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 minicells that lack chromosomal DNA. Suppression of septation occurs at potential division sites located adjacent to a cryptic prophage in a gene that is normally not expressed and is thought to be part of the minD gene products. MinC is believed to be the proximate inhibitor can also be activated by the product of minE (4), a gene that is normally not expressed and is thought to be part of a cystic 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 carboxy-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 stability.

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**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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**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− ΔminCDE) (lanes a, c, e, g, i, and k), RC7 (lon ΔminCDE) (lanes b, d, f, h, j, and l), PB114 (lon− ΔminCDE) (lanes m), MS114 (lon ΔminCDE) (lanes n), PB114(ΔDB164) (lon− ΔminCDE P_{minD}) (lanes o, q, s, and u), or MS114(ΔDB164) (lon ΔminCDE P_{minD}) (lanes p, r, t, and v) containing the P_{minD} ΔminCDE plasmids pBB201 (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_{minD} ΔminC19) (lanes m and n) or containing the P_{minD} ΔminCDE plasmid pJPB120m4 (minC24) (lanes o and p), pJPB120m5 (minC25) (lanes q and r), pJPB120m6 (minC27) (lanes s and t), or pJPB120m7 (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.

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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 MinC-D-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 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 MinC-D-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).

Plasmids containing seven mutant minC alleles under control of P<sub>Plac</sub> or the constitutive P<sub>metA</sub> promoter were introduced into isogenic lon<sup>+</sup> 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 Bsu36I/PstI fragment of pDB201 (P<sub>metA</sub>-minC) with PstI and SalI fragments from minC mutant plasmids (6) pJBP120m4 (minC24), pJBP120m12 (minC28), and pJBP120m11 (minC27). Plasmids pMS1 (minC36) and pMS2 (minC37) were prepared by digesting the pBlue-Script derivative pDB201 (minC<sup>+</sup>) with BspEI or PstI, 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> extract from strain PB114/pDB201 (ΔminCDEP<sub>metA</sub>-minC<sup>+</sup>) 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<sup>+</sup> 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 near normal levels in lon mutant cells was accompanied by restoration of function of the MinC-D-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).

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Region 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.

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REFERENCES