Bacterial Chromosome

Secondary article

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Under conditions favouring most active growth, bacteria replicate their DNA and divide with generation times as short as 20–25 minutes; similary rapid rates are practically unknown in other taxa. In contrast to the typical eukaryotic cell, DNA transcription, translation and replication occur simultaneously and at nearby locations. The highly dynamic processes lead to a reticulation of DNA and RNA, permitting optimization of mRNA interaction (number of contacts) with ribosomes. Increased order in the chromatin seems to occur only with very much slower overall growth.

Article Contents

Introduction

During the 1950s, a deoxyribonucleic acid (DNA)-specific cytological staining (of the Feulgen type) of chemically-fixed bacteria revealed in the light microscope, one, two or even four strongly stained 'patches' per cell. In cells of higher organisms, this staining was known to be confined to the nucleus and defined the chromatin and the chromosomes (Gr. *khroma* colour). This shared staining pattern gave rise to the terms 'bacterial chromosomes', 'nucleoids' or 'nuclear equivalents', all meaning these same, coloured patches. After removing the ribonucleic acid (RNA) by digestion with ribonuclease to leave only DNA for staining, these results were further confirmed. More recently, the staining with DNA-specific fluorescent dyes, such as 4′,6-diamidino-2-phenylindole (DAPI), substantiates these earlier results and provides an easier approach. With such dyes, it is now even possible to stain the DNA of live cells without disturbing their growth (**Figure 1**). Owing to the limited resolution ($\sim 0.2\,\mu m$), it is impossible to define exactly the shape of nucleoids in light micrographs, particularly because the size and shape of these patches is extremely variable and dependent on the preparation methods and growth media used.

At about the same time, toward the middle of the twentieth century, bacterial mutants (variants) were discovered, which 'breed true', i.e. in which the overwhelming majority of the descendants of an individual mutant cell carry the altered character. In the decade following Watson and Crick's double helix proposal (1953) of a linear genetic code, the widespread acceptance of the DNA molecule as the support for the cell's genetic information displaced the earlier belief that proteins fulfilled this role and led to a great increase in research on DNA, so that knowledge of bacterial genetics progressed rapidly.

Microbial taxonomy has been revolutionized through the use of new techniques of DNA and RNA sequence analysis. By comparing, for example, the detailed base sequence of 16S ribosomal RNAs, Woese proposed that there were two branches of the former bacteria: the Bacteria and the Archaea, of which the latter seem to share more with Eukarya than the former (as discussed later in this entry). As very few ultrastructural

