Lack of Regulation of the Modification-Dependent Restriction Enzyme McrBC in *Escherichia coli*

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Received 19 October 2001/Accepted 13 December 2001

Restriction alleviation (RA) by the type I restriction enzyme *EcoKI* is caused by treatments that damage DNA. RA is due to proteolysis of the *EcoKI* HsdR subunit by the ClpXP ATP-dependent protease. Here we show that the modification-dependent enzyme McrBC is not subject to RA, although it is moderately sensitive to ClpAP.

Bacteria that have been treated with agents that damage DNA can become temporarily deficient in their ability to restrict incoming phage or plasmid DNA, a phenomenon known as restriction alleviation (RA) (4, 15). Recently, the molecular explanation for RA of the type I restriction enzyme *EcoKI* has been found (9): it depends on proteolysis of the HsdR subunit of *EcoKI* by the ATP-dependent protease ClpXP (3) under RA conditions. Proteolysis plays a key role in the regulation of diverse metabolic processes and in the cell's responses to environmental stimuli (12). As HsdR is the *EcoKI* subunit that catalyzes DNA cleavage, RA is readily explained. RA is biologically meaningful, because bacteria with damaged DNA are likely to generate unmodified restriction enzyme recognition sites by DNA repair synthesis. Such unmodified sites would be lethal in the presence of an active restriction enzyme.

McrBC from *Escherichia coli* is a member of a class of restriction enzymes that recognize and cleave DNA carrying modification patterns imprinted by foreign hosts. It cuts DNA with a modified cytosine (mC; 5-methyl-cytosine or 5-hydroxymethyl-cytosine) preceded by a purine, and cleavage requires pairs of such sequences separated by 40 to 3,000 bp (14). Because unmodified DNA is not a substrate for this enzyme, DNA damage and subsequent repair cannot be a biological cause of RA of McrBC. Reports of RA on McrBC action are conflicting (2, 7, 8). However, the three reports used different host bacteria and substrates: mC-containing λ phages (8), T4gt phages (2), and mC-containing plasmid DNA (7). From these studies, it was concluded that the mechanism of RA for *EcoKI* must be basically different from that for McrBC (8).

Here we have reinvestigated RA of McrBC by using a set of Clp mutants of *E. coli* W3110, the closest to the ancestral *E. coli* K-12 of all laboratory strains (1). A *clpX* mutant strain was created by P1 transduction of the $\Delta clpXI::Kan$ locus (6) into W3110 (strain SSN1 [11]). At the same time, a transduction of the $\Delta clpPI::cm$ allele (10) was carried out, resulting in a *clpP*-deficient strain. The *clpA* strain was an MC4100 derivative (RH7189) (5).

Under the conditions described by Dharmalingam and Gold-

berg (2), restriction by McrBC assayed with T4 and T4gt phages was unaffected by UV irradiation of the host cells (Fig. 1). *EcoKI* restriction assayed with λ phage in the same experiment was alleviated (reduced) about 100-fold, confirming the results of Makovets et al. (9) (Fig. 1). Similar results were obtained when 2-aminopurine was used as the DNA-damaging agent (results not shown). Thus, bacteria that show RA for *EcoKI* do not show it for McrBC.

The possibility remained that the McrBC enzyme was a target for one of the Clp proteases but that residual McrBC levels remained high enough to provide efficient restriction. Figure 2 depicts the results of Western blot analysis that shows that the amount of the McrB subunit in a wild-type cell is greatly reduced in the stationary phase of growth compared to the amount found in exponentially growing bacteria. This decline that occurs in the stationary phase is not seen in cells carrying mutations in the *clpP* or *clpA* genes but is found in cells with mutations in the *clpX* gene. Thus, McrB is a substrate for the ClpAP protease, at least in the stationary phase of growth.

Treatment of exponentially growing cells with 2-aminopurine to induce RA does not stimulate proteolysis of McrB but does lead to proteolysis of the HsdR subunit of *EcoKI*, as described by Makovets et al. (9) (Fig. 3). As shown in Fig. 3,

FIG. 1. RA after exposure to UV irradiation. Wild-type *E. coli* strain W3110 was irradiated with increasing doses of UV light. Restriction was determined by using lambda phages for *EcoKI* (▲) and T4gt phages for McrBC (●).

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FIG. 2. Results of Western blot analysis with anti-McrB antibodies. The *E. coli* strains were grown in Luria-Bertani broth, and samples were taken at 2 and 24 h. The relevant genotype of each strain is indicated at the top. wt, wild type.

HsdR is stable in the *clpP*- and *clpX*-deficient strains, confirming that it is the ClpXP protease that is responsible for RA (9). Under these conditions, McrB levels remained constant, a result which is in agreement with the lack of RA activity described above.

From these studies, we conclude that RA of *EcoKI* is crucial for the survival of the cell after treatment with a mutagen, since the bacteria with damaged DNA are likely to generate unmodified restriction enzyme recognition sites by the DNA repair mechanism. In contrast to this, unmethylated DNA is not a substrate for McrBC restriction, abolishing the need for RA of this restriction system. Earlier studies suggested that W3110, the *E. coli* strain used in these studies, may carry an as-

yet-uncharacterized restriction system active against T-even phages (13). This may explain some of the differences between our results and those of previous reports (2, 7, 8). However, as McrB is moderately sensitive to the ClpAP protease, this may also help to reconcile results concerning RA in previous publications (2, 7, 8).

We thank Andreas Kuhn and Elisabeth A. Raleigh for materials used in this study.

This work was supported by the Swiss National Science Foundation.

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FIG. 3. Results of Western blot analysis of *E. coli* extracts treated with anti-*EcoKI* (upper panel) and anti-McrB (lower panel) antibodies. Treatment with (+) or without (-) 2-aminopurine is indicated. The relevant genotype of each strain used is indicated at the top. wt, wild type.