Visualization of prokaryotic DNA in a regularly condensed chromatin-like fiber*

(electron microscopy/chromosome structure/bacteriophage λ/Escherichia coli)

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ABSTRACT  Electron microscopy of disrupted Escherichia coli cells under certain conditions revealed loops of a fiber 120 Å in diameter which were attached to the cell envelope and showed a 130 Å repeating beaded substructure. These fibers were detected only when the cells were lysed in 0.15 M NaCl solutions directly on the electron microscope supporting films and if the dehydration steps began within 2 min of lysis. Under these conditions examination of cells lysogenic for phage λ after superinfection with λ wild type or deletion mutants disclosed short loops of a 120 Å diameter fiber free of the cell envelope. Because the contour length of these loops was proportionate to the DNA content of the superinfecting λ phage, it was concluded that the fibers contained DNA condensed 6.5-fold in blocks of about 250 base pairs.

Packaging DNA is a problem common to all cells. In higher cell nuclei the histones stabilize DNA in a condensed structure termed chromatin. This nucleoprotein complex can be isolated and visualized as a network of fibers with a 120 Å diameter and a regularly repeating bundled substructure (2-4). Comparison of the size of a bacterial cell and the total length of DNA it contains makes some condensation of its DNA likely. But prokaryotic cells do not possess histones, and consequently it is of interest to ask how their DNA might be kept in a condensed state in the cell; no such condensation has yet been observed. Prokaryotic cells do contain large amounts of Mg++ and the small basic polyanines (5), and these alone or together with proteins could complex DNA. Assuming that such a condensed or complex state exists within the cell but is labile upon lysis, a technique for lysing bacteria in physiologic salt solutions directly on the electron microscope supporting film and for preparing samples for electron microscopy has been developed to approach a visualization of the structure of the putative condensed DNA. Observations presented here with Escherichia coli and phage λ indicate that immediately after lysis, prokaryotic DNA can be found in fibers having physical parameters remarkably similar to those found for the eukaryotic unit fiber (3, 4, 6). The significance of this finding to studies of higher cell chromosome structure and the relation of these bacterial fibers to the state of DNA in vivo are discussed.

MATERIALS AND METHODS

Growth of Bacteria and Phages. E. coli K12 strains Hfr C71 and 5274 from the J. Lederberg collection were grown in H broth (7). E. coli K12 159T" (λ cts857 Sam 7) (8) was grown in TY medium with 0.1% maltose (9). Phage were a gift from Dr. Ronald Davis of this department.

Gentle Cell Lysis and Electron Microscopy. Mid-logarithmic (2 × 10⁸/ml) to stationary (2 × 10⁹/ml) cell cultures were harvested by centrifugation and resuspended at 10¹⁰ cells per ml in 0.15 M NaCl, 0.05 M Tris-HCl (pH 7.5), 1 mM EDTA (buffer A) at 0°. Egg white lysozyme (1 mg/ml in buffer A) was added to 25 μg/ml, and after 5–10 min at 0° the cells were collected by centrifugation and resuspended to the original cell concentration in 0.15 M NaCl, 0.01 M Tris-HCl (pH 7.5), 1 mM EDTA (buffer B) without lysozyme. Aliquots were diluted 100-fold with buffer B and placed in 0.1 ml wells of a plastic micro titer plate. Glow discharged carbon electron microscope supports (10) were immersed in the wells for 4 min, rinsed with buffer B, and placed in wells filled with buffer B containing 1% Triton X-100. The micro titer plate with the supports was then warmed from 0° to 37° for 1–5 min. The supports were removed, dehydrated, and shadowed with tungsten (10), the washes beginning with 50% ethanol. Electron micrographs were taken with a Philips EM 300 and dimensions measured directly on the original micrographs by optically projecting them onto an electronic digitizer tablet.

RESULTS

Visualization of E. coli cells disrupted on the electron microscope supporting film

Several images of E. coli cells treated as described above are in Fig. 1. Prominent in these micrographs were fibers arranged in loops apparently attached to the disrupted cell envelopes. The fibers had a diameter of about 120 Å and showed an axial repeat of about 130 Å along much of the fiber length, giving them a beaded appearance (Table 1, Fig. 1A). The beads often appeared to be connected by short filaments (which will be called thin filaments) of a diameter indistinguishable from that of purified DNA mounted under the same conditions. Masses of the 120 Å fibers were also seen unattached to the envelopes. The three E. coli K12 strains examined all showed these basic features. Lysis of E. coli cells in solutions containing 0.15 M NaCl by brief (0.5–2 min) incubation at 37° (stopped by start of dehydration) with 1% Triton X-100 consistently gave structures as shown in Fig. 1A, B, and D. Upon incubation for longer times at 37° the beaded 120 Å fibers were not observed, but rather long branched ropes of variable diameter and thin filaments were found (Fig. 1C). When the bacteria were lysed in 1 M NaCl, 0.01 M NaCl, or ionic detergent solutions only the branched ropes and thin filaments were observed even at short incubation times. To aid the characterization of these fibers, phage λ DNA in E. coli was examined.

