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Nucleosomelike Structures Associated with Chromosomes of the Archaebacterium *Halobacterium salinarium*

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Chromosomes of the halophilic archaebacterium *Halobacterium salinarium* were examined by electron microscopy after being spread onto water. The major part of the chromosomal DNA was associated with protein particles with diameters of 9.4 nm, arranged tandemly along the DNA fibers. Thus, the primary structure of the chromosome resembles that of eucaryote chromosomes.

Eucaryotic chromosomal DNA is associated with basic proteins, histones, forming a "beads with strings"-like structure (15). The beadlike structure, designated a nucleosome, is composed of histone octamers and 146 base pairs of DNA and is 11 nm in diameter (9, 17). On the other hand, most regions of procaryotic chromosomal DNA are fundamentally free from histones or histonelike proteins (4), although the bacteria do contain histonelike proteins which

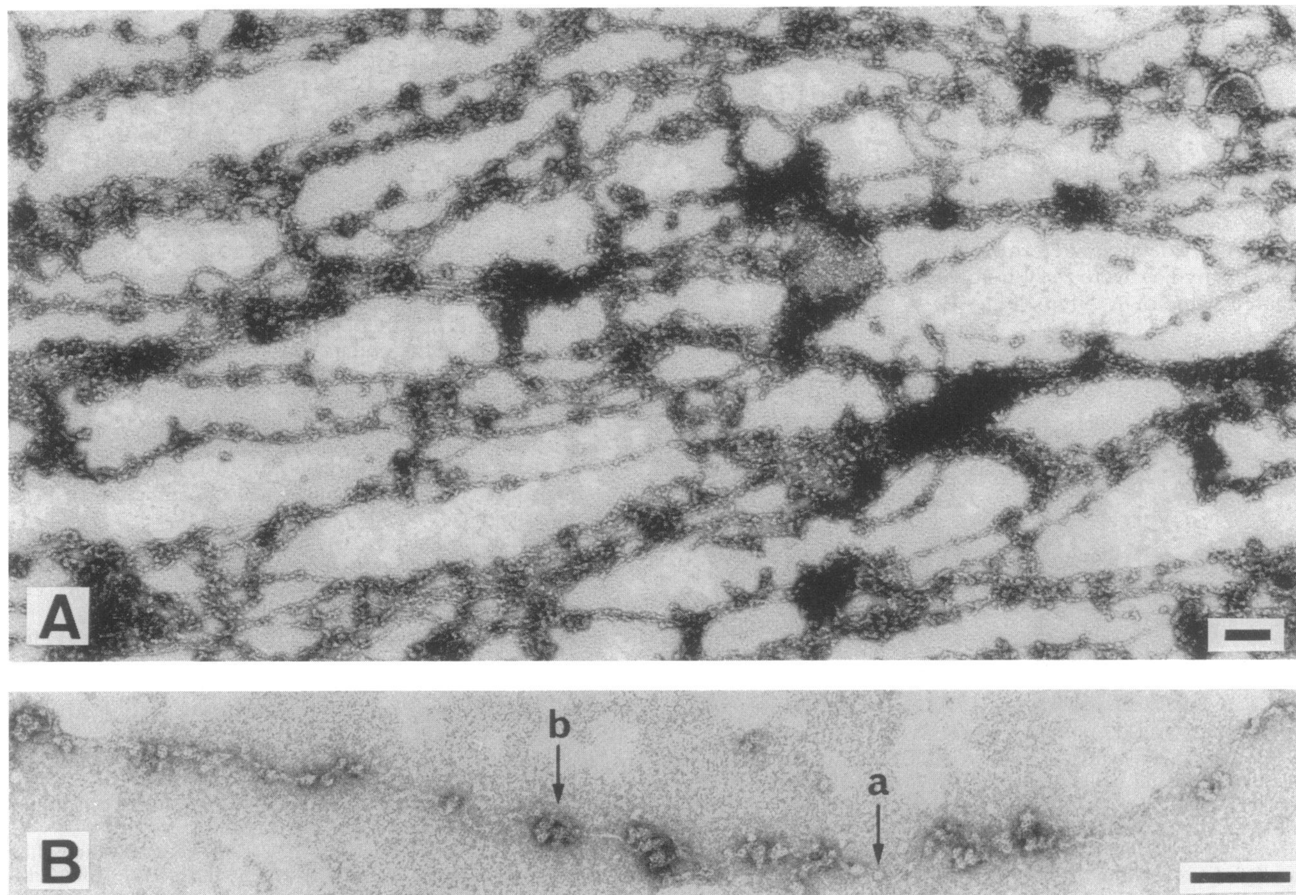

FIG. 1. Electron micrographs of chromosomal fibers visualized by negative staining with uranyl acetate. Specimens were prepared by method 1. a and b, Typical nucleosomelike particles and aggregates of particles, respectively. Bars, 100 nm.

