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Nature of DNA and RNA Degradation in *Escherichia coli* Induced by Colicin E₂

Sir:

Colicin E₂, one of the bacteriocins produced by *Enterobacteriaceae*, induces remarkable degradation of DNA¹⁾ and RNA²⁾ in sensitive strains of *Escherichia coli*. What kinds of DNase and RNase are responsible for these degradations are not yet known. Endonuclease I and RNase I are known to be the main degradative enzymes for DNA and RNA in *E. coli* cells. However, genetic mutants defective for these enzymes, *E. coli* 1100 (endonuclease I⁻) and *E. coli* Q 13 (RNase I⁻), also showed considerable degradation of their own nucleic acids due to colicin E₂, which seemed to exclude the possibility of these enzymes being involved in the colicin action (Fig. 1).³⁾

Nature of DNA and RNA degradation induced by colicin E₂ was studied by zonal sedimentation analysis. Colicin E₂ was prepared according to the procedure described by Beppu and Arima.⁴⁾ *E. coli* K 12 W 2252 *thy*⁻ (*met*⁻, *λ*^s) prelabeled with ³H-thymidine was challenged with colicin E₂ at high multiplicity (about 50) and cells sampled at appropriate intervals were lysed by treatments with lysozyme-EDTA and sodium dodecyl sulfonate.⁵⁾ The lysate was ultracentrifuged

FIG. 1. Time Course of DNA and RNA Degradation.

Colicin E₂ was added to the growing culture at 37°C 30 min after chasing of cells pre-labeled with ³H-TdR (A) or ¹⁴C-uracil (B). Samples were taken into equal volumes of cold 10% TCA and residual radioactivities were counted.

- *E. coli* W 2252 *thy*⁻
- *E. coli* 1100
- ▲—▲ *E. coli* B
- *E. coli* Q 13
- *E. coli* 1100, no colicin E₂ addition
- *E. coli* Q 13, no colicin E₂ addition

through alkaline sucrose density gradient (5~20%) and fractionated. Radioactivity in each fraction was measured by liquid scintillation spectrometer. As shown in Fig. 2, band of DNA shifted toward the top rapidly after the colicin challenge (average MW of DNA was calculated⁶⁾ to decrease to 70% after 3 min and 35% after 7 min). Sedimentation analysis at neutral pH also showed rapid decrease of average MW of DNA at early stage.

Seven minutes after the colicin challenge,

1) M. Nomura, *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 315 (1963).

2) K. Nose and D. Mizuno, *Biochem. Biophys. Acta*, **119**, 639 (1966).

3) A. Yanai, T. Beppu and K. Arima, presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April 4, 1969.

4) T. Beppu and K. Arima, *J. Bacteriol.*, **93**, 80 (1967).

5) E. M. Kutter and J. S. Wiberg, *J. Mol. Biol.*, **38**, 395 (1968).

6) J. Abelson and C. A. Thomas, *J. Mol. Biol.*, **18**, 262 (1966).

FIG. 2. Alkaline Sucrose Density-gradient Profile of ^3H -TdR Labeled *E. coli* W 2252 *thy*⁻ DNA at Various Stages during Degradation.

releasing of radioactivity into the cold acid-extractable fraction reached only 5% of total activity originally present in DNA. These observations suggest that early stage of DNA degradation due to colicin E_2 is endonucleolytic.

Zonal sedimentation analysis of RNA-degradation due to colicin E_2 was carried out with *E. coli* Q 13 preliminary labeled with ^{14}C -uracil. Cells were lysed by lysozyme-EDTA-SDS, and RNA was extracted by phenol method and ultracentrifuged through 1.5~15% sucrose density-gradient.⁷⁾ As shown

FIG. 3. Sucrose Density-gradient Profile of ^{14}C -Uracil Labeled *E. coli* Q 13 RNA at Various Stages during Degradation.

in Fig. 3, both 23s and 16s ribosomal RNA were degraded but residual RNA retained considerable size even 60 min after the colicin challenge.

These data suggest that some degradative enzyme(s), probably endonucleolytic one ex-

7) E. H. McConkey, "Methods in Enzymology," Vol. XIII, Academic Press, 1967, p. 620.

cept endonuclease I and RNase I, may be responsible for the early stage of nucleic acid degradation induced by colicin E₂.

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