Stability of the *Escherichia coli* Division Inhibitor Protein MinC Requires Determinants in the Carboxy-Terminal Region of the Protein

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Certain mutations in the C-terminal region of the *Escherichia coli* division inhibitor protein MinC cause loss of function of the division inhibitor by making MinC more sensitive to degradation by Lon protease, implying a possible role for the C-terminal region in regulating the stability and cellular concentration of MinC.

The gene products of the *min* gene cluster of *Escherichia coli*—MinC, MinD, and MinE—are required to ensure that the division septum is properly placed at the midpoint of the cell. In the absence of normal function of the *min* gene products, septation occurs at potential division sites located adjacent to the cell poles, leading to the formation of small, spherical minicells that lack chromosomal DNA. Suppression of septation at the polar sites is normally accomplished by the action of a septation inhibitor whose function requires the *minC* and *minD* gene products. MinC is believed to be the proximate cause of the inhibition of septation by the MinCD division inhibitor, with MinD acting as an activator of the septation-inhibitory activity of the MinC protein (5). The MinC division inhibitor can also be activated by the product of *dicB* (4), a gene that is normally not expressed and is thought to be part of a cryptic prophage in *E. coli* K-12 strains (1). The MinCD division inhibitor is given specificity for polar sites by the MinE topological specificity protein (3).

A number of *minC* mutants have been identified in which the ability to respond to MinD has been lost, as shown by the observation that overexpression of MinD no longer leads to division inhibition (6). Most of the mutations are located in the 3′ region of *minC*, resulting in amino acid changes or truncations in the region between amino acids 160 and 200 of the 232-amino-acid MinC protein. This led to the suggestion that this region of MinC participates in interaction with MinD (6).

In this communication we report that mutations in the car-

boxy-terminal region of *minC* lead to instability of the MinC protein as shown by a marked decrease in MinC concentration in cells that contain a functional Lon protease. Restoration of the normal MinC concentration by growth in Lon⁻ cells was associated with a return of the division inhibition that is normally seen when *minC* is coexpressed with *minD*. Thus, determinants in the carboxy-terminal region of MinC impart protection against intracellular degradation, and the loss of function of the mutant MinC proteins reflects changes in their

FIG. 2. MinC immunoblot analysis in *lon*⁺ and *lon* mutant cells. Immunoblot analysis (5) was performed on 0.05 A_{600} U of strain PB115 (*lon*⁺ Δ*minCDE*) (lanes a, c, e, g, i, and k), RC7 (*lon* Δ*minCDE*) (lanes b, d, f, h, j and l), PB114 (*lon*⁺ Δ*minCDE*) (lane m), MS114 (*lon* Δ*minCDE*) (lane n), PB114(λDB164) (*lon*⁺ Δ*minCD*/P*lac*-*minD*) (lanes o, q, s, and u), or MS114(λDB164) (*lon* Δ*minCDE*/P*lac*-*minD*) (lanes p, r, t, and v) containing the P*lac*-*minC* plasmids pDB201 (*minC*⁺) (lanes a and b), pMS706 (*minC24*) (lanes c and d), pMS755 (*minC27*) (lanes e and f), pMS709 (*minC28*) (lanes g and h), pMS1 (*minC36*) (lanes i and j), pMS2 (*minC37*) (lanes k and l), or pCL45 (P*min*-*minC19*) (lanes m and n) or containing the P*aadA*-*minC* plasmid pJPB120m4 (*minC24*) (lanes o and p), pJPB120m5 (*minC25*) (lanes q and r), pJPB120m11 (*minC27*) (lanes s and t), or pJPB120m12 (*minC28*) (lanes u and v). Lane w contained purified MinC (100 µg). Cells were grown for 5 h in the presence of 1 mM (lanes a to l) or 0.2 mM (lanes o to v) IPTG (isopropyl-β-D-thiogalactopyranoside). Arrowheads indicate the position of authentic MinC.
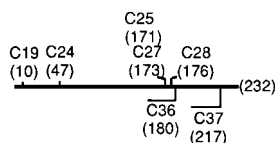

FIG. 1. *minC* mutations. Missense *minC* alleles are shown above the line representing the 232-amino-acid *minC* gene. Alleles leading to premature termination are shown below the line. Sites of amino acid replacement or premature termination of translation are indicated by amino acid position in parentheses. The *min36* and *min37* gene products also contain 11 and 1 additional amino acids from the cloning vector at their C termini, respectively.