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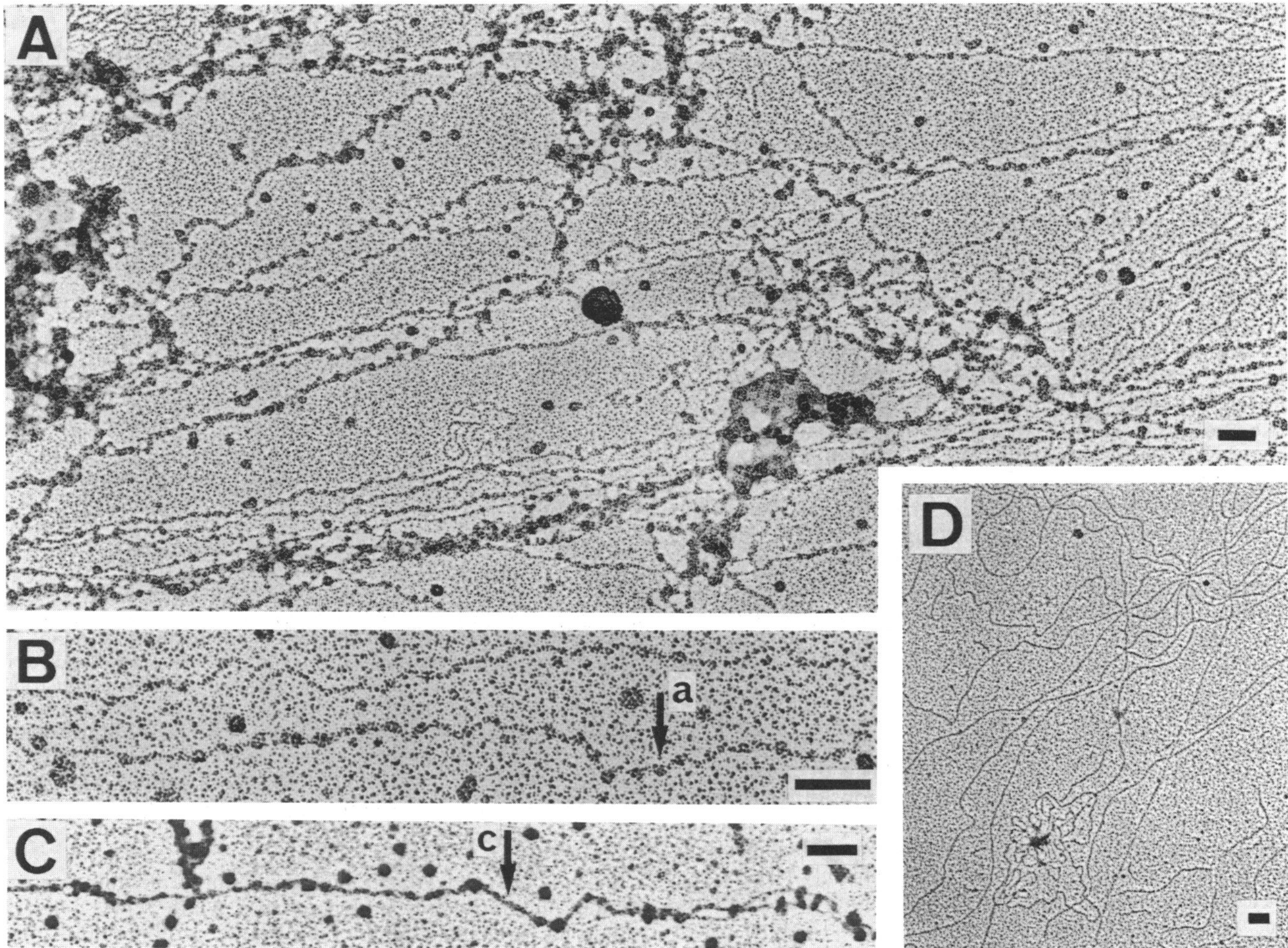