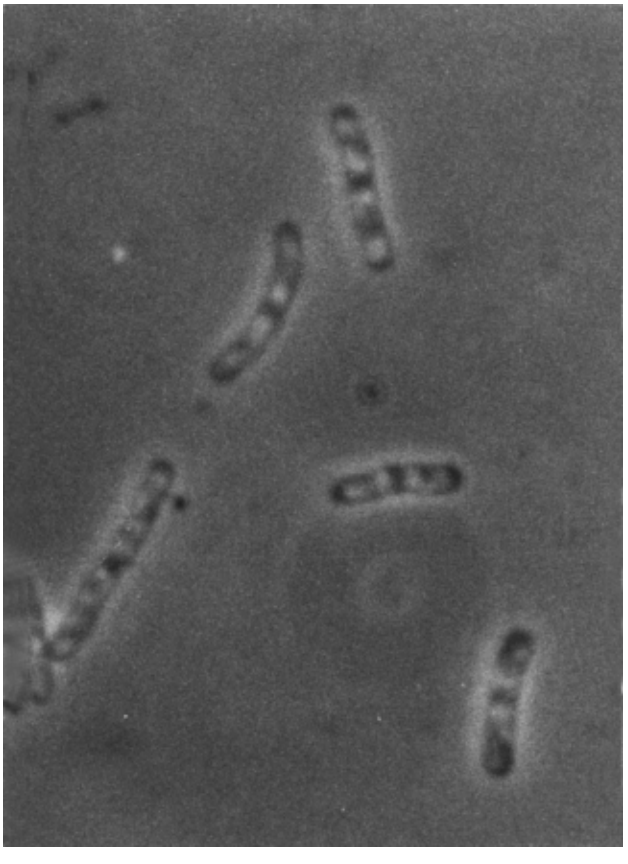

Figure 1 *In vivo*, fluorescently stained, *Escherichia coli*. DAPI is added to a culture of exponentially growing cells in an amount determined experimentally for each strain and medium; being low enough not to inhibit growth. Viewed under near blue ultraviolet fluorescence and phase contrast. The latter is necessary for visualizing the bacterial 'body'.

(i.e. microscopic) studies have yet been made on the nucleoids of archaea, we concentrate mainly on the results of the more thoroughly studied bacteria, and *Escherichia coli* in particular.

Functions, Size and Nature of the Bacterial Chromosome and its Packing into the Cell

Taking great care to minimize shearing, Cairns (1963) isolated the radioactive isotopically labelled DNA of an *E. coli* cell and spread it onto a photographic plate to record autoradiographic images. He found large circles with a 1300-μm circumference. He also demonstrated the process of replication of such circles, neatly demonstrating that the genetic apparatus of a bacterium consists of a single linear molecule of DNA in the form of a (closed) circle. This conclusion was confirmed independently, by genetic methods.

Further, a set of essential observations were made by Miller (1973) using electron microscopy: DNA was isolated from metabolizing cells in such a way that the synthesized messenger RNA (mRNA) transcript remained attached to the DNA and formed tree-like patterns, 'Christmas trees', with an increasing length of the 'twigs' towards one end. When comparing nuclear DNA of eukaryotes with that isolated from bacteria, Miller found an essential difference: within the bacteria, the twigs of mRNA were studded with ribosomes, illustrating previous biochemical findings that transcription and translation occur simultaneously in bacteria, on nearby sites. In contrast, in eukaryotes, the mRNA has to become detached from the DNA and to move out of the nucleus into the cytoplasm before translation can begin.

To accommodate the 1300-μm long chromosome into an *E. coli* cell of 2–3-μm length and some 0.8-μm diameter is often erroneously considered as a remarkable feat requiring a high degree of condensation and compaction. To quantify these parameters, the packing density was defined by Woldringh and Nanninga as the local concentration of DNA ($mg\,mL^{-1}$). Estimates of some $20\,mg\,mL^{-1}$ were found for the DNA of actively growing bacteria (1.5×10^{-14} g mass of DNA of chromosome and 1.4×10^{-12} mL volume of an *E. coli* cell with two nucleoids). When similar calculations were made for eukaryotic DNA in the interphase (in which DNA undergoes replication) nucleus of a liver cell, ignoring differentially stained eu-and heterochromatin, $20–40\,mg\,mL^{-1}$ was also obtained. Metaphase chromosomes, however, are some ten times more condensed, at $200–300\,mg\,mL^{-1}$, and DNA in bacterial viruses can even reach 800 to $1000\,mg\,mL^{-1}$! These chromosomes are associated with nonreplicating, condensed chromosomes taking up positions on the equatorial plane of the (three-dimensional) spindle apparatus. We have to conclude that the condensation of the bacterial chromosomes is comparable to that of the eukaryotic interphase nucleus and thus does not require any procedure of an exceptional nature.

