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About the organisation of condensed and decondensed non-eukaryotic DNA and the concept of vegetative DNA (a critical review)

E. Kellenberger

Department of Microbiology, Biozentrum, University of Basel, Klingelbergstr. 70, CH-4056 Basel, Switzerland

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Experiments are reviewed that allow one to assign naturally occurring DNA-containing plasmas to either of two classes by virtue of their sensitivity to aggregation upon dehydration in organic solvents. The interphase nuclei of higher cells are relatively insensitive, while the DNA plasmas represented by bacterial nucleoids, vegetative bacteriophage and the chromosomes of dinoflagellates are sensitive. In higher cells the bulk of DNA is organised with histones in the form of nucleosomes. In prokaryotes and in the pool of vegetative phage DNA the most abundant histone-like protein HU is not associated with the bulk DNA, but localised in the border region with ribosomes where transcription and translation occur. These experimental results strongly suggest that the two classes of DNA plasmas are distinguishable by a low (1:10) or high (1:1) protein-to-DNA ratio. The hypothesis is formulated that the vegetative DNA (replicating and transcribing), throughout the living world, is nucleosome-free; during evolution, nucleosomes would have been introduced as a simple and adequate means for compacting the resting DNA. Condensation of DNA does not occur with prokaryotic nucleoids, but does take place when DNA is withdrawn from the vegetative phage pool to become packaged into phage heads. Dinoflagellate chromosomes are rather condensed although structurally different from eukaryotic chromosomes (e.g., those from *Euglena*) and are much more aggregation-sensitive.

1. Introduction of the problems and definitions

In the typical, eukaryotic cells of plants and animals, one observes cyclic variations in the local concentration of nuclear DNA: decondensed chromatin of the interphase condenses into chromosomes of the metaphase. We define the local concentration as the matter per unit volume contained in adequately small volumes which must be smaller than the cellular structure itself (nucleus, chromosome) but sufficiently large that it contains several segments of a DNA filament. The local concentration is therefore a complementary measure of the water content of a cytosol or cytogel. Either quantity can be calculated from the other when the 'dry density' (reciprocal of the specific volume) of the matter considered (e.g., the DNA) is known.

For the interphase nucleus of a hepatocyte we calculate an average DNA concentration of 20–40 mg/ml on assuming values of $6–12 \times 10^{-12}$ g DNA per cell [1] and a volume of 2.8×10^{-10} ml per nucleus [2,3] and when neglecting possible concentration differences between hetero- and euchromatin. Estimations of the local concentrations of DNA in metaphase chromosomes are much more difficult and it is to be hoped that the new possibilities provided by ratio contrast in STEM will soon be used to determine such concentrations [4]. For the time being, one should be on the safe side when stating that the local concentration of DNA in a condensed chromosome is more than 10-times greater.

In the 'nucleoid' of prokaryotic cells cyclic

Correspondence address: E. Kellenberger, Department of Microbiology, Biozentrum, University of Basel, Klingelbergstr. 70, CH-4056 Basel, Switzerland.

variations in the local concentration of DNA do not occur or are incomparably smaller than those of eukaryotic cells. In *Escherichia coli*, the local DNA concentration is of the order of 20 mg/ml [5]. No substantial variation in this value occurs even when, as a result of external action, the 'nuclear material', which is rather dispersed during exponential growth [6], becomes confined to