FIG. 2. Electron micrographs of chromosomal fibers visualized by shadowing. Specimens were prepared by method 2 (A, B, and C). In panel D, the chromosomal fraction prepared as described in the text was treated with proteinase K at a final concentration of 200 μg/ml in the presence of 50 mM Tris hydrochloride (pH 7.5), 75 mM NaCl, and 5 mM EDTA. After incubation for 30 min at 30°C and the addition of 0.1 volume of 1-μg/ml cytochrome *c*, the preparations were spread onto a water surface and the electron microscopic specimen was prepared as described in the text for method 2. a and c, Typical nucleosomelike particles and an aggregate of fibers, respectively. Bars, 100 nm.

form nucleosomelike particles in vitro (2, 18). Thus, this difference in primary structure of the chromosomes is a characteristic property, in addition to the presence of the nuclear membrane, distinguishing eucaryotes from procaryotes.

A series of bacteria known as archaebacteria seem to be procaryotes, although many properties of these bacteria differ from findings in most procaryotes (5). Based on an analysis of rRNA, the archaebacteria are considered one of the three kingdoms (eubacteria, archaebacteria, and eucaryotes) of cellular organisms (6), although another classification has recently been proposed (11).

A histonelike protein, HTa, was first isolated from *Thermoplasma acidophilum*, a species which could be considered an archaebacterium (19). HTa formed a nucleosomelike structure about half the size of eucaryotic nucleosomes (20). Similar histonelike proteins have been isolated from the nucleoprotein complex of several kinds of archaebacteria (3, 7, 10, 16). These findings suggest that the archaebacterial chromosome resembles that of eucaryotes rather than that of most other procaryotes. However, it was recently shown that some DNA-binding proteins isolated from the acidothermophilic archaebacterium *Sulfolobus acidocaldarius* do not form the nucleosomelike structure but do form another type of structure (13). Thus, investigations showing the chromosomal structure directly are warranted.

In the present study, we examined the chromosomal structure of a halophilic archaebacterium with an electron microscope and found that the major part of the chromosomal DNA is associated with nucleosomelike particles.

A halophilic archaebacterium, *Halobacterium salinarium* (IAM 13166), was obtained from the Institute of Applied Microbiology, University of Tokyo, and cultured on agar, as described elsewhere (14). Colonies of the bacterium were collected with a looped needle, dipped into 1.0 ml of 50 mM Tris hydrochloride (pH 7.5)–1 mM EDTA–0.1 M NaCl, and allowed to stand for a few minutes. This procedure led to bacterial lysis, since the bacterium grows in an extremely high concentration of salts (14). After lysis, the bacterial DNA was gathered with the needle to form an opalescent aggregate and left to stand for 5 min, with gentle shaking. This procedure was repeated twice after the buffer solution was changed, and the aggregate was then pipetted gently with a Pasteur pipette until the aggregate dissolved uniformly into the buffer solution. From this solution, samples were prepared for electron microscopy by using the follow-

ing two methods (unless otherwise stated) and were observed under a Hitachi electron microscope H-600 at 75 kV.

For method 1, formaldehyde at a final concentration of 1.0% was added to the DNA solution and the preparation was then left to stand for 30 min for fixation. A portion of the fixed DNA solution was spread onto a water surface, and the DNA was picked up with glow-discharged, carbon-coated grids. After the DNA was stained with 1.0% aqueous uranyl acetate and air dried, electron microscopic observations were made.

For method 2, cytochrome *c* to a final concentration of 0.05% was added to the DNA solution. After the solution was spread onto a water surface, DNA was picked up with carbon-coated grids, stained with 1.0% uranyl acetate-acetone, and rotary shadowed with Pt-Pd (4:1) at an angle of $\tan^{-1}$ (1/10). This procedure was followed by electron microscopic observation. *Escherichia coli* chromosomes and isolated rat liver chromatin (1) were also examined as controls.