Is the packing of the chromosome the result of a folding into a defined three-dimensional structural pattern that is determined by specific interactions with 'condensing principles', i.e. such as specific, crosslinking proteins? Or is it a more 'mobile' randomly distributed structure, reflecting simply those specific metabolic functions actively undergoing transcription-translation as well as those involved in the replication of the chromosome for a given cell environment and growth phase? Highly relevant are recent physical chemistry experiments involving 'spontaneous' condensation of DNA as a consequence of increased ion concentrations and of 'crowding' effects. The latter can be simulated *in vitro*, e.g. by addition of polyethylene glycol (PEG). For the first it is too frequently forgotten that intracellular concentrations of small molecular weight solutes in growing bacteria are generally 2–3 times higher than those of the medium; they are responsible for the cell turgor pressure. Na^+, generally more abundant extracellularly, is replaced intracellularly by K^+, and Cl^- by negatively charged amino acids (like glutamic acid) and other small molecular organic substances, probably reflecting relative levels in the conditions in which bacteria evolved. Intracellular, positively charged Mg^{2+}, Ca^{2+}, putrescine and spermidine are important as neutralizing partners of the negatively charged phosphate groups of DNA, which are not occupied by the histone-like, basic proteins. They form associations with lower dissociation constants than with the monovalent potassium ions. In experimental conditions *in vitro*, resembling intracellular conditions, the 50-μm long bacteriophage T2 DNA condenses into individual particles that, with fluorescent staining, are visible even by light microscopy. Taking into account the light microscope's limited resolution, the radius of the particles is estimated to be about 0.3 μm, i.e. more than 10 times smaller than the length of the 'artificially' unravelled DNA. With similar experiments, but using electron microscopy, the 'condensates' had a size comparable to that of mature phage.

The foregoing findings strongly suggest that achieving the low packing densities encountered *in vivo* in growing bacterial cells ($20\,mg\,mL^{-1}$), and in the DNA pool of replicating and transcribing 'vegetative phage', requires no particular, highly specific condensing mechanisms. Indeed, one purpose of bound proteins might be precisely the prevention of spontaneous aggregation (condensation) of DNA, which, when strong enough, as in eukaryotic metaphase chromosomes, obviously inhibits replication and transcription.

The other space-saving organization characteristic of DNA is that of compaction by supercoiling. This is discussed below.

New Views of the Bacterial Chromatin Using Cryofixation and Freeze-substitution

Electron microscopy of thin sections, with a resolution estimated not to be below some 5 nm, i.e. 40 times that of the light microscope, should have been able to provide the fine details of the bacterial chromosome that were lacking. In early studies these expectations were only partially fulfilled. Thin-section techniques, developed for the study of eukaryotic cells and tissues, achieved revolutionary progress that laid a basis for modern cell biology. The same cytological techniques of fixation and embedding applied to bacteria yielded disappointing results: the bacterial DNA was in the form of randomly shaped aggregates within an otherwise empty zone, at that time named the 'nuclear vacuole'. The absence of a membrane around this vacuole was later confirmed for bacterial nucleoids in whatever form they were observed. Improved fixation prevented the aggregation of the DNA during dehydration in ethanol so that the chromatin, the 'DNA-containing plasm', appeared in the form of fine fibrils whose thickness was compatible with that expected of DNA. What was troubling to those involved in such studies was the strong variation of shape of the 'nucleoid', which depended on the cytoplasmic fixatives used: with osmium tetroxide fixation it is rather strongly confined; whereas after aldehyde fixation it comprises a group of many, much smaller 'vacuoles'. A fundamental change came with the introduction of cryotechniques.

Water-containing biological material can be frozen so rapidly that its water is transformed into the vitreous state, which is amorphous, i.e. noncrystalline. Such frozen and still hydrated material can be cryosectioned; however, the slices yielded are not as thin as those obtained from current embedding in plastic resins. Techniques for staining cryosections with heavy metals are not yet available and image contrast is therefore too low to reveal fine detail. Fortunately, the frozen material at low temperature can also be freeze-substituted by organic solvents, such as acetone or methanol. At temperatures around $-80^{\circ}C$, while still solid, the ice is dissolved slowly into the solvent. The ice is thus progressively replaced by the solvent, which can in turn be substituted by the liquid resin. When this is subsequently rendered solid, it can be subjected to thin slicing or microtomy. This procedure of cryofixation and freeze-substitution (CFS) is limited to rather thin layers of material because the maximum depth of vitreous ice is only some 10–20-μm thick. The method is thus ideal for several layers of 0.8-μm bacteria.