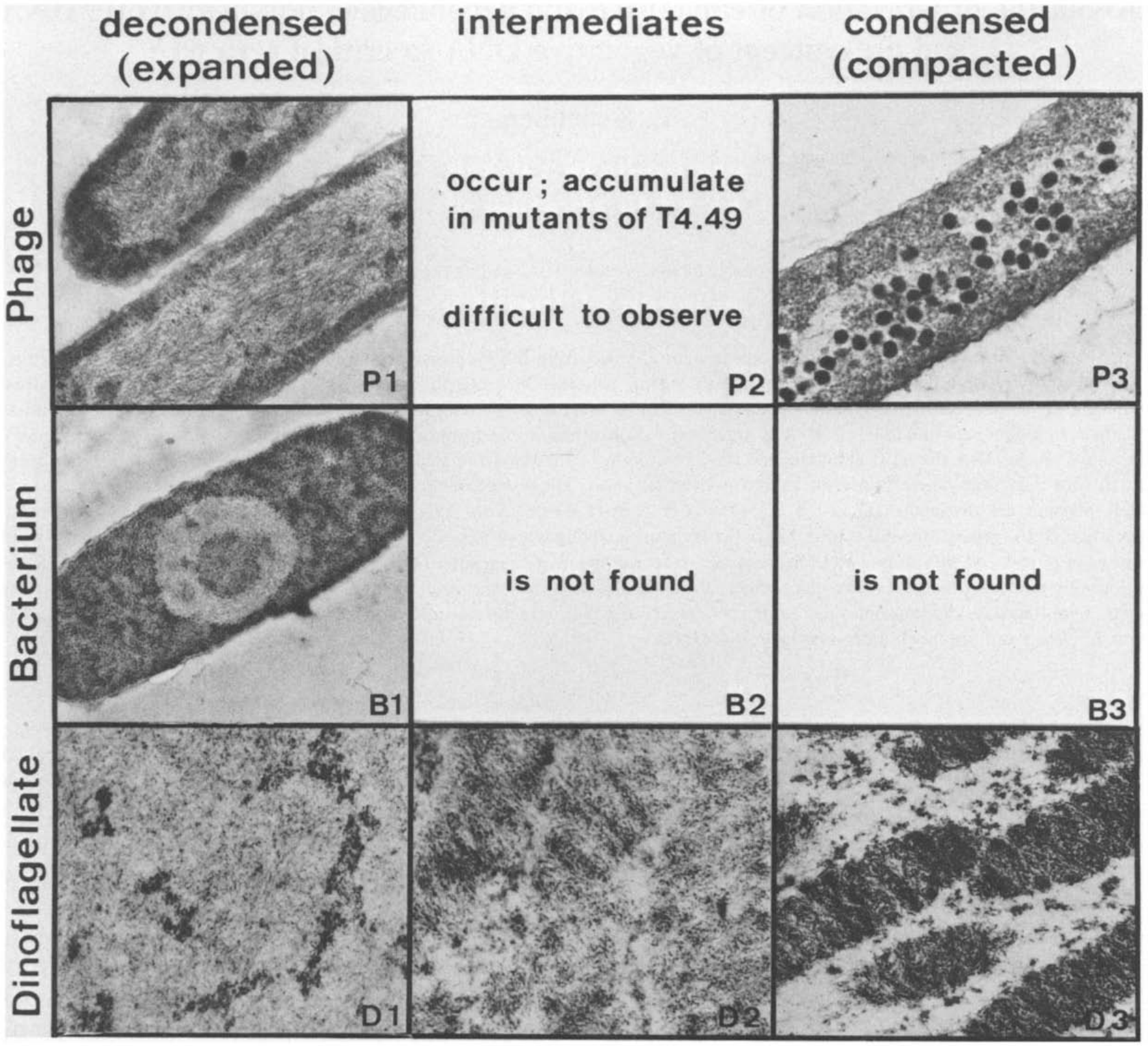


Fig. 1. Different non-eukaryotic organisms compared with respect to the occurrence of physiological condensation. DNA-containing plasmas are shown from phages, bacteria and dinoflagellates in comparable states of decondensation (expansion) or condensation. What we compare are the apparent local concentrations of the DNA plasmas, as discussed in the text. For bacteria, no condensation has been observed, but the degree of dispersion of the DNA plasma within the cytoplasm could vary strongly according to the physiological conditions [6]. The present bacterium has been treated with chloramphenicol so as to show a confined, non-dispersed state of the nucleoid. Even in this confined, non-dispersed state, the DNA is no more compact that that in more dispersed states. The pool of replicating and transcribing phage DNA is of about the same degree of compaction as the bacterial nucleoid or expanded dinoflagellate chromosome. The DNA packed into the virion is as highly concentrated as the most compact chromosome of dinoflagellates. In both cases intermediates are found. Sections of partly full phages do not appear distinct from sagittal, partial sections of completely full phages unless particular technical tricks are employed. Such sections are therefore not shown.

restricted globular organelles (fig. 1), such as occurs in the case where in metabolising cells new protein synthesis is inhibited [7]. It is interesting to note that the DNA concentration of *E. coli* is comparable to that of the interphase nucleus of a hepatocyte.

A 20–50-fold condensation occurs when bacteriophage T4 DNA of the vegetative pool is packaged into phage heads. Upon infection with bacteriophages of the T-even type, the host DNA becomes completely degraded while the injected phage DNA is replicated and transcribed. This DNA forms the so-called 'vegetative DNA pool' of which the local concentration is virtually identical with that of the nucleoid, namely, 20–50 mg/ml. When packaged into phage heads, the local concentration now reaches 0.8 g/ml [5]. Packaging can be prevented by appropriate mutants of the phage, or by inhibiting protein synthesis 8 min after infection. In these cases the DNA pool becomes greatly enlarged and is therefore easily observable on thin sections with an electron microscope (fig. 1).

In exponentially growing *E. coli* cells the doubling time is approx. 25 min. In such cells, no cyclic variations in macromolecular syntheses occur, as is easily measurable by the labeling produced by short radioactive pulses and autoradiography [8]. The relative amount of unlabeled cells is a direct measure of the period of time in which the considered macromolecular syntheses would have ceased. A short, transient period of cessation of DNA synthesis occurs only with 'poor' growth media, where the doubling time is substantially increased (for references, see ref. 9). Prokaryotic and vegetative phage DNAs are thus in a continuous state of metabolic activity, in strong contrast to that of eukaryotic cells for which the metabolically active period is less than 1/10 of the generation time. In the subsequent text we shall use the term *vegetative DNA* synonymously to *metabolically active DNA*.

In the early 1960s the hypothesis had been formulated that vegetative-DNA of any origin might be similarly organised. Histones were then known to be DNA partners of eukaryotic cells, however, the organisation of chromatin into nucleosomes was not discovered until much later [10]. Today, there is general agreement that the *bulk of eukaryotic DNA, whether condensed or decondensed, is organised in the form of nucleosomes.*

Much less agreement is found when scrutinizing the literature as to whether or not nucleosomal DNA is able to transcribe – and replicate – without nucleosomes being removed (for references, see ref. 11). Recently, very elegant and convincing experiments performed by Lorch et al. [12] have provided results showing that it is very likely that the nucleosomes dissociate transiently during passage of the transcriptional complex, in order to re-form afterwards. Eukaryotic nucleosomes may thus play no active role in transcription; they serve to provide for a certain degree of compaction in the bulk DNA. We shall therefore call them *bulk nucleosomes*. Their role is to increase the local concentration of DNA; this concentration is likely to be lowest for vegetative DNA.

Cytochemical (staining) methods demonstrated some time ago that bacteria and dinoflagellates contained much less basic ('histone-like') protein partners of the DNA than typical prokaryotic cells (for references, see refs 13 and 14). The DNA-containing plasmas of these organisms also showed much greater sensitivity towards preparation method-induced phenomena of aggregation when observed in electron microscopy [15,16].

We therefore put forward the hypothesis that the DNA-containing plasmas belong to one of two classes, eukaryotic and non-eukaryotic, whereby we assumed the same type of structure for vegetative DNA. In eukaryotic cells, vegetative DNA is present only for very short periods and involves various small regions of the genome at very different times, and therefore is not easily detectable by electron microscopy. In bacteria, however, a very large part of the DNA must always be in the vegetative state. The same is likely to hold true for vegetative phage DNA. It could thus be that these DNA plasmas, which are readily observable in electron microscopy, might be representative of vegetative DNA in general.

Below we shall summarize the available experimental evidence in favor of this hypothesis.

2. Different aggregation sensitivities of various DNA-containing plasmas as a consequence of dehydration (and fixation)

The observation of thin sections in electron microscopy is about the only tool that allows the investigation of in situ structures in the cell. Potentially, cryosections observed when still frozen-hydrated [17] would be most adequate. Unfortunately, it is not yet possible to produce the very thin sections that are required for high resolution. In this respect, sections from resin-embedded cells are more appropriate. Here, the cellular water is replaced by organic liquids which, at the last step, are cured into a solid resin. During this dehydration most biological macromolecules in solution, such as nucleic acids and proteins, will precipitate by forming more or less coarse aggregates. Every biochemist knows that aqueous macromolecular solutions begin precipitating when ethanol or acetone is added at concentrations between 40 and 60%. Coarse precipitation is prevented by prior fixation: by means of chemical cross-linkers, like aldehydes or osmium tetroxide, the biological matter is transformed into a gel, which, in many cases, is then sturdy enough to withstand dehydration without massive collapse into aggregates.