Figures 1A and 2A show typical structures in a part of the bacterial chromosome. Methods 1 (Fig. 1) and 2 (Fig. 2) were used mainly to examine the size of molecules and to visualize DNA, respectively. Essentially, the same fibrous structures with particles were visualized by both methods, although the diameters of the fibers or particles were not the same, and more free DNA was observed in specimens prepared by method 2. Figures 1B and 2B and C show details in the stretched regions of the chromosome. These figures reveal that the chromosome is primarily composed of particles and connecting fibers (Fig. 1B). The fiber seems to correspond to non-protein-associated regions of DNA, since the diameter of the corresponding fiber in Fig. 2B was the same as that of the isolated free DNA and the fiber was digested with DNase I (not shown). Uniformly sized particles were frequently arranged in tandem along the DNA fiber (Fig. 1B and 2B). The particles on a DNA fiber often aggregated, forming a knoblike structure (Fig. 1B). Two or more DNA fibers with particles were sometimes associated lengthwise and looked then like thick fibers with particles (Fig. 2C for two fibers and Fig. 1A and 2A for larger numbers of fibers). Considering these structures, it seems that all the chromosomal structure seen in Fig. 1A and 2A is primarily composed of similar particles and DNA fibers. On the other hand, such particles in the *E. coli* chromosomes were never found when lysozyme-treated *E. coli* was directly spread onto a water surface (not shown).

The diameter of the particles was estimated to be 9.4 ± 1.2 nm (n = 203), while that of the nucleosomes from rat liver (measured as a control) was 10.3 ± 0.8 nm (n = 205) (Fig. 3). Thus, the particle is comparable in size to the eucaryotic nucleosome.

The DNA fibers with the particles showed a nucleoprotein-type light absorption spectrum with a maximum at 260 nm (not shown). Proteinase treatment of the chromosomal fraction yielded particle-free DNA fibers (Fig. 2D). The DNA fibers with the particles were found when 1.0 M NaCl was present in the buffer solution (not shown). These results demonstrate that the particle is composed of protein and DNA and that the association of the particles is relatively stable. Thus, the particle differs from an unstable *E. coli* chromatinlike fiber (8), a beaded structure generated by ethanol dehydration (12), or the in vitro nucleoprotein complex of *E. coli* histonelike protein HU (2) but resembles the eucaryotic nucleosome (9). In preliminary experiments, we observed similar particles on the chromosomal DNA from other genera of archaebacteria including methanogens,

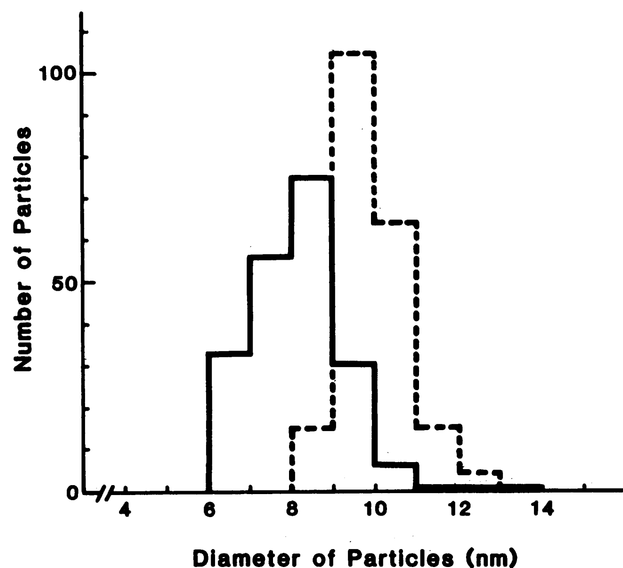

FIG. 3. Size of the nucleosomelike particles. Chromosomes of *H. salinarium* and rat liver chromatin were negatively strained with uranyl acetate and prepared for electron microscopy as described in the text for method 1. The diameters of monomers of particles were measured from prints magnified 200,000-fold, and the size distribution was plotted. Solid and dotted lines represent nucleosomelike particles from *H. salinarium* and nucleosomes from rat liver chromatin, respectively. Averages ± standard deviations were 9.4 ± 1.2 (n = 203) and 10.3 ± 0.8 (n = 205) nm for chromosomes of *H. salinarium* and rat liver chromatin, respectively.

Methanococcus vannielii, and the acidothermophilic archaebacterium *Sulfolobus acidocaldarius*. We therefore consider that the archaebacterial chromosome is commonly composed of DNA and protein and forms the nucleosomelike particle.

We found no constancy in the length of the DNA fiber between the particles (Fig. 1B and 2B), a finding differing from that obtained with the eucaryotic chromosomes (9). Furthermore, a considerable amount of particle-free DNA was always detected (Fig. 2A). This result resembles that from most other procaryotic chromosomes: a particle-free DNA was always observed when other procaryotic chromosomes were isolated by the same procedure. These results suggest that the archaebacterial chromosome has aspects of both eucaryotic and procaryotic chromosomes. However, further investigations are required to elucidate this point of view.

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