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In situ distribution of *Eco*RI methylase and restriction endonuclease in cells of *Escherichia coli Bs 5*

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Specific IgG antibodies were raised in rabbits against purified *Eco*RI methylase and restriction endonuclease. Post embedding labeling experiments, using the protein A-gold technique, were made with paraformaldehyde-glutaraldehyde fixed cells, embedded in Lowicryl K4M resin at low temperatures. Labeling with methylase-specific antibodies showed 60–70% of gold particles in the cytoplasm and 30–40% at the cell envelope, whereas the use of restriction enzyme-specific antibodies led to a distribution of 10–30% in the cytoplasm and 70–90% in the cell envelope. The results coincide with the proposed function of the enzymes: in the cytoplasm methylase protects the cells' own DNA from self-destruction, and the restriction endonuclease cuts foreign DNA when entering the cell.

*Eco*RI methylase; *Eco*RI restriction endonuclease; Immunoelectron microscopy; Low-temperature embedding; Protein A-gold labeling

1. INTRODUCTION

Restriction endonucleases have gained great importance in molecular genetics as tools to analyze DNA samples and to isolate and transfer genes. Their original destination in the cell is regarded to be the protection of the organism against foreign DNA, where the restriction enzyme has the task of cutting foreign DNA that enters the cell. On the other hand, the modification enzyme has to protect the cell against self-destruction. *Eco*RI is a type II restriction enzyme. In this type two different proteins show either restriction or modification activity. Both enzymes of the *Eco*RI system have been purified and characterized with respect to structure and function [1–7]. Immunocytochemical techniques have proven to be valuable methods for locating proteins at their original position inside cells [8–11]. Recently we have shown that the majority of the molecules of the *Eco*RI restriction endonuclease within *Escherichia coli Bs 5* are located in the periplasmic space [11]. Here, we describe the different distribution of the modification methylase and the restriction endonuclease inside the bacterial cell and relate the results to their proposed function.

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2. MATERIALS AND METHODS

2.1. *Materials*

Lyophilized protein A, protein A-Sepharose CL-4B, and calibration proteins were purchased from Pharmacia (Uppsala), the Lowicryl K4M kit from Chemische Werke Lowi (Waldkraiburg, FRG), and the DNA-cellulose from Sigma (Deisenhofen, FRG). All other chemicals were bought from Merck (Darmstadt).

2.2. *Enzyme preparations*

*Eco*RI restriction endonuclease was isolated from *E. coli Bs 5* (DSM 686) as described by Rubin and Modrich [3]. A probe of *Eco*RI methylase,

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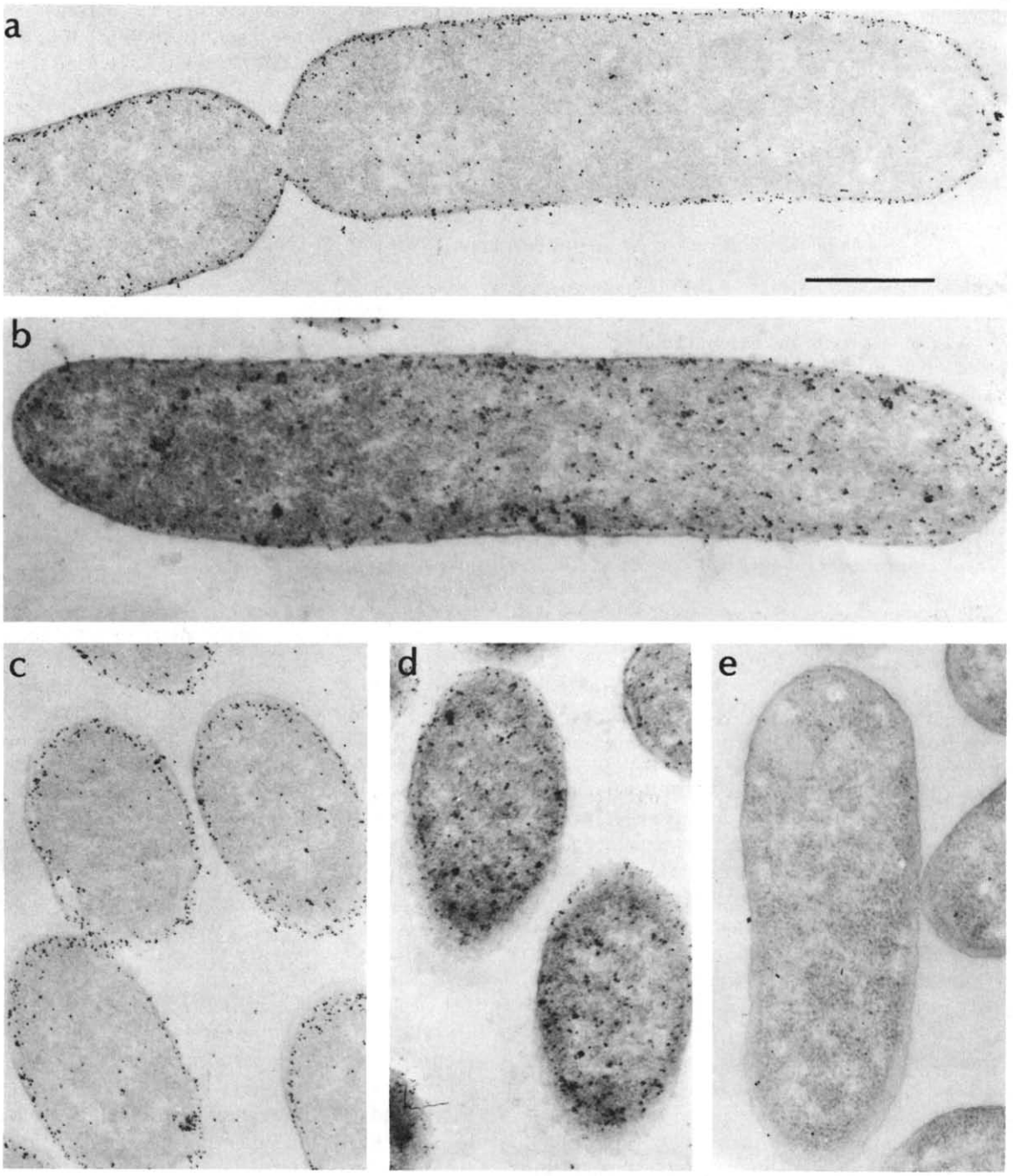

Fig.1. Protein A-gold labeling on ultrathin sections of low-temperature embedded *E. Bs 5* cells using *Eco*RI restriction endonuclease-specific antibodies (a,c), *Eco*RI methylase-specific antibodies (b,d) or preimmune serum as a control experiment (e). Bar, 0.5 μm.

isolated from an overproducing strain [12], was kindly provided by Dr P. Modrich (Durham, NC). In order to obtain a purified methylase sample from this probe for antiserum preparation, the enzyme was bound to DNA-cellulose in a column and eluted with three different NaCl concentrations (0.25, 0.5, 0.75 M).

2.3. *Preparation of antiserum, and electron microscopy*

Specific IgG-antibody preparation and purification, protein A-gold preparation, embedding of the bacteria and the labeling experiments were done as described [11]. Antibody specificity was demonstrated with the Ouchterlony double-immunodiffusion test [13] and with the golden blot technique [14].

Electron micrographs were taken with a Philips EM 301 electron microscope at calibrated magnifications, and at an acceleration voltage of 80 kV.

3. RESULTS AND DISCUSSION

Prior to further purification, the methylase sample showed several bands on an SDS-polyacrylamide gel. Therefore, the protein had to be purified before injection into a rabbit. Absorption chromatography on DNA-cellulose turned out to be an easy method to separate the methylase from other proteins. The purified samples, showing only one band at 39 kDa, were used for antibody preparation.