A considerable number of years ago, we had observed together with Antoinette Ryter that most

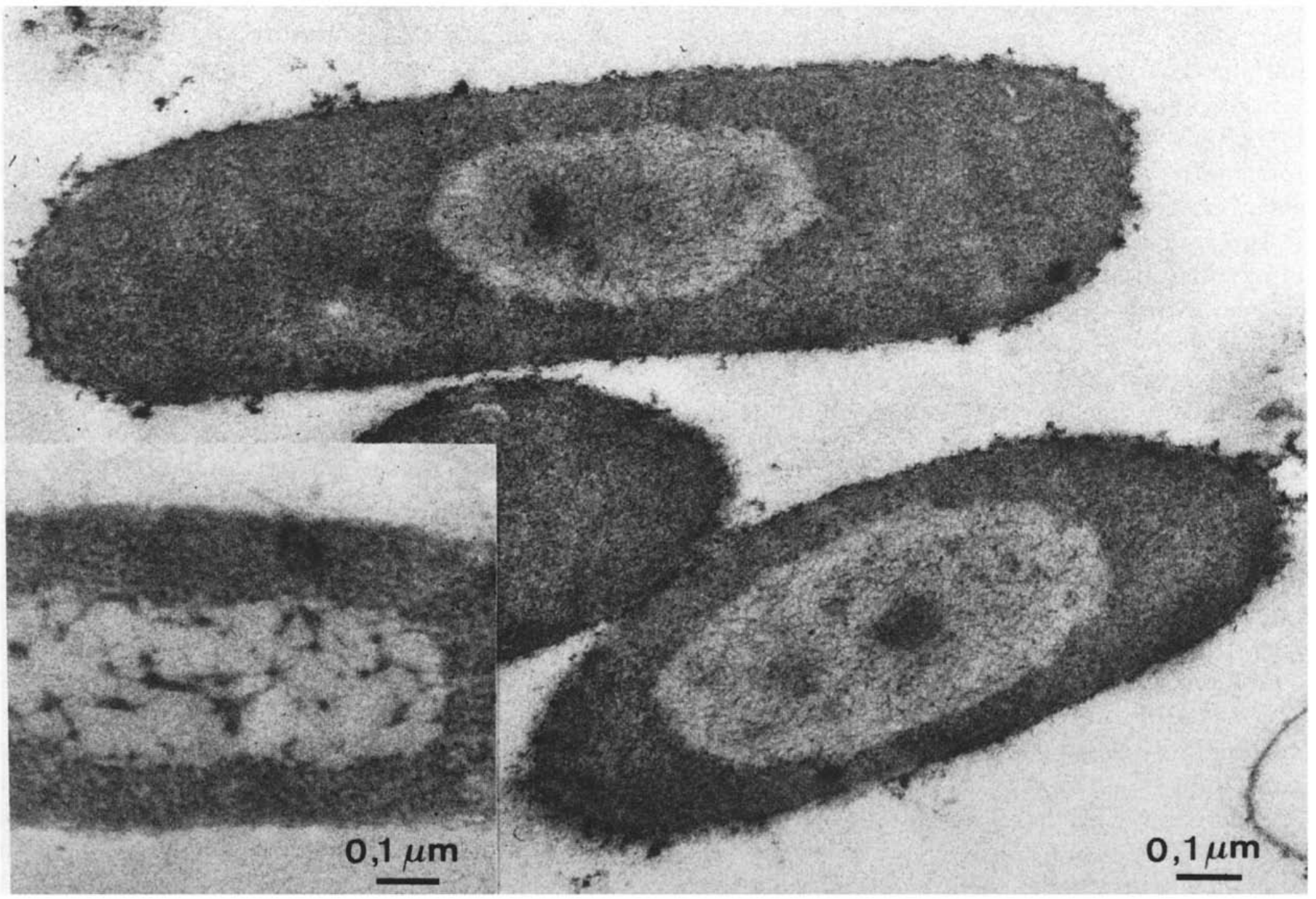


Fig. 2. *E. coli* B cultivated in tryptone to which 25 mg/ml of chloramphenicol was added when the bacteria had reached a concentration of 3×10^8 cells/ml. After 2.5 h, the bacteria were fixed under R-K conditions and embedded in Vestopal W. Bacteria show the typical response to this treatment, namely, the vesicular form of the nucleoid. Its DNA plasma is composed of thin filaments. (Inset) Nucleoid of the same bacteria which, however, after fixation and before dehydration, were submitted to EDTA treatment leading to aggregation.

of the currently used formulations for fixing tissues and cells produced rather coarse aggregates when processing bacterial nucleoids or the phage DNA pool, as shown in fig. 2 [18,19]. Purely empirically, we finally succeeded in establishing the so-called R-K conditions for OsO_4 fixation [18] which preserved these DNA-containing plasmas as a finely stranded fibrous structure.