* A portion of this work was described in the Proceedings of the 1975 ICN-UCLA Symposium (ref. 1).
Visualization of $\lambda$ DNA in the condensed state

If extrachromosomal DNA of phage $\lambda$ were found to be condensed, then it would be reasonable to assume that bacterial DNA is condensed in the same manner. $E. coli$ cells were infected with phage $\lambda$ under conditions where $\lambda$ replication is blocked by repressor (11). A $\lambda$ lysogen 159T- ($\lambda$ cI857 Sam7), expressing an active $\lambda$ repressor (growth and superinfection at 32°), was superinfected (multiplicity of infection = 1 to 2) with either $\lambda$ Sam7 phage (wild-type length) or
with λ b221 (a 22% deletion), grown for 45 min to allow covalent closure of the linear λ DNA (12), and then disrupted by the lysis procedure. After 1–2 min of incubation at 37° the cells appeared as before (Fig. 1A, B, and D), but in addition small closed loops of 120 Å diameter fibers were seen. These loops were not found on examination of uninfected cells and they were free of the cell envelopes (Fig. 2C). As with the cell-associated 120 Å fibers, they were relaxed, and a beaded substructure with short, thin filament bridges could often be observed (Fig. 2C). Beyond 2 min of incubation at 37° the small closed loops were changed in appearance and were indistinguishable from purified supercoiled λ DNA mounted under identical conditions (Fig. 2D). At a multiplicity of infection of 10 (λb221), many more loops were found, and more than 90% of them had contour lengths twice that found at superinfections of low multiplicity of infection. Frequently these appeared as two joined loops (Fig. 2F).

The fibers of the small closed loops were 120 ± 20 Å in width and the contour lengths measured 2.5 ± 0.2 μm after λ Sam7 superinfection and 1.9 ± 0.2 μm after superinfections at low multiplicity of infection with λb221 (Table 1); the latter contour was shorter by 25%. The conclusion that these loops contain DNA molecules is buttressed by the fact that λb221 is a 22% deletion. Therefore, each λ Sam7 DNA molecule which contained 4.65 × 10⁴ base pairs (13) was shortened 6.5 ± 0.5-fold in the 2.5 μm loop (from the 15.8 μm length of the extended DNA, calculated from the base pair content and the B helical conformation). Between 175 and 210 subunits of 130 Å (0.013 μm) length would sum to give a 2.5 μm length, and thus each subunit would contain about 220 to 265 base pairs of DNA. The corresponding values for λb221 would be a packing ratio of 6.7 ± 0.5 and about 230 to 290 base pairs per repeat (Table 1).

**DISCUSSION**

The primary condensation of DNA in higher cell nuclei gives rise to a 120 Å diameter fiber described as a regularly repeating chain of 200 to 250 base pair bundles joined by short thin bridges (3, 4, 14). Germond et al. (15) have reconstructed a similar 120 Å beaded fiber from purified simian virus 40 DNA and histone. This study has attempted to probe the possibility that DNA condenses in prokaryotic cells even though histones are absent. The results presented here show that under certain conditions prokaryotic DNA can be found in condensed fibers having physical parameters similar to those of the eukaryotic 120 Å fiber (Table 1). These condensed prokaryotic fibers were observed only if the cells were lysed in physiologic salt solutions on the electron microscope supporting film and only if the washing and ethanol drying steps began within 1–2 min of cell lysis. It is important, therefore, to ascertain whether they existed as such within the cell or were a result of the events of lysis and preparation for electron microscopy.

It has been found that after treatment with glutaraldehyde the eukaryotic DNA-histone fibers are not changed after a variety of chemical and physical manipulations. However, in studies to be reported elsewhere little protein was found bound to E. coli or λ DNA after extraction by the methods used here, and glutaraldehyde treatment did not stabilize the E. coli fibers against disruption upon prolonged incubation at 37°. Structural changes during drying, particularly by the use of ethanol, therefore were of particular concern. Compacted states of DNA can be generated by ethanol dehydration. None of the discrete condensations described by Lang (16), however, had an appearance even close to those of the fibers visualized here nor did they show any evidence of a beaded substructure. In our experiments, when ethanol dehydration was replaced with simple blotting on filter paper, the same beaded fibers were observed. Finally, these condensed fibers lost their compacted structure upon prolonged incubation on the grid while the dehydration steps remained the same; hence it would seem that this structure could not have been a simple product of the dehydration. Purified DNAs in many different salt solutions carried through the cell lysis procedure have not yielded 120 Å beaded fibers. I believe, therefore, that the drying technique has not led to the appearance of the structures seen in the lysate.