Cryofixation is not simply a temperature reversible immobilization, but has been demonstrated to create physical crosslinks, producing a gel that resists subsequent aggregation in organic, water-miscible solvents, even when they are at room temperature.

Sections of *E. coli* and *Bacillus subtilis* processed by CFS reveal additional features: the ribosomes are clearly visible and are not distributed equally throughout. Many ribosome-free spaces of variable size are apparent (**Figure 2**) that contain fine globular material, which replaces the fibrillar one visible within the nucleoids obtained with the previously employed Ryter–Kellenberger (R-K)-technique. The fibrillar material was directly identified, by appearance as containing DNA. For the rather globular content of the ribosome-free spaces of CFS-prepared bacteria, indirect identification had to be made by immunocytochemistry. For DNA, a new immunostain was discovered which showed convincingly that most, if not all, ribosome-free spaces contained double-stranded DNA (Bohrmann *et al*., 1991). According to Miller's experiments, referred to previously, the DNA-dependent

Figure 2 Serial, longitudinal sections of *Escherichia coli*, prepared by cryofixation and freeze-substitution (CFS) for the electron microscope. Five of a series of 11 thin sections, taken from the middle part of the cell, are shown. The nonuniform distribution of ribosomes can be distinguished. The bacterial chromatin is in the ribosome-free spaces, as shown by immunostaining (Bohrmann *et al.* 1991). Bar, 0.5 μm.

RNA polymerase must be associated with the metabolically active DNA; this was confirmed by immunolabelling (Dürrenberger *et al*., 1988). Even by more 'old-fashioned' methods (osmium tetroxide fixation), the site of RNA synthesis was found, by autoradiography, to be outside the bulk of the nucleoid (Ryter and Chang, 1975). The very flexible stem and the twigs of Miller's Christmas trees are distributed all over the cell within the ribosome-free spaces in such a manner that a maximum number of ribosomes can become involved in protein synthesis. According to a proposal of C. Robinow, University of Western Ontario, Canada, this form of the nucleoid, with excrescences reaching far into the cytoplasm, was described as 'coralline'.

The problem with these new CSF findings was that, in cell sections, the nucleoid no longer appeared to be confined to a central location, as was the case with osmium tetroxide fixation, or with the observation of whole cells by electron microscopy, or, in light microscopy, after staining or by phase contrast. Knowing that a good thin section is at the most 50-nm thick means also that, cut longitudinally, a cell of *E. coli* is sectioned into about 15 consecutive serial sections. By reconstructing a cell from these serial sections, the problem might be resolved. The ribosome-free spaces of 11 serial sections were transferred to slightly coloured transparent foil and carefully cut out. The package of superimposed transcripted sections was then photographed by a low-resolution (pinhole) camera and printed (**Figure 3**). The low-resolution picture of the reconstituted cell looks exactly like some of the phase contrast light micrographs taken from a culture of the same strain. This experiment demonstrates that the ribosome-free spaces are not entirely randomly distributed within the cell; there is an increased amount in the central region, corresponding to what is observed in the light microscope with live, entire cells. Indeed, centrally located, larger ribosome-free spaces had frequently been observed in near-equatorial sections. These central areas – the 'bulk' – are considered to represent those parts of the chromatin, which are not (at a given moment) involved in gene expression. When the protein synthesis is inhibited, e.g. by treatment with sublethal doses of chloramphenicol, or by amino acid starvation of an amino acid-requiring strain, the chromatin assembles into a near sphere, frequently showing a ribosome-free central core. This typical spherical nucleoid is observed whatever the method of preparation/microscopy technique used. Its physical structure and generation are still not understood.