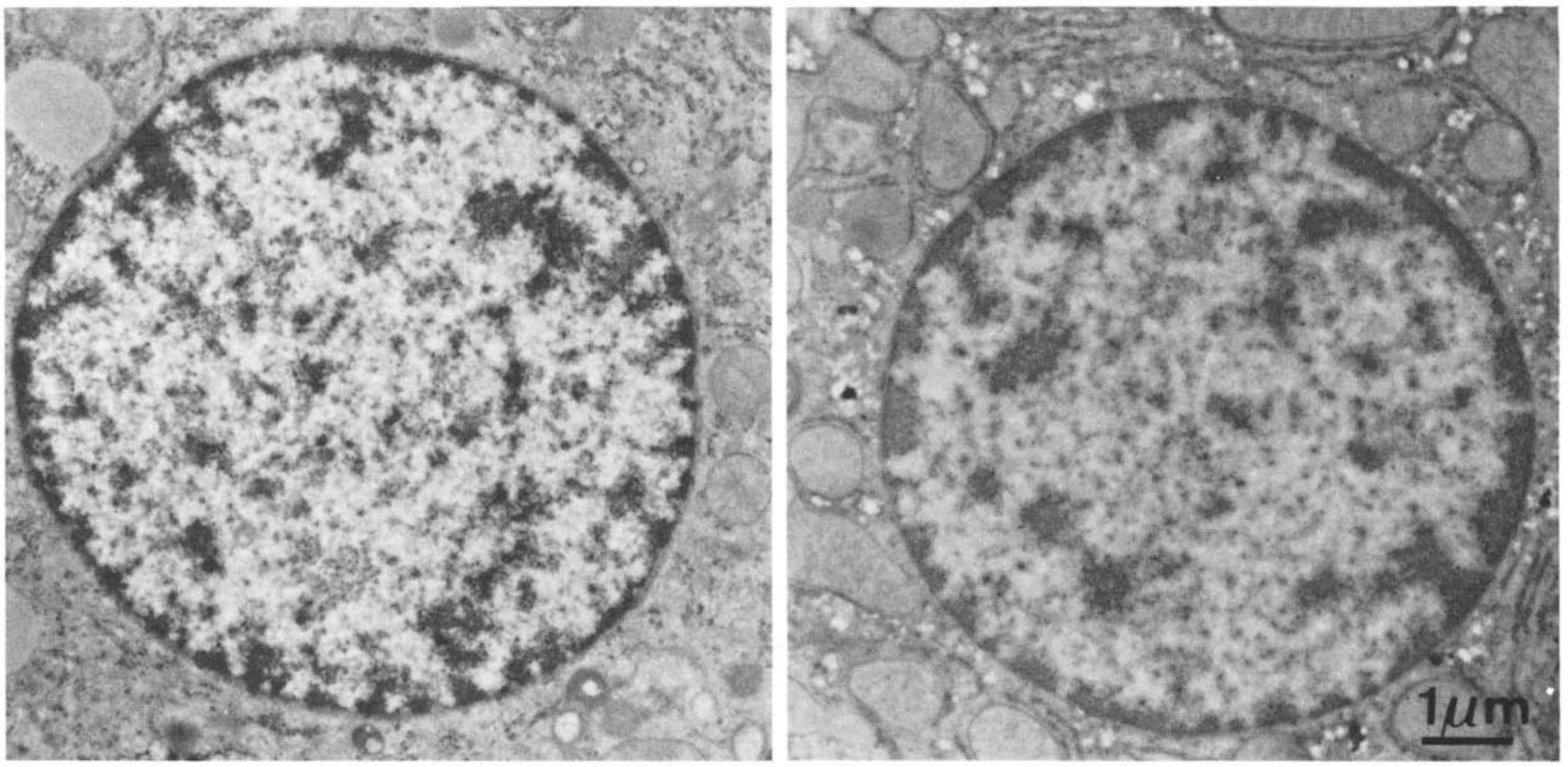


Fig. 3. Interphase nucleus of rat liver cells. (a) After fixation with 2% glutaraldehyde and 1% osmium tetroxide, embedded in Epon 812. (b) Same fixation but subsequently treated with 0.25 M EDTA for 1 h. Sections are stained with uranyl acetate and lead according to Millonig [61]. On comparing the micrographs we observe no distinct structural changes although the staining differs. On comparison with fig. 2, one clearly sees that the DNA-containing plasma of the interphase nucleus is relatively insensitive towards aggregation. (Experiments and micrographs by W. Villiger.)

Amino acids and/or small peptides, Ca^{2+} and a pH around 6 are required. The fine strandedness is further improved with post-fixation in uranyl acetate (0.5% in acetate-veronal buffer). If this post-fixation is replaced by treatment with 0.25 M EDTA in the same buffer (EDTA was formerly introduced under the trade name 'Versene'), then strong aggregation again occurs. This test demonstrated the strong sensitivity of DNA plasmas of nucleoids (ref. 18 and fig. 2), phage T4 DNA pools (ref. 19 and fig. 5) and of dinoflagellate nuclei (ref. 20 and fig. 4). It was equally positive with artificial solutions of DNA [21]. To our surprise, we observed no comparable aggregation with interphase nuclei of eukaryotic cells (with B. Blondel in the early 1960s, unfortunately as a 'negative' result which was never published). Recently, with W. Villiger, we have repeated this test on hepatocytes and confirmed the previous finding of a very low sensitivity of the hepatocyte (fig. 3) as compared to dinoflagellates (fig. 4) or other non-eukaryotes. The test was applied by other authors to mitochondrial nucleoids [22] and blue-green algae [23] and high sensitivity was demonstrated.

3. In vitro experiments on gelation

By means of in vitro experiments in the test-tube we tried to understand more about the mechanisms underlying this high sensitivity, with the hope of contributing towards elucidation of the structure and properties of vegetative DNA. From in vitro experiments with the pure sodium salt of DNA [15,21,24], it was shown that the absence of coarse aggregates was always correlated with macroscopically visible gelation, which occurs with OsO_4 when applied under R-K conditions, with uranyl acetate, indium chloride and potassium permanganate. With aldehydes and other formulations for OsO_4 fixation, no gelation could be produced, fully explaining their failure to prevent later aggregation during dehydration. The sodium salt of pure DNA, commercially available nucleohistone and nucleoprotamine were studied [15,21,24]. We also attempted to use eukaryotic chromatin, carefully extracted by Markus Noll. The commercial nucleohistone and nucleoprotamine showed behavior which was not different from that of the other DNAs. With chromatin, however, the same experiments were not feasible since, at the required concentration, this chromatin was already so viscous as to approximate the form of a gel. Instead, we found that cross-linking with a fixative almost completely inhibited the swelling and contraction of isolated, membrane-free nuclei, which are under the control of magnesium. We tentatively concluded that the insensitivity to aggregation of eukaryotic chromatin was due to its high relative protein content [24]. The observed aggregation sensitivity of commercial nucleohistone was explained through protein determinations which demonstrated the protein content to be less than half of that normally associated with chromatin.

All these in vivo and in vitro observations agree in showing that in reality only the typical chromatin of eukaryotic cells from plants and animals shows an exceptional lack of sensitivity towards solvent-induced aggregation. This could be caused by the high protein content of this eukaryotic chromatin, which would favor efficient cross-linking with various fixatives, including aldehydes. All other non-eukaryotic DNA-containing plasmas would be less rich in proteins. This relatively low protein content would explain the lack of gelation and consecutive aggregation during dehydration with organic liquids.

