12. J. Bacteriol. **174:**8016–8022.
16. **Gottesman, S.** 1989. Genetics of proteolysis in *Escherichia coli*. Annu. Rev. Genet. **23:**163–198.

16a. **Gottesman, S.** Personal communication.

17. **Gottesman, S., and V. Stout.** 1991. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-23. Mol. Microbiol. **5:**1599–1606.
18. **Gottesman, S., P. Trisler, and A. Torres-Cabassa.** 1985. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. J. Bacteriol. **162:**1111–1119.
19. **Hackstadt, T., and J. C. Williams.** 1981. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. Proc. Natl. Acad. Sci. USA **78:**3240–3244.
20. **Hassett, D. J., W. A. Woodruff, D. J. Wozniak, M. L. Vasil, M. S. Cohen, and D. E. Ohman.** 1993. Cloning and characterization of the *Pseudomonas aeruginosa sodA* and *sodB* genes encoding manganese- and iron-cofactored superoxide dismutase: demonstration of increased manganese superoxide dismutase activity in alginate-producing bacteria. J. Bacteriol. **175:**7658–7665.

20a. **Jubete, Y., M. R. Maurizi, and S. Gottesman.** Personal communication.

21. **Kelly, T., J. P. Dillard, and J. Yother.** 1994. Effect of genetic switching of capsular type on virulence of *Streptococcus pneumoniae*. Infect. Immun. **62:**1813–1819.
22. **Mallavia, L. P., J. E. Samuel, and M. E. Frazier.** 1991. The genetics of *Coxiella burnetii*: etiologic agent of Q fever and chronic endocarditis, p. 259–284. *In* J. C. Williams and H. A. Thompson (ed.), Q fever: the biology of *Coxiella burnetii*. CRC Press, Inc., Boca Raton, Fla.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Myers, W. F., O. G. Baca, and C. L. Wisseman, Jr.** 1980. Genome size of the rickettsia *Coxiella burnetii*. J. Bacteriol. **144:**460–461.
26. **Parker, C. T., A. W. Kloser, C. A. Schnaitman, M. A. Stein, S. Gottesman, and B. W. Gibson.** 1992. Role of the *rfaG* and *rfaP* genes in determining the lipopolysaccharide core structure and cell surface properties of *Escherichia coli* K-12. J. Bacteriol. **174:**2525–2538.
27. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:**319–353.
28. **Rubens, C. E., L. M. Heggen, R. F. Haft, and M. R. Wessels.** 1993. Identification of *cpsD*, a gene essential for type III capsule expression in group B streptococci. Mol. Microbiol. **8:**843–855.
29. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Simpson, J. A., S. E. Smith, and R. T. Dean.** 1988. Alginate inhibition of the uptake of *Pseudomonas aeruginosa* by macrophages. J. Gen. Microbiol. **134:**29–36.
31. **Sisk, W. P., J. G. Chirikjian, J. Lautenberger, C. H. Jorcyk, T. S. Papas, M. L. Berman, R. Zagursky, and D. L. Court.** 1986. A plasmid vector for cloning and expression of gene segments: expression of an HTLV-I envelope gene segment. Gene **48:**183–193.
32. **Sozhamannan, S., and D. K. Chattoraj.** 1993. Heat shock proteins DnaJ, DnaK, and GrpE stimulate P1 plasmid replication by promoting initiator binding to the origin. J. Bacteriol. **175:**3546–3555.
33. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124:**307–316.
34. **Takiff, H. E., S. M. Chen, and D. L. Court.** 1989. Genetic analysis of the *rnc* operon of *Escherichia coli*. J. Bacteriol. **171:**2581–2590.
35. **Ueguchi, C., M. Kakeda, H. Yamada, and T. Mizuno.** 1994. An analogue of the DnaJ molecular chaperone in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**1054–1058.
36. **Weiss, E., and J. W. Moulder.** 1984. The rickettsias and chlamydias, p. 701–704. *In* N. R.Krieg and J. G. Holt (ed.), Bergey's manual of determinative bacteriology. The Williams & Wilkins Co., Baltimore.
37. **Yura, T., H. Mori, H. Nagai, T. Nagata, A. Ishihama, N. Fujita, K. Isono, K. Mizobuchi, and A. Nakata.** 1992. Systematic sequencing of the *Escherichia coli* genome: analysis of the 0–2.4 min region. Nucleic Acids Res. **20:**3305–3308.
38. **Zuber, M., T. A. Hoover, and D. L. Court.** 1995. Cloning, sequencing and expression of the *dnaJ* gene of *Coxiella burnetii*. Gene **152:**99–102.
39. **Zuber, M., T. A. Hoover, B. S. Powell, and D. L. Court.** 1994. Analysis of the *rnc* locus of *Coxiella burnetii*. Mol. Microbiol. **14:**291–300.