There was no cross-reaction between purified methylase-specific IgG antibodies and endonuclease protein or between endonuclease-specific IgG antibodies and methylase protein, either in the Ouchterlony double-immunodiffusion test, or with the golden blot technique.

The results of the labeling experiments are depicted in fig.1. Endonuclease-specific antibodies resulted in labeling of ultrathin sections mainly at the cell envelope (fig.1a,c), whereas methylase-specific antibodies bound all over the cell, resulting in a uniform distribution of the gold particles (fig.1b,d). It has been shown previously that most *Eco*RI endonuclease molecules are located in the periplasmic space [11], and statistical analyses confirm these data. An amount of 10–30% of the endonuclease has been found in the cytoplasm and 70–90% in the cell envelope. The *Eco*RI methylase distribution, however, amounted to 60–80% in the cytoplasm and 30–40% in the area close to the cell envelope. Thus, as most of the methylase protein is located in the cytoplasm, newly synthesized DNA can be rapidly modified. Also most of the gold particles near the cell envelope are found on the inner side of the cytoplasmic membrane indicating that the given percentage in the cytoplasm represents a minimal value.

The diffusion rates for proteins in the periplasmic space are lower than in the cytoplasm (1000-fold lower than in water and 100-fold lower than in the cytoplasm [15]). Therefore, the uniform distribution and the large amount of the restriction endonuclease in the periplasmic space increase the protection of the cell and lower the probability of foreign DNA entering the cell uncleaved.

The distribution of these enzymes as demonstrated here, however, is not absolutely necessary for protection of the cell's own DNA and cleavage of foreign DNA. This scope should also be attained, although not quite as efficiently, by a uniform distribution of both enzymes by the fact that DNA methylated only in one strand (the old one) is also protected from cleavage. Thus it remains to be seen whether all type II restriction systems are distributed similarly to the case investigated.

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REFERENCES

[1] Modrich, P. and Roberts, J.R. (1982) in: Nucleases (Linn, S.M. and Roberts, R.J. eds) pp. 109–154, Cold Spring Harbor, NY.
[2] Pingoud, A., Urbanke, C., Alves, J., Ehlbrecht, H.-J., Zabeau, M. and Gualerzi, C. (1984) Biochemistry 23, 5697–5702.
[3] Rubin, R.A. and Modrich, P. (1980) Methods Enzymol. 65, 96–104.
[4] Halford, S.E. and Johnson, N.P. (1983) Biochem. J. 211, 405–415.

[5] Johannssen, W., Schuette, H., Mayer, H. and Mayer, F. (1984) Arch. Microbiol. 140, 265–270.
[6] Rubin, R.A. and Modrich, P. (1977) J. Biol. Chem. 252, 7265–7272.
[7] Young, T.S., Kim, S.H., Modrich, P., Beth, A. and Jay, E. (1981) J. Mol. Biol. 145, 607–610.
[8] Bendayan, M., Roth, J., Perrelet, A. and Orci, L. (1980) J. Histochem. Cytochem. 28, 149–160.
[9] Rohde, M., Mayer, F. and Meyer, O. (1984) J. Biol. Chem. 259, 14788–14792.
[10] Roth, J., Bendayan, M. and Orci, L. (1978) J. Histochem. Cytochem. 26, 1074–1081.
[11] Kohring, G.-W., Mayer, F. and Mayer, H. (1985) Eur. J. Cell Biol. 37, 1–6.
[12] Cheng, S.-C., Kim, R., King, K., Kim, S.-H. and Modrich, P. (1984) J. Biol. Chem. 259, 11571–11575.
[13] Ouchterlony, O. (1949) Acta Pathol. Microbiol. Scand. 26, 507–515.
[14] Brada, D. and Roth, J. (1984) Anal. Biochem. 142, 79–83.
[15] Brass, J.M., Higgins, C.F., Foley, M., Rugman, P.A., Birmingham, J. and Garland, P.B. (1986) J. Bacteriol. 165, 787–794.