To what extent are the above conclusions in accord with other kinds of evidence? Units of DNA replication the size

| Table 1. Physical parameters of the 120 Å condensed prokaryotic fibers and the 120 Å eukaryotic chromatin fiber |
|---|---|---|
| E. coli | Sam7 b221 | Eukaryotic 120 Å fiber (from the literature) |
| Diameter (Å) | 120 ± 20 | 120 ± 20 | 80–100 (2) |
| | 120 ± 20 | 110 ± 5 (4) | 100–140 (3) |
| Axial repeat (Å) | 130 ± 20 | 130 ± 20 | 110 ± 10 (4) |
| | 130 ± 20 | 110 (28) | |
| Contour length (µm) | 2.5 ± 0.2 | 6.3 ± 0.5:1 | 7.0 ± 0.5:1 (4) |
| Packing ratio | 1.9 ± 0.2 | 6.7 ± 0.5:1 | 6.8:1 (14) |
| Axial repeat (base pairs) | 220–265 | 205 ± 15 (6) | |
| | 230–290 | 170–200 (4) | |
| Superhelix density | –0.07 (22) | –0.07 (24) | –0.07 (SV40) (23) |

Dimensions of E. coli and λ fibers were measured directly on the electron micrographs. The increase in diameter due to added tungsten metal was estimated from the fractional increase in apparent diameters of M13 phage and tobacco mosaic virus when rotary shadowed under these conditions as contrasted to their measured diameters determined by negative staining with phosphotungstic acid. Values presented were based on 200 measurements of E. coli and λ fiber diameters and axial repeats taken on 20 different micrographs and measurements of 50 different λ loop contours. Axial repeat and packing ratio calculations for λ were based on a value of 4.65 × 10⁴ base pairs for λ Sam7 DNA (13) and a 22% deletion for λb221. DNA lengths were calculated from these values, assuming the B helical conformation. Superhelical densities have been changed to agree with a 36° ethidium bromide unwinding angle (25). SV40, simian virus 40.
of a single bead (200 to 250 base pairs) have been found in studies of DNA replication in eukaryotic cells (17, 18), in certain phages (19), and more recently in *E. coli* (20). Also the reported properties of DNA in the *E. coli* folded chromosome (nucleoid) are consistent with this study. As isolated in 1 M NaCl and 0.2% deoxycholate solutions (21, 22), the nucleoid was shown to be gathered into 50 to 100 independent domains of supercoiling. As described here, cells lysed in such solutions did release branched ropes that were similar in appearance to high molecular weight supertwisted DNAs prepared under these conditions.

Finally, the great similarity in superhelical densities of covalently closed circular simian virus 40 (23) DNA, *λ* DNA (24, 25), and DNA of the *E. coli* folded chromosome (22) would suggest that DNA folding in prokaryotic cells and in eukaryotic nuclei may be the same if, indeed, the superhelically condensed DNA fibers. An example of 120 Å condensed fibers of *E. coli* Hfr C71 cells apparently stretched during mounting (A) was selected to show the beaded nature of the 120 Å fiber and thin filaments connecting the beads. Normally, the fibers appear as in (B), an enlargement of a portion of Fig. 1D. (C) A closed 1.9 μm loop found near a disrupted cell (upper left) after superinfection with *λ* b221. (D) The typical appearance of purified (24) supertwisted *λ* Sam7 DNA mounted by this technique in buffer B containing 1% Triton X-100. (E) A closed 2.5 μm 120 Å fiber loop found after *λ* Sam7 superinfection. In (F) a structure found after superinfection at high multiplicity of infection (X b221) is shown, apparently two attached 1.9 μm loops. (G) A loop as in (E) but seemingly stretched as in (A). Tungsten rotary shadowed, shown in reverse contrast. Bars equal 0.5 μm except for (B), which is 0.1 μm.
ocal turns reflect an ordering of DNA in the condensed fiber and arise from their subsequent covalent closure in that state. In recent studies (4) the minichromosomes of simian virus 40 were visualized as relaxed in the condensed state (as were the λ and E. coli loops here), and upon removal of the histones yielded extended supertwisted DNA (as did the λ and E. coli loops upon prolonged incubation at 37°C).†

What factors might stabilize DNA in a condensed state? The histone-like protein recently discovered in E. coli by Rouviere-Yaniv and Gros (26) was found to be present in too small an amount to fully complex E. coli DNA as do the histones in eukaryotic nuclei. Indeed, a specific search for such a class of proteins failed to detect any appreciable amount (27). This fact and the observed lability of the condensed fibers suggest that the DNA may be complexed with small molecules such as Mg++ and polyamines and perhaps a very heterogeneous class of weakly bound proteins.

Further studies will be required to fully elucidate the nature of the condensed fibers and their putative relation to the condensed state of DNA in vivo. The lysis technique itself, however, should provide a useful tool in structural studies of bacterial and phage life cycles.

Many laboratories including our own are currently investigating the role of histones in the condensation of DNA in the eukaryotic beaded 120 Å fiber. This report provides an example in which DNA has been found in a condensed state with physical parameters strikingly similar to those of the histone-complexed eukaryotic fibers, but in the apparent absence of the histones. This observation seems to suggest that the template for this structural organization may lie in the nature of the DNA molecule itself.

† Note Added in Proof. Superinfections with λ phage having defective cohesive ends yielded 2.5 μm linear fibers 120 Å in diameter with the beaded appearance. Thus, supertwisting must have resulted from DNA being closed in this conformation and could not have provided a force stabilizing the structure.

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References: