The Localization of Deoxyribonucleic Acid in *Escherichia coli*.*

By Lucien G. Caro, Robert P. van Tubergen, and Frederick Forro, Jr.‡ (*From the Department of Biophysics, Yale University, New Haven, Connecticut.*)§

The purpose of this study was to localize deoxyribonucleic acid in a bacterial cell. Tritium-labelled thymidine was employed to identify the DNA and radioautography was chosen as the method of detection. To insure a high percentage of labelling, a thymine-requiring strain of *Escherichia coli*, the strain 15 T⁻, was used (1). When whole cells are studied the localization of the label is limited by the resolution of the autograph procedure which, even for tritium, is about 1.5 micron, the same order of magnitude as the cell itself. This difficulty was overcome by studying the distribution of label among thin cross-sections of the cells. The thinness of the sections is then the determining factor in establishing the precise location of isotopic material in the cell.

Materials and Methods

Electron Microscopy.—Cultures of *E. coli* 15 T⁻ were grown exponentially for 6 to 7 generations at 23°C. in M-9 synthetic medium (2) containing 4 micrograms of thymidine per ml. The cells were fixed for 15 minutes at room temperature in 1 per cent OsO_4 buffered at pH 7.0 with acetate-veronal buffer and containing 0.6 M sucrose, followed by 4 hours in 10 per cent formaldehyde in the same buffer (3). They were then dehydrated and embedded according to standard techniques (4). Sections were cut on a Porter-Blum microtome and floated on a mixture of 20 per cent acetone and 1 per cent lanthanum nitrate in water (9). Lanthanum nitrate acts as a stain for certain cellular components and increases the over-all contrast. The sections were examined in an RCA EMU-3B microscope.

Radioautography.—Small cultures (0.05 ml.) were grown in M-9 medium containing tritium-labelled thymidine from Schwarz Labs, Inc. Approximately 0.05 ml. of 2 per cent agar at 40°C. was added to the culture, mixed thoroughly, and allowed to gel for a few minutes. This procedure insured a good dispersion of the cells for subsequent radioautography. Fixation and embedding were carried out as described for electron microscopy. Examination in the electron microscope showed that the addition of the agar did not introduce morphological variations. Sections were cut at 0.25 micron, mounted on a clean glass slide, dried, and dipped into a 1 per cent solution of collodion in amyl acetate. The sections were then covered with a strip of Kodak autoradiographic emulsion and exposed for varying lengths of time (5). After photographic processing they were mounted in 50 per cent glycerin and covered with a thin cover slip. Without the collodion treatment the film became detached from the section during the final stages of processing. The amount of label present in a given cross-section of a cell was estimated by counting the number of exposed photographic grains associated with it, correcting for tracks whenever it seemed necessary. Phase contrast was employed in locating sections of cells; bright field, for counting grains.

RESULTS

Electron Microscopy.—*E. coli* 15 T⁻ shows only a small number of morphologically distinct cellular components (Figs. 1 and 2). These are:

(*a*) A cell wall composed of two dense layers, deeply stained by lanthanum nitrate, 40 to 50 A in thickness, separated by a lighter layer of similar thickness.

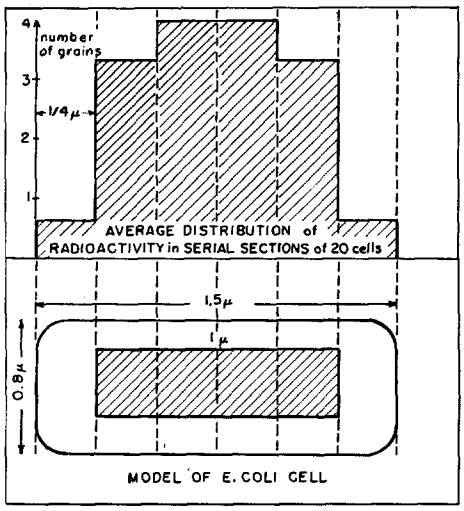
(*b*) A cytoplasm or ground substance containing numerous small dense granules 75 to 100 A in diameter. Also throughout this region are larger granules (350 to 700 A) located mostly at the poles of the cell. These bodies appear only after lanthanum nitrate staining. Their nature is unknown.

(*c*) A central, roughly cylindrical, electron-transparent region along the axis of the cell, containing only a dispersed network of very fine fibrils of various sizes. These can be as thin as 20 to 25 A. In some cells the fine fibrillar material disappears, to be replaced by thicker strands. This region has been described by various authors (6, 7) as the nuclear region, mostly on the basis of a comparison with the chromatin bodies seen in the light microscope (8). Bradfield (11) reached a similar

* Supported in part by research grants from the John A. Hartford Foundation and from the United States Public Health Service (Grant No. C-2768).

‡ The authors wish to thank Dr. Harold J. Morowitz and Dr. Ernest C. Pollard for valuable suggestions and advice.

§ *Received for publication, April 17, 1958.*



TEXT-FIG. 1. Average distribution of radioactivity in serial sections.

conclusion, using a modified Feulgen technique. A more direct identification was deemed worth while.

Dimensions of the cell derived from an examination of a large number of micrographs are as follows:

Length of cell—1.56 microns ranging from 1.10 microns to 2.20 microns (for dividing cells).

Width of cell—0.80 micron.

Length of nuclear region—⅔ length of cell—1.0 micron.

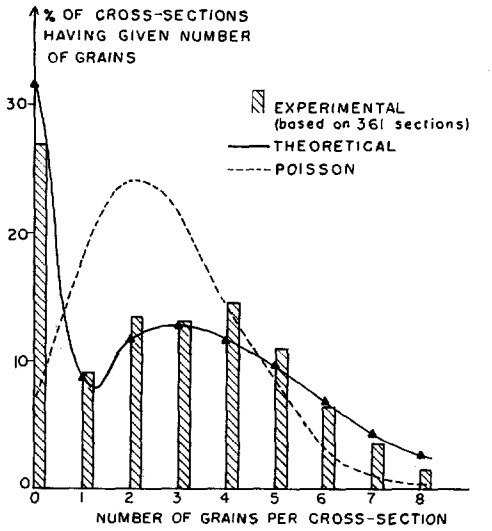
From these data a model was drawn of the *E. coli* cell, to be used in an analysis of the results (Text-fig. 1).

Radioautography.—(*a*) *Serial sections.* The maximum number of serial sections obtained was five. Only those cells were selected for analysis that were oriented in a direction perpendicular to the plane of the sectioning and the first section of which contained a full ¼ micron section through the tip (that is to say, the first section was not a grazing one). These cells were then followed through at least three sections, representing 0.75 microns or ½ the length of the average cell. The results for 20 cells are shown in Table I. Text-fig. 1 shows a block diagram of the distribution of label in serial sections, obtained by averaging these results and assuming symmetry for the other half of the cell. By comparison with the model of an *E. coli* cell, we can see that the longitudinal distribution of radioactivity follows fairly closely that of nuclear material. As might be expected from this result, grazing longitudinal sections seem to be devoid of activity.

TABLE I

Distribution of Grains in Serial Sections of 20 Cells

First section	Second section	Third section
1	5	2
0	6	5
0	5	4
1	1	2
0	4	9
0	5	2
1	4	2
0	3	4
0	3	5
1	4	5
0	0	4
0	4	6
3	3	6
0	1	3
0	2	3
0	4	5
3	3	1
1	2	4
1	3	4
0	4	3



TEXT-FIG. 2. Distribution of grain among individual sections.

(*b*) *Distribution of grains among individual sections.* In order to collect more extensive data, the less exacting procedure of examining randomly selected, rather than serial, sections was adopted. Sections exposed for varying lengths of time were

examined and the distribution of grains among single cross-sections of cells was plotted for each field. For comparison the expected distribution of radioactivity was calculated, assuming the label to be distributed uniformly in the nuclear region as shown in the model of *E. coli* cell in Text-fig. 1. The expected distribution was arrived at by the following reasoning:

Consider a number of sections having equal amounts of label. The number of radioactive decays and therefore of photographic grains associated with single sections should follow a Poisson distribution (10). If a is the average number of decays per section, the probability that a given section will have i grains is $P_i = \frac{a^i e^{-a}}{i!}$. This applies to only one class of sections having a given amount of isotope. Let P_f be the probability that a given section belongs to that class. If there are n classes containing varying amounts of isotope, the total probability that a section will have a number of grains i becomes

$$P_i = \sum_{f=1}^{n} P_f \frac{a_f^i \cdot e^{-a_f}}{i!},$$

in which a_f is the average amount of label for each class. The activity of the whole cell was established for each exposure by finding the average grain count for a large number of sections and by multiplying this by the average number of sections it takes to make a whole cell.

To simplify calculations it was assumed that sections could be divided into three classes: sections containing no nuclear region, sections through the nuclear region and containing, therefore, 25 per cent of the total nuclear region, and sections containing ½ that amount or 12.5 per cent. This last class represents an average of the sections cutting partially through the nuclear region. It was found that a more elaborate analysis brought little change to the shape of the final curve. The probability for each class was evaluated from the model and the distribution of radioactivity calculated according to the formula shown above.

Text-fig. 2 shows the results obtained for a 14 day exposure. In the same figure is drawn a Poisson distribution curve representing the hypothetical case of a uniform distribution of radioactivity in the cell. The observed distribution fits reasonably well the theoretical one, based on the model shown in Fig. 1. Exposures of 3.5 days and 9 days yielded similar results.

It is worth noting that if the grain count is plotted in the second and third sections in serial sections, that is those sections which are now known to go through the nuclear region, a distribution quite close to a Poisson curve is obtained. This would show that, within the limits of resolution imposed by the technique, the axial distribution of radioactivity in the nuclear region is fairly uniform.

This study presents some evidence that the DNA in log phase *E. coli* 15 T⁻ prepared in a manner similar to that used in electron microscopy is restricted to an axial region occupying ⅔ the length of the cell. This region is seen to coincide with what has been described as the nuclear region in electron micrographs of various bacteria.

SUMMARY

Thin sections of *E. coli* 15 T⁻ grown in a medium containing tritium-labelled thymidine were radioautographed. The distribution of radioactivity among cross-sections of cells was studied. The label was found to occupy the same position as the "nuclear region" seen in electron micrographs of cells prepared in a similar manner.