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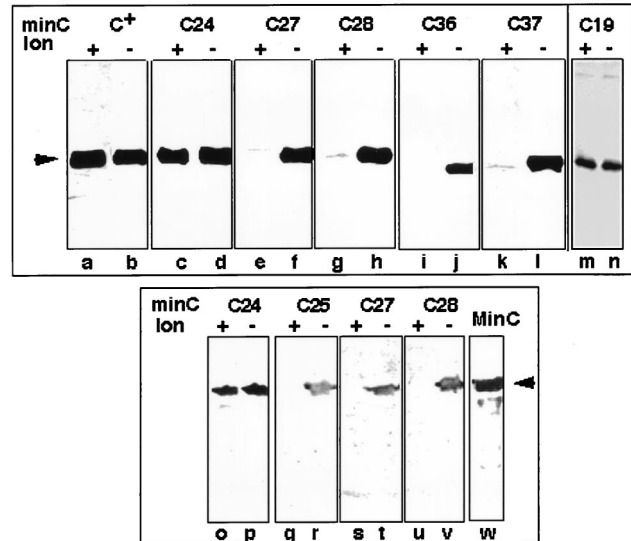
TABLE 1. Phenotypes of *minC* mutants in *lon*[+] and
lon mutant cells in the presence of MinD[a]

Plasmid	Genotype	Phenotype	
		lon[+] host	*lon* mutant host
pJBP120m4 (6)	P_{aadA}-*minC24*	Sep[−]	Sep[−]
pJPBm5 (6)	P_{aadA}-*minC25*	Min[−]	Sep[−]
pJPBm11 (6)	P_{aadA}-*minC27*	Min[−]	Sep[−]
pJPBm12 (6)	P_{aadA}-*minC28*	Min[−]	Sep[−]
pDB201 (3)	P_{lac}-*minC*[+]	Sep[−]	Sep[−]
pMS706	P_{lac}-*minC24*	Sep[−]	ND
pMS755	P_{lac}-*minC27*	Min[−]	ND
pMS709	P_{lac}-*minC28*	Min[−]	Sep[−]
pMS1	P_{lac}-*minC36*	Min[−]	Min[−]
pMS2	P_{lac}-*minC37*	Min[−]	Min[−]
pCL45 (6)	P_{min}-*minC19*	Min[−]	Min[−]

[a] The indicated *minC* plasmids were present in either a *lon*[+] (PB115 [*lon*[+] Δ*minCDE*]) or *lon* mutant (RC7 [*lon* Δ*minCDE*]) host. For the P_{aadA}-*minC* plasmids, the host was lysogenic for λDB164 (P_{lac}-*minD*) (3). For the P_{lac}-*minC* plasmids, the host also contained pDB211 (P_{aadA}-*minD*). Cells were grown overnight in the presence of 0.2 mM IPTG before examination by phase-contrast microscopy (2). In the absence of IPTG or in hosts containing the vector plasmids pBluescript or pGB2 the phenotypes were Min[−], reflecting the Δ*minCDE* genotype of the host strains. ND, not done because stable transformants could not be obtained, even when transformants were grown in the presence of glucose. Min[−], the culture contained large numbers of spherical minicells plus cells that were up to 6 times as long as normal cells, Sep[−], essentially all cells were long filaments at least 10 times as long as normal cells.

cellular concentrations rather than loss of specific sites that interact with the MinD activator protein.