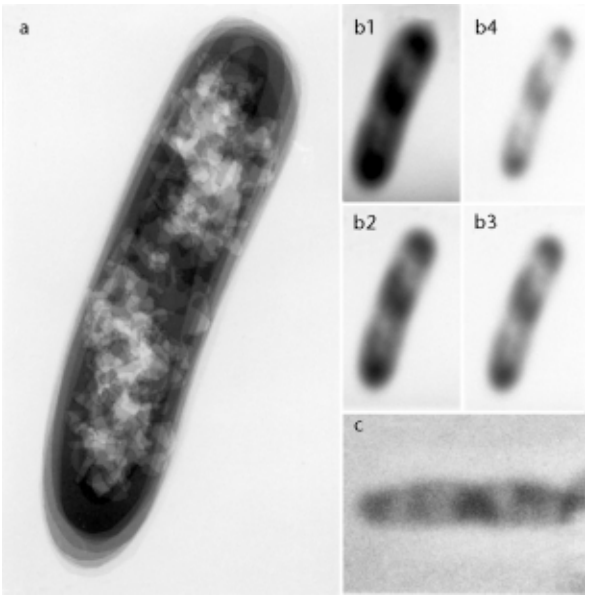

Figure 3 Serial sections, of which five are shown in **Figure 2**, are schematically redrawn on coloured foils and, with the ribosome-free spaces carefully cut out, superimposed to form a package that is a reconstruction of the whole cell. A sharp photographic image of this reconstructed cell is given in (a). The out-of-focus pictures (b1) to (b4) are obtained with a pinhole camera; these prints, on hard-grade paper, differ from each other only by the exposure time. (c) A light microscope phase contrast micrograph is shown. According to the concentration of the surrounding refracting material, phase contrast images vary exactly as do those of (b1) to (b4). They demonstrate how the apparent size of the 'nucleoid' is determined by optical parameters. To a somewhat lesser degree, this is also true for the varying intensities of the fluorescent staining. From Bohrmann *et al.* (1991) with permission.

The Bacterial Chromatin is Supercoiled

Double-stranded DNA, depending upon whether it is under torsion or not, shows differential binding of psoralen. The amount of this substance detected as being bound is directly related to the degree of supercoiling. Pettijohn and Pfenniger (1980) have systematically applied this technique to cells of *E. coli*. They found negative supercoiling, i.e. a torsional stress that facilitates opening of the double strands, such as is needed for replication and transcription. We will see further (below) that the DNA of thermophilic bacteria is, in contrast, positively supercoiled, so as to inhibit their DNA strands from opening; this would obviously counter the destabilizing effect of the high temperatures of their environment.

Left-hand torsion, applied to a thread, will be relaxed when the thread is put into the form of a left-handed solenoid (**Figure 4**). This is what we do when we fold our garden hose and is what happens when the DNA is wound around the protein cores of the eukaryotic chromatin forming nucleosomes. In this restrained form, the chromatin is at rest, not subject to twisting forces.

When a circular DNA molecule is supercoiled, then the circle is reduced (collapsed) into an elongated plectonemic supercoil (**Figure 4**). While the solenoid allows for a substantial shortening ('compacting') of a DNA thread, this is possible only to a very limited degree with the plectonemic form. It is still not known in which of the two

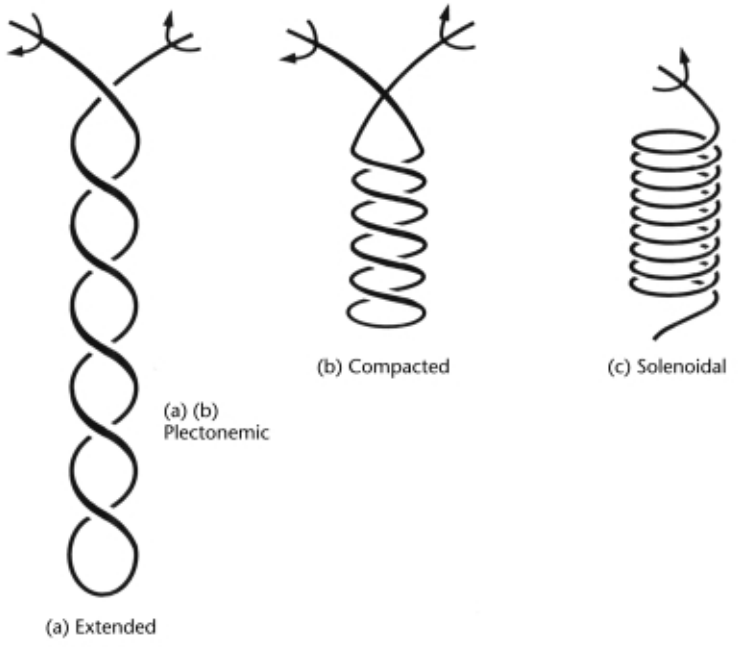

Figure 4 Proposed compaction forms of DNA. The same length of DNA is shown in the form of the loose (a) and compacted (b) plectonemic supercoiling. In (c) it is in a solenoidal form. The compaction ratio of the length of the stretched DNA molecule to that of the supercoiled, compacted form is about 9 in (b) and (c), but only 3 in (a). By normalizing the dimensions of the figure such that two windings of (c) correspond to those of a eukaryotic nucleosome, the bending (curvature) is then 0.23 for these and 0.25 and 0.26 for (b) and (a) respectively. The loose plectonemic form and its derivatives have been and still are extensively studied by electron microscopy and sedimentation rates. For technical reasons, low ionic concentrations are preferred for the electron microscopic studies of DNA. Under these conditions it is strongly charged and neighbouring fibres repulse each other, so as to produce the loose structure. In the absence of a sufficient amount of adequate basic proteins as partners, 'naked' DNA shows neither the compacted form (b) nor (c). As yet, solenoidal compaction has been observed only with nucleosomes, where the DNA is wound around a solid core of histones.

forms the bacterial chromatin is supercoiled. Both forms would lead to a rather microglobular appearance on high resolution electron micrographs if preserved as such. A fibrous appearance, obtained after chemical fixation, can be readily accounted for as chemically induced relaxation of the supercoil.

The enzymes responsible for supercoiling are the topoisomerases, frequently in combination with other histone-like proteins. Topoisomerases I and II are of particular importance and were discovered first. Topoisomerase I is able to introduce torsion by opening only one strand of DNA, whereas topoisomerase II (known as gyrase in bacteria) is able to break both. By immunolabelling, topoisomerase I was localized to the same area as that of RNA polymerase.

Ruth Kavenoff once succeeded in producing very elegant electron micrographs of isolated bacterial nucleoids (reported in most general reviews on bacterial chromatin), which showed some hundred loops emerging laterally from a sort of scaffold. Most of these loops were randomly bent and only few appeared in the form of plectonemic supercoils. None was solenoidally coiled. The whole arrangement showed a surprising resemblance to the model of the eukaryotic chromosome proposed by Laemmli: after dissolution of chromatin, the intact scaffold remained behind. Topoisomerase II was demonstrated to be situated on the scaffold and to be involved in the attachment of the chromatin loops. Considerable efforts were developed to apply Laemmli's biochemical and immunochemical procedures also on the Kavenoff-type of prokaryotic nucleoids. Unfortunately, only two groups achieved something approaching her work in quality, although much less convincing, having lost most of the supercoil of the loops and without succeeding in adding any biochemical and immunological identifications.

Probably stimulated by the pictures of Kavenoff, Pettijohn and coworkers demonstrated the existence of independent chromosomal segments or domains, possibly in the form of loops. By irradiating the bacterial chromosome *in vivo* with gamma rays, they demonstrated that the supercoiling of each individual segment is independently lost through the radiation-induced single-strand nicks produced in each segment (Lyderson and Pettijohn, 1977). Speculatively, an attractive model of the bacterial nucleoid is based on these findings and by analogy with the model of Laemmli: the loops are formed by a crosslinking by gyrase

and other proteins, together forming a scaffold, exactly as demonstrated for the eukaryotic chromosome. Although a negative result, and thus not published, it is worth mentioning that, by immunocytochemistry, gyrase was not found to be enriched in the centre of the globular, chloramphenicol-induced nucleoid (mentioned above), which, *in vivo*, is the strongest observation in favour of this putative model. Although the hypothesis of the loops has not yet been demonstrated, it is interesting to note that some repeat sequences in *E. coli* (BIME-2) have strong correlations with the binding and activity of gyrases (Espéli and Boccard, 1997).