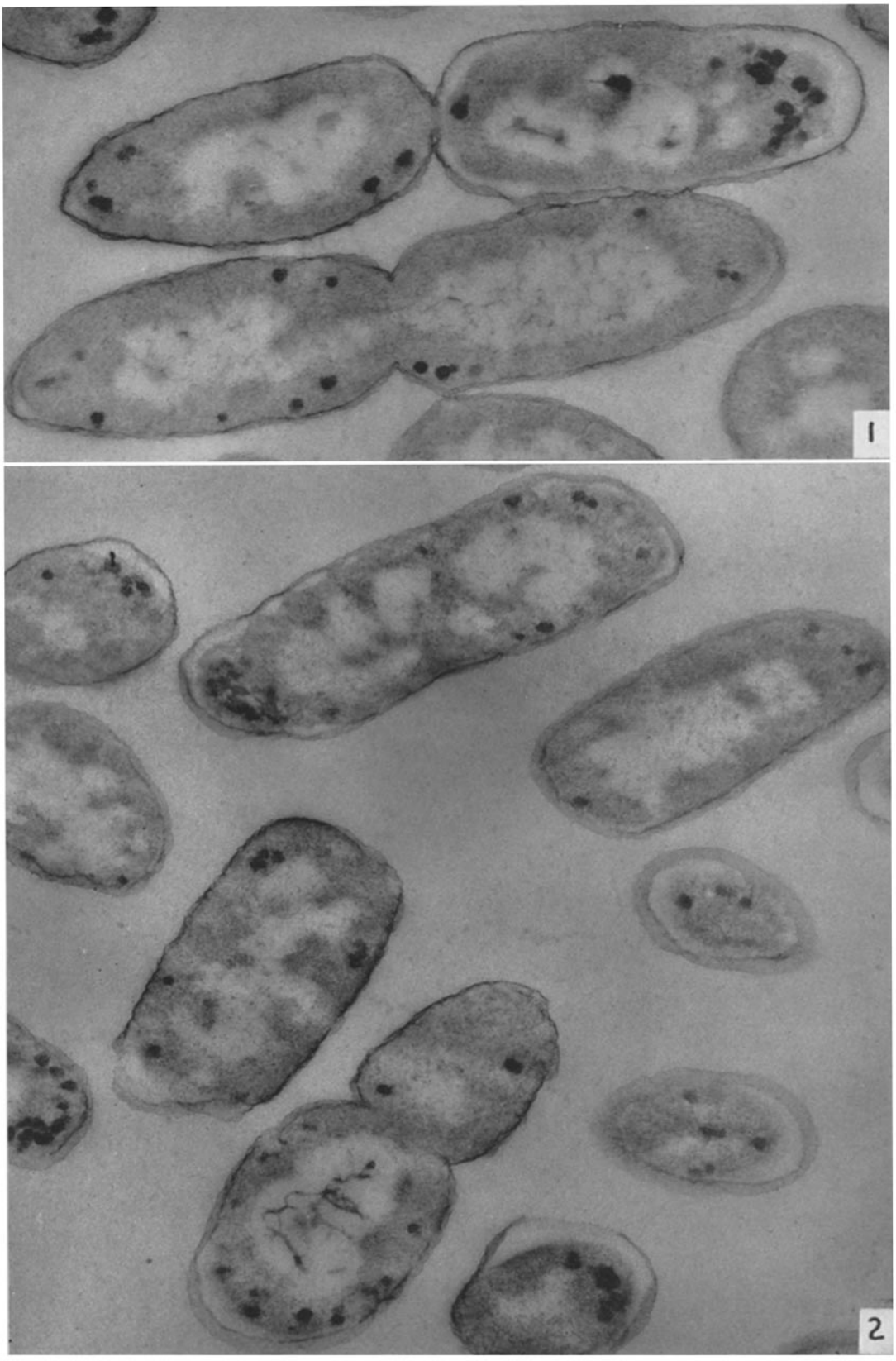
BIBLIOGRAPHY

1. Painter, R. B., Forro, F., Jr., and Hughes, W. L., Jr., *Nature*, 1958, **181**, 328.
2. Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolton, E. T., and Britten, R. J., *in* Studies of Biosynthesis in *Escherichia coli*, Washington, D. C., Carnegie Institution of Washington, 1957.
3. Niklowitz, W., *in* Electron Microscopy, Proceedings of the Stockholm Conference, September 1956, 115.
4. Palade, G. E., *J. Exp. Med.*, 1952, **114**, 427.
5. Doniach, I., and Pelc, S. R., *Brit. J. Radiol.*, 1950, **23**, 184.
6. Chapman, G. B., and Kroll, A. J., *J. Bact.*, 1957, **73**, 63.
7. Maaløe, O., and Birch-Andersen, *in* Bacterial Anatomy, Sixth Symposium of the Society for General Microbiology, Cambridge, 1956, 261.
8. Robinow, C. F., *Bact. Rev.*, 1956, 20, 207.
9. Gibbons, I. R., and Bradfield, J. R. G., *in* Electron Microscopy, Proceedings of the Stockholm Conference, September 1956, 121.
10. Lindsay, R. B., and Margenau, H., *in* Foundations of Modern Physics, New York, John Wiley and Sons, 1936.
11. Bradfield J. R. G., *Nature*, 1954, **173**, 184.

EXPLANATION OF PLATE 239

FIG. 1. Two dividing cells. The cell wall, deeply stained by lanthanum nitrate, occasionally appears as a double structure. The dense bodies appearing at the periphery of the cell are also stained by lanthanum nitrate. The nuclear region occupies the central axial region of the cell. It contains fine fibrils (bottom cell) or clumps of fairly dense material (top cell). × 42,000.

FIG. 2. Cross-sections and longitudinal sections of a variety of cells. Note that the length of the nuclear region remains approximately proportional to the length of the cell. × 42,000.



(Caro *et al.*: DNA in *E. coli*)