Plasmids containing seven mutant *minC* alleles under control of P_{lac} or the constitutive P_{aadA} promoter were introduced into isogenic *lon*[+] and *lon* mutant strains that contained a chromosomal deletion of the *minCDE* gene cluster. Five of the mutations were missense mutations (*minC19*, *minC24*, *minC25*, *minC27*, and *minC28*); two were nonsense mutations that led to formation of truncated MinC proteins (*minC36* and *minC37*) (Fig. 1). Plasmids pMS706, pMS709, and pMS755 were prepared by replacing the 579-bp *Bsu*36I/*Pst*I fragment of pDB201 (P_{lac}-*minC*) (5) that originates within *minC* with the corresponding *Bsu*36I/*Pst*I fragments from *minC* mutant plasmids (6) pJPB120m4 (*minC24*), pJPB120m12 (*minC28*), and pJPB120m11 (*minC27*). Plasmids pMS1 (*minC36*) and pMS2 (*minC37*) were prepared by digesting the pBluescript derivative pDB201 (*minC*[+]) with *Bst*EII or *Pst*I, respectively, and then treating with T4 DNA polymerase or Klenow polymerase and religating the blunt ends to give 3′ *minC* deletion mutations.

Mutations that led to changes in the C-terminal region of the protein (*minC25*, *minC27*, *minC28*, *minC36*, and *minC37*) were associated with a marked decrease in the MinC immunoreactive band in *lon*^{+} cells compared with *lon* mutant cells (Fig. 2). The results were the same both in the absence (Fig. 2, lanes a to n) and presence (Fig. 2, lanes o to v) of MinD. In contrast, in the two mutants in which the mutations were located in the amino-terminal region of the protein (*minC19* and *minC24*), the cellular concentrations of MinC were similar in *lon*[+] and *lon* mutant hosts.

For quantitation, the cellular concentrations of the mutant proteins were estimated by comparing the intensity of the mutant MinC band with that of the MinC band in serial dilutions of a MinC[+] extract from strain PB114/pDB201 (Δ*minCDE*/P_{lac}-*minC*[+]) grown and immunoblotted under the same conditions. This showed a 93 to 97% reduction in MinC concentration in cells expressing the C-terminal MinC mutations in a *lon*[+] background as compared with a *lon* mutant background.

In the case of missense mutants in the carboxy-terminal

region of MinC (*minC25*, *minC27*, and *minC28*), the restoration of MinC concentration to near normal levels in *lon* mutant cells was accompanied by restoration of function of the MinCD-mediated division inhibition system, as shown by the formation of nonseptate filaments when the mutant alleles were coexpressed with *minD* (Table 1 and Fig. 3f to h). In contrast, in *minC* mutants in which immunoblotting showed that MinC concentration was unaffected by the *lon* status of the host (*minC19* and *minC24*), the phenotypes were also unaffected by the *lon* status of the host (Table 1 and Fig. 3).

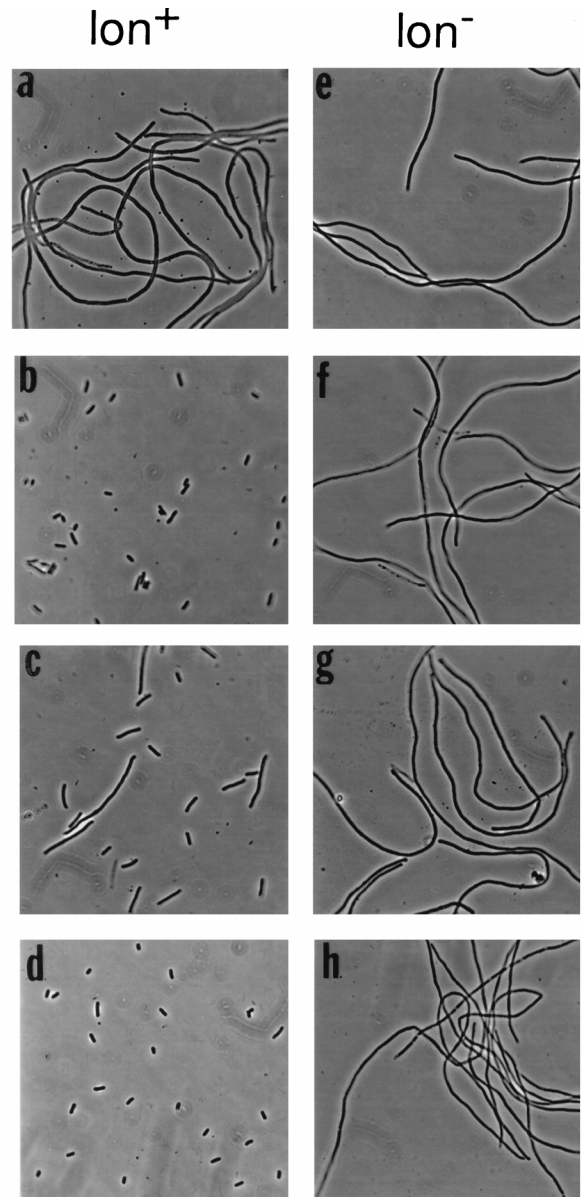We conclude that determinants in the carboxy-terminal re-