Recently, we have discovered that aggregation is very strongly reduced on treatment at low temperatures as shown in fig. 5 for the vegetative pool of phage T4 [16]. This finding led us to perform in situ immunocytochemical studies on the intracellular location of putative DNA partners, as described in section 4.

4. Histone-like proteins of bacteria are not organised like typical nucleosomes of bulk DNA

Over the preceding decade, some nine 'histone-like' proteins have been isolated and characterised [25–31]. They are defined as very basic, acid-soluble proteins which are able to bind to DNA. Only one excellent paper relates the relative amounts of

Table 1

Isoelectric points, molar ratios in S100 extracts and references for purified nucleoid proteins (courtesy of Yamazaki et al. [31])

Molecular mass (kDa)	Isoelectric point	Molar ratio	Corresponding protein
28	7.3	7	–
27	6.4	1	H protein [28]
26	8.0	1	22 kDa protein [29]
24	9.8	1	–
22	7.0	2	–
17	9.6	4	HLPI [30], protein 1 [27], BH1
17	7.3	4	–
14	not determined	3	–
9	9.8	100	HLPII [30], HU [25], BH2

all these proteins as determined via a single method in the same laboratory [31]. These determinations are listed in tables 1 and 2. Not all published values agree with these data; in particular, for protein H greater amounts are claimed on the basis of peroxidase staining of immunoblots [32] which, unfortunately, is a method of debatable virtue. From table 1 one may observe that protein HU [25] is the most abundant histone-like protein, at least 10-times more so than any other. This DNA-binding protein, of which two nearly identical forms exist (HU_1 and HU_2), occurs with some 40 000 copies per cell of *E. coli* which corresponds to about one dimer per 200 base-pairs [33]. An identical protein was isolated from *Bacillus globigii* [34] and a similar protein was found in yeast mitochondria [35]. Protein NS (NS_1 and NS_2) was discovered as bound to native 30 S ribosomes [36]. It is released when the 70 S complex is formed. By

Table 2

Comparison of affinities of proteins to DNA (courtesy of Yamazaki et al. [31])

Samples of 5 μg (in 100 μl) of each purified protein were applied to a mini-DNA-cellulose column (column size 200 μl). The column was allowed to stand for at least 5 min to allow sufficient reconstitution of the DNA-protein complex, and then washed with 300 μl buffer A. The proteins were then eluted stepwise with 300 μl of 100, 150, 200, 250, 300, 350 mM and 1 NaCl in buffer A. The protein in each fraction was precipitated by the addition of an equal volume of 40% trichloroacetic acid, and analyzed by SDS-polyacrylamide gel electrophoresis. The presence of protein in eluates is indicated by a plus sign.

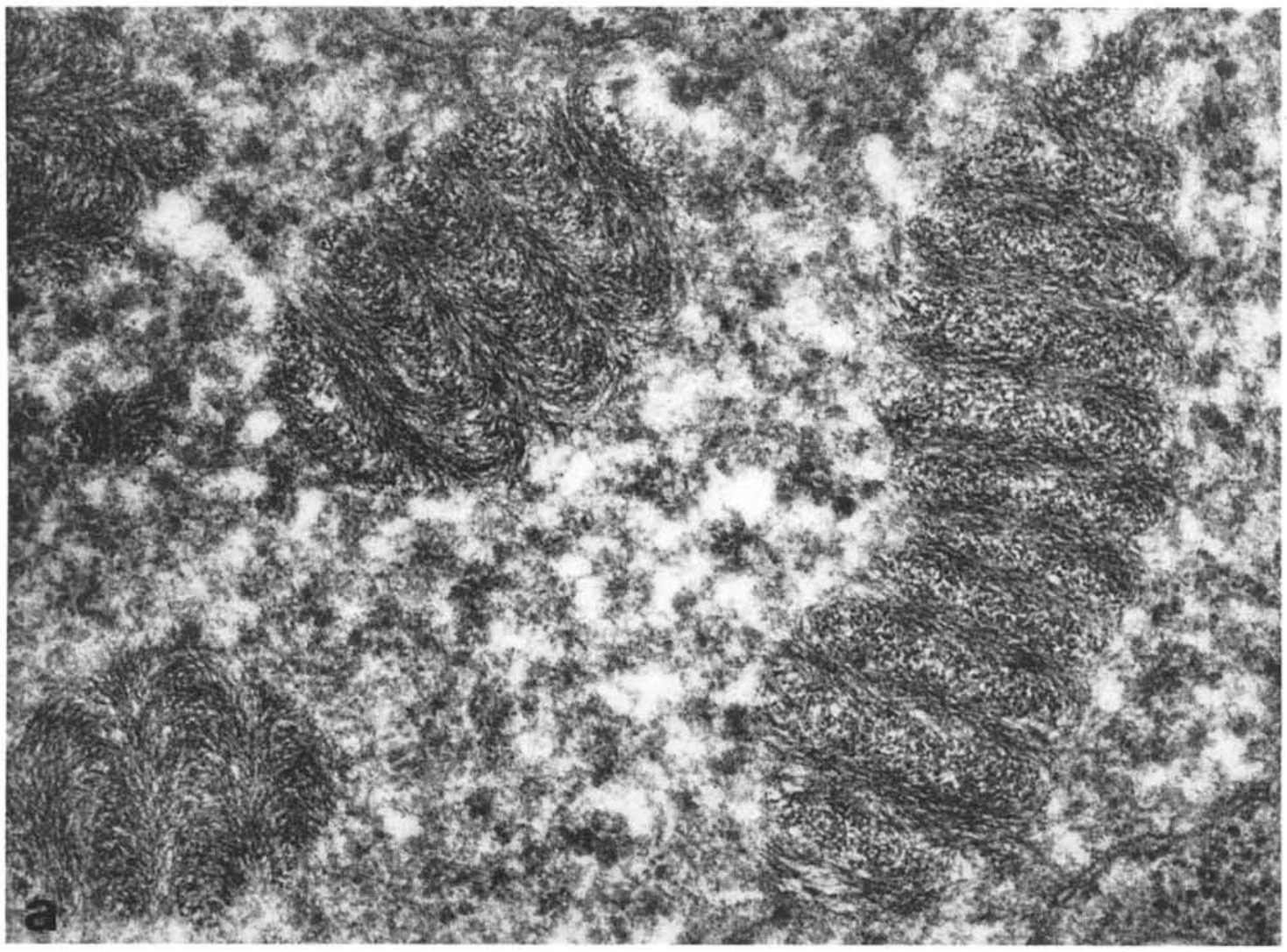
Protein	Protein detected in fraction with NaCl at (mM)						
	50	100	150	200	250	300	350
28 kDa	–	–	+	+	–	–	–
27 kDa	–	–	+	–	–	–	–
22 kDa	–	+	+	–	–	–	–
17 kDa (a)	–	–	–	+	+	–	–
17 kDa (b)	–	+	+	–	–	–	–
9 kDa	–	–	–	+	+	–	–
RNA holopolymerase	–	–	–	–	–	+	+