The reverse gyrase of thermophilic bacteria generated interest in recent years (Bouthier de la Tour *et al*., 1998), as have the various helicases.

Histones, Histone-like Proteins and Nucleosomes

The name 'histone-like' was chosen because of the chemical properties shared with the eukaryotic histones, mainly their relatively small size, basicity, DNA-binding properties and their acid solubility. Implicitly, there is a tendency to assume that they have similar functions to those of the eukaryotic histones, namely to cause the solenoidal coiling of DNA by forming its central solid core. It is well known, however, that the relative amounts of these histone-like bacterial proteins are much too low to be able to organize all the chromatin of a cell into nucleosomes, as a similar structure to that of the eukaryotes, by restraint of supercoiling to a comparable degree. It is generally agreed that nucleosomes, if they exist at all in bacteria, must be highly fragile, accounting for their alternative designation as compactosomes. This fragility is obviously the reason why they have never yet been isolated. In the electron microscope, something resembling the eukaryotic nucleosomes had been assembled *in vitro* with a large excess of the protein HU. When *in vivo* the histone-like proteins are bound to DNA, they exert functions that are metabolically very active, quite distinct from the role of the histones of the eukaryotic nucleosomes in quiescence. In common with the latter, they also modify the migration rate of small circular DNA during electrophoresis, a phenomenon which is in agreement with a beaded structure but there is no proof for it.

For the stated reasons, a structural role for the histone-like proteins could only be inferred indirectly. By immunocytochemistry, protein HU was found to be localized in the area of RNA synthesis, i.e. on the nucleoid projections and not in the, supposedly inert, bulk (Dürrenberger *et al*., 1988). By using permeabilized cells, some authors could introduce large amounts of HU into the cell and found, by fluorescence microscopy, that the bulk of chromatin also contained HU. For us this observation confirmed that, in the native cell, the bulk of the DNA is not saturated with HU to form nucleosomes, whereas the well-known DNA-binding property of HU is again validated. The other major histone-like protein of *E. coli*, H-NS, was also found mostly on the border between the bulk of the nucleoid and the cytoplasm. In a strain overproducing H-NS, it was chiefly present in the bulk of the nucleoid (for references see Spurio *et al*., 1992), leading to the same conclusions as discussed above for HU when present in excess. Of interest is the additional observation that, in the H-NS excess situation, spherical nucleoids appear. In overproducing conditions the cells lose viability, and the synthesis of macromolecules, in particular of proteins, is inhibited. The authors' interpretation of these observations is that H-NS plays a decisive role in the confinement of the chromosome into the spherical shape. Why then does direct inhibition of protein synthesis also lead to the same or similar form of nucleoid?

Recent structural studies of eukaryotic histones by X-rays and nuclear magnetic resonance imaging led to the discovery and definition of a typical substructure, the 'histone fold'. As far as is known from the still limited number of investigations, this fold was not found in histone-like proteins of bacteria described above, but was present, to our great surprise, in DNA-binding proteins of most of the archaea that have so far been carefully investigated. The presence of 'real' histones, as defined by the presence of this typical fold, is correlated with other features that are distinct from those of the bacteria (Li *et al*., 1999): (1) nucleosomes are relatively stable and can be isolated; (2) the chromatin is resistant to aggregation during dehydration involved in the preparation for thin sections, and therefore must be an HP-chromatin, i.e. a chromatin rich in associated proteins.

These distinctive differences between bacteria and archaea should stimulate the careful investigation of archaea with modern methods, with a view to obtaining clearer ideas about our unicellular forebears.

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