FIG. 3. Phenotypes of *minC* mutants in the presence of MinD in *lon*[+] and *lon* mutant cells. Representative examples are shown; other results are summarized in Table 1. The *lon*[+] Δ*minCDE* hosts were PB114 (λDB164 P_{lac}-*minD*) (a to c) and RC3F^{−}/pDB211 (P_{aadA}-*minD*) (d). The *lon* Δ*minCDE* hosts were MS114 (λDB164) (e to g) and MS114/pDB211 (h). The strains contained *minC* plasmid pJPB120m4 (P_{aadA}-*minC24*) (a and e), pJPB120m11 (P_{aadA}-*minC27*) (b and f), pMS709 (P_{lac}-*minC28*) (c and g), or pJPB120m5 (P_{aadA}-*minC25*) (d and h). Cells were grown overnight in the presence of 0.2 mM IPTG (isopropyl-β-D-thiogalactopyranoside) prior to examination by phase-contrast microscopy (2).

gion of MinC are required to maintain the protein in a state that is resistant to the *lon* protease since alteration of individual amino acids in the C-terminal domain or removal of the C-terminal 15 or 52 amino acids was associated with a marked reduction in cellular concentration of MinC in *lon*^{+} cells. The reduction of MinC concentration, in turn, appeared responsible for the previously observed loss of the ability to respond to MinD activation. Therefore, the loss of the ability of the mutant MinC proteins to support the MinCD-mediated division inhibition reaction cannot be used to support the view that this region is directly implicated in interactions with MinD. The results instead imply a role for the C-terminal region in regulating the stability and cellular concentration of MinC. It is not known whether this reflects a role in MinC folding or whether it indicates the presence of a site in the C-terminal region that is involved in other interactions that affect the susceptibility of MinC to intracellular turnover.

REFERENCES

1. **Béjar, S., F. Bouché, and J.-P. Bouché.** 1988. Cell division inhibition gene *dicB* is regulated by a locus similar to lambdoid bacteriophage immunity loci. Mol. Gen. Genet. **212:**11–19.
2. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1988. Isolation and properties of *minB*, a complex genetic locus involved in correct placement of the division site in *Escherichia coli*. J. Bacteriol. **170:**2106–2112.
3. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*. Cell **56:**641–649.
4. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1990. Central role for the *Escherichia coli minC* gene product in two different cell division-inhibition systems. Proc. Natl. Acad. Sci. USA **87:**1129–1133.
5. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1992. Roles of MinC and MinD in the site-specific septation block mediated by the MinCDE system of *Escherichia coli*. J. Bacteriol. **174:**63–70.
6. **Mulder, E., C. L. Woldringh, F. Tétart, and J.-P. Bouché.** 1992. New *minC* mutations suggest different interactions of the same region of division inhibitor MinC with proteins specific for *minD* and *dicB* coinhibition pathways. J. Bacteriol. **174:**35–39.