Table 3

Gold particle counts on immunolabeled bacterial sections (courtesy of M. Dürrenberger)

As described in detail in a forthcoming paper (Dürrenberger, Björnsti, Hobot, Uetz and Kellenberger, manuscript in preparation) the micrographs of labeled thin sections were covered with an adequately spaced grid of small squares. The size of the squares is chosen such that it can contain up to about three ribosomes. In each square the presence of ribosomes and gold is determined.

	Gold on bulk DNA	Bulk DNA without gold	Gold on ribosomal areas	Ribosomal areas without gold
Growing *E. coli*	1.23 ± 0.77	18.81 ± 3.7	29.93 ± 4.2	50.04 ± 5.08
T4.31$^-$-infected *E. coli*	0.5 ± 0.02	13.83 ± 2.4	36.06 ± 1.8	49.58 ± 3.4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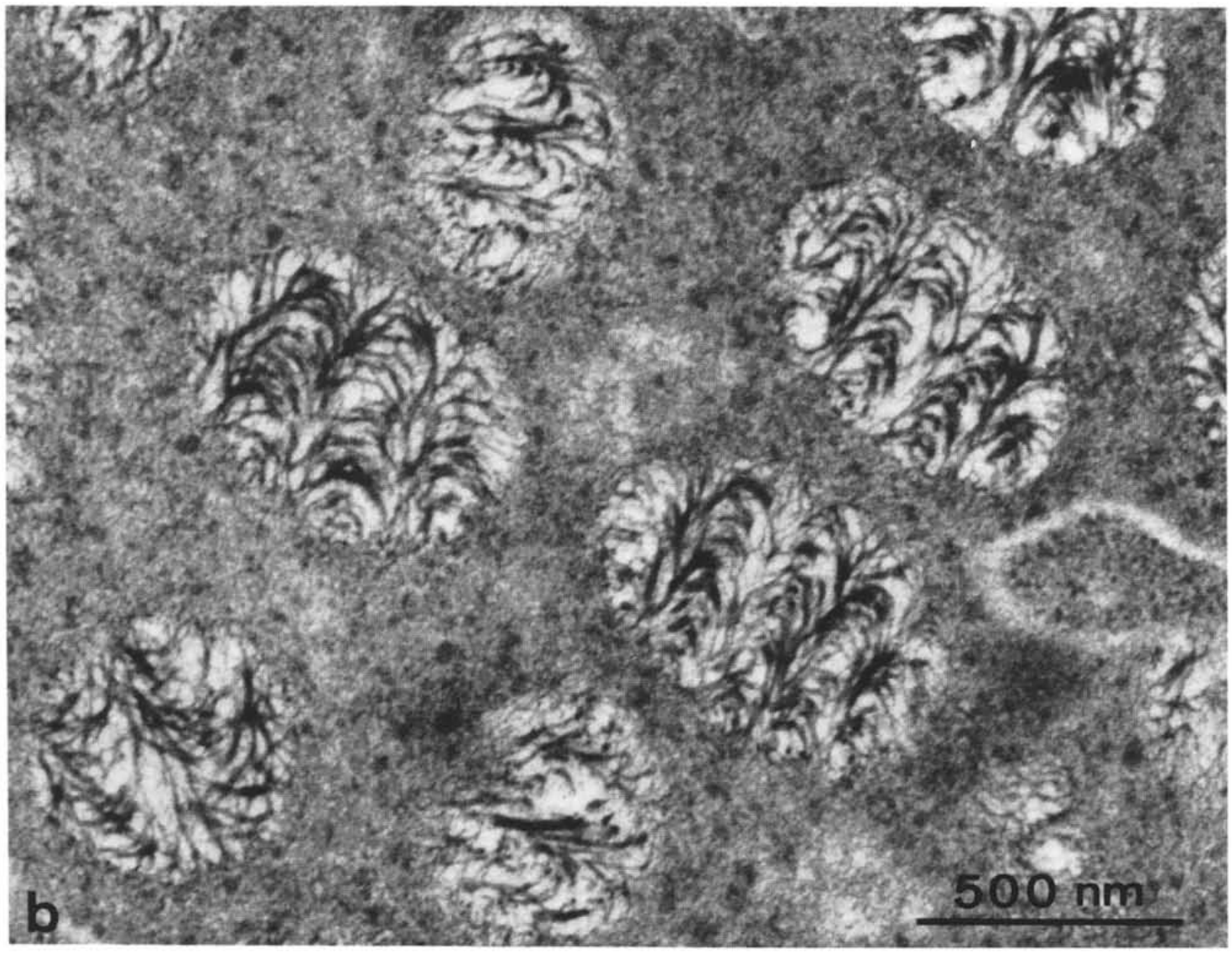
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500 nm

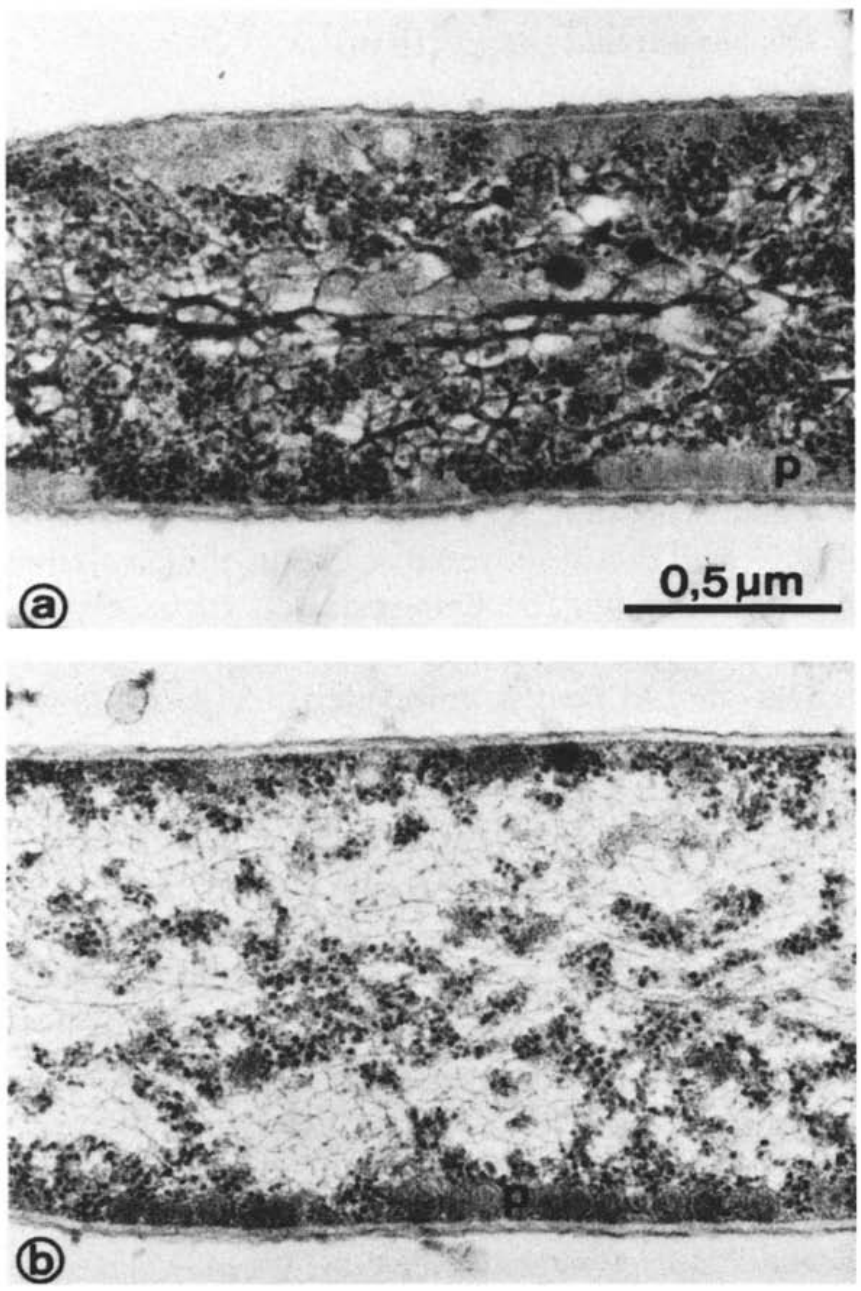

Fig. 5. *E. coli* cells after infection with a mutant (21⁻) of bacteriophage T4 that is arrested at the prohead (p) stage. Polyheads (ph) are also visible in some cells. The replicating phage DNA (vegetative pool) is presented as strands which appear coarse in panel a, already finer in panel b and so fine as to be nearly undetectable in panel c. Same magnification for panels a–c. Individual preparations of the three samples differed: (a) fixation in 2% glutaraldehyde for 60 min, thereafter 'classically' treated at room temperature by dehydration in ethanol and embedding in Epon. The sections were stained with uranyl acetate and lead citrate. (b) In this case, after the same fixation, the sample was dehydrated by 'progressive lowering of the temperature' (PLT) and then embedded in Lowicryl K4M. Sections are stained as in panel a. (c) The sample was not fixed but rapidly frozen by the slam procedure at liquid helium temperature, substituted into acetone containing 2.5% OsO_4 at 190 K over 64 h and then embedded in Epon and stained as in panels a and b.

means of sequencing, protein NS was found to be identical with HU [37,38].

In a recent investigation we showed that HU is present in sufficient amounts to warrant reliable in situ location by immunolabeling on sections from resin-embedded cells [39]. Details of the location study with all controls that exclude hiding of antigenic sites by the bound DNA will be presented in a forthcoming paper (Dürrenberger, Björnsti, Hobot, Uetz and Kellenberger, manuscript in preparation); the preliminary results, which are quite valid, are available [7]. Statistical evaluations of protein A-gold-labeled antibody binding (table 3) unambiguously show that HU is not associated with the bulk DNA, but is present in those regions where DNA is simultaneously transcribed and translated [40]. HU is thus possibly involved in these functions and might be bound to both the DNA and 30 S ribosomes. The conclusion that is drawn is that HU certainly does not possess the function of forming nucleosomes with

Fig. 4. Chromosomes of the dinoflagellate *Crypthecodenium cohnii* are aggregation-sensitive. In panel a they are shown with a minimum of aggregation, as obtained after fixation in mixtures of aldehydes followed by OsO_4 and uranyl acetate; in panel b they are shown with strong aggregation as obtained after aldehyde fixation alone. The sections were stained with uranyl acetate and lead as in fig. 3. The less relevant details of procedures are as follows: (a) Glutaraldehyde, 1.25%; paraformaldehyde, 2%; OsO_4, 2%; uranyl acetate, 0.5% in phosphate buffer. Dehydration in ethanol at room temperature. Embedding in Epon with thermal polymerisation. (b) Glutaraldehyde, 0.5%; paraformaldehyde, 3%. Dehydration in ethanol by progressive lowering of the temperature. Embedding in Lowicryl HM20. Polymerised at −35 °C. (Cultures by G. Vernet and M. Maeder; specimen preparation and electron microscopy by M. Maeder.)

metabolically inactive, 'resting' DNA, as is the main function of typical eukaryotic nucleosomes. One is obviously free to define bacterial nucleosomes as being atypical, extremely unstable particles which disappear upon isolation, as has been proposed by Broyles and Pettijohn [41]. In our in situ location studies we have employed cryofixation, i.e., rapid freezing of cells not previously chemically fixed by slamming them onto a liquid helium-cooled copper block [6]. The ice is then substituted at −80 °C by acetone containing 3% glutaraldehyde [42]. Being insoluble in organic solvents, it is highly unlikely that HU moves away from the bulk DNA so as to collect on the ribosomes. In a detailed paper (Dürrenberger, Björnsti, Hobot, Uetz and Kellenberger, in preparation) we present experimental results which show that physiological conditions exist under which the HU is *not* associated with ribosomes, despite using the same technique of cryosubstitution. Also, for other reasons, cryosubstitution might be considered as being far more gentle than any biochemical isolation procedure.

In the same paper we also demonstrate an identical location for HU with respect to the vegetative DNA of phage, namely, its absence in the bulk and presence over the ribosomes near to the pool (table 3).

The location of HU is therefore quasi-identical with that of single-stranded DNA [42] and DNA-dependent RNA polymerase (unpublished data).

Location studies with the other histone-like proteins listed in table 1 have not yet been attempted because of the much lower amounts present. If, however, these quantitations turn out to be incorrect, further attempts at immunocytochemical location should be made. Another approach should also be pursued: it is indeed most likely that in bacteria the DNA is not in the form of the sodium salt, but rather is neutralised by magnesium, putrescine and spermidine [43]. We have therefore proposed to isolate protein partners by binding them to these DNA salts [7]. The first experimental results indicated some differences from the pattern of proteins isolated on 'normal' DNA columns; they encourage further attempts at isolation of more specific protein partners.

5. Discussion and perspectives

According to our experimental results the bulk DNA of bacterial nucleoids and of the vegetative phage DNA pool does not contain the histone-like protein HU. Since HU is the most abundant representative of this group of proteins (ref. 31 and tables 1 and 2), hopes of finding the bulk DNA of these DNA-containing plasmas to be organised in the form of nucleosomes are vanishing. However, it remains possible that new nucleosomal proteins [44,45] will be discovered or that the available quantitations will be demonstrated to be experimentally underestimated.

One should bear in mind that nucleosomes are not the only possibility for supercoiling of DNA. Other potential ways can easily be imagined when involving topoisomerases and other proteins (replicating and transcribing complexes) that are able to introduce superhelical twists into the DNA molecule. The interesting data of Pettijohn's group [41,46–48], obtained from either psoralene cross-linking in vivo and in vitro or digestion studies, can also be explained by other types of supercoiling. None of these experiments have revealed the well-known period of 180–250 bp so typical for the bulk nucleosomes of eukaryotes [49]. The absence of this period is obviously one of the reasons for postulating atypical, unstable nucleosomes [41].

Our experimental results suggest that HU is involved in transcription. Since in bacteria transcription and translation appear to be interdependent (see p. 288 of ref. 9) one might even presume that HU binds both ribosomes and DNA; attachment of the 30 S ribosome to the DNA could be mediated by HU, such that the messenger-ribosome complex is formed immediately upon synthesis of the mRNA. On forming the 70 S ribosome, the DNA would then be released from it together with HU.

The belief in the existence of true nucleosomes in prokaryotes is based on the observation by electron microscopy of nucleosome-like structures on DNA released from bacteria [50]. Despite numerous efforts and the use of adequate specimen preparation methods, other workers were not able to repeat the experiment [51]. Since it has been shown that beautiful 'nucleosomes' can be

produced by ethanol treatment [52] with completely protein-free DNA, such an artefact can no longer be excluded. The observation from electron microscopy that nucleosome-like particles are formed when DNA is reacted in vitro with protein HU [53] at a 1 : 1 ratio does not provide a convincing argument, not only because the natural abundance of HU is about 10-times lower, but also because it has never been shown that eukaryotic nucleosomes are readily produced with only one of the core histones!

The possible absence of typical bulk nucleosomes in bacterial nucleoids and vegetative phage pools is obviously strongly corroborated by their belonging to the class of aggregation-sensitive DNA plasmas. As is strongly suggested by our results from in vitro studies on fixation and gelation (section 3), this different physico-chemical behavior is expected to be the consequence of a smaller proportion of protein partners; the DNA concentration is indeed very similar for the phage pool, the nucleoid and eukaryotic interphase nuclei (20–40 mg/ml). The phage pool and nucleoid are those containing the largest proportion of vegetative DNA. In dinoflagellates (for references, see ref. 13) with doubling times of several hours and of which various species contain widely differing amounts of DNA (of which the lowest content is still about 400-times greater than that of *E. coli*) the proportion of vegetative DNA is expected to be much smaller. The many chromosomes of these unicellular organisms are nevertheless aggregation-sensitive (refs. 20 and 54 and fig. 4). At the level of the optical microscope, the dinoflagellates are very similar to algae, such as *Euglena*; both also have a nuclear membrane. However, *Euglena* appears to be comparatively aggregation-insensitive as are animal cells. Dinoflagellates with two nuclei exist, one of each type [55,56]. Upon isolation two distinct types of chromatin filaments were observed, according to their origin from the two types of nuclei present. Those arising from the eukaryotic nucleus showed distinct nucleosomes, while those from the other nucleus did not [57]. These cells with two nuclei, one of each type, are obviously a most appropriate specimen for future tests.

It is noteworthy that dinoflagellates also contain only small quantities of histone-like proteins (for references, see refs. 13 and 14), again explaining why their DNA plasmas belong to the aggregation-sensitive class. Immunocytochemical location studies with their histone-like proteins have been initiated in several laboratories.

From the evolutionary standpoint [14], it is quite understandable that replication, transcription and translation would have originated prior to the nucleosomal compaction mechanism. At this early stage, no basic structural distinction between bulk and vegetative DNA would have existed. To disentangle the two genomes of a bacterium after replication, strong condensation was not yet necessary. Bacteriophages, however, already use a more than 30-fold condensation for the packaging of DNA into the phage head, although no nucleosomal compaction is involved. Nucleosomal compaction of the viral DNA inside the virion has hitherto only been found in that group to which SV40 belongs [58,59].

For the dinoflagellates some experimental evidence has been presented for a possible condensation-decondensation [20,60], although the predominant belief is that chromosomes remain permanently in the condensed state. One would then have to assume that only loops of DNA would emerge from the condensed chromosome for performing its metabolic activities. Such a form of vegetative DNA could possibly be detected by studying the locations of ss- and ds-DNA as well as of histone-link proteins. Such immunocytochemical studies on dinoflagellates are currently in progress in several laboratories.

Acknowledgements

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