Volume 4 Number 8 August 1977 Nucleic Acids Research

Histone-like proteins in the purified Escherichia coli deoxyribonucleoprotein

A. J. Varshavsky, S. A. Nedospasov, V. V. Bakayev, T. G. Bakayeva and G. P. Georgiev

Insitute of Molecular Biology, Academy of Sciences of the USSR, Vavilov Street 32, Moscow B-312, USSR

Received 9 May 1977

ABSTRACT

Analysis of E.coli chromosomes isolated under conditions similar to those used for isolation of eukaryotic chromatin has shown that: 1) The proteins of highly purified E.coli deoxyribonucleoprotein are mainly in addition to RNA polymerase two specific histone-like proteins of apparent molecular weight of 17,000 and 9,000 (proteins 1 and 2, respectively). 2) Proteins 1 and 2 occur in approximately equal molar amounts in the isolated E.coli chromosome, and their relative content corresponds to one molecule of protein 1 plus one molecule of protein 2 per 150-200 base pairs of DNA. 3) There are no long stretches of naked DNA in the purified E.coli deoxyribonucleoprotein suggesting a fairly uniform distribution of the proteins 1 and 2 along DNA. 4) The protein 2 is apparently identical to the DNA-binding protein HU which was isolated previously /1/ from extracts of E.coli cells. 5) Digestion of the isolated E.coli chromosomes with staphylococcal nuclease proceeds through discrete deoxyribonucleoprotein intermediates (in particular, at ~120 base pairs) which contain both proteins 1 and 2. However, since no repeating multimer structure was observed so far in nuclease digests of the E.coli chromosome, it seems premature to draw definite conclusions about possible similarities between the nucleosomal organization of the eukaryotic chromatin and the E.coli chromatin structure.

INTRODUCTION

It has recently become clear that the isolated chromatin of either higher or lower eukaryotes has a repeating("subunit") structure resembling a string of closely packed beads ("nucleosomes") /2-21/. Each nucleosome comprises about 200 base pairs of DNA wound around a histone core. The core is a histone octamer which consists of four kinds of histones (H2a, H2b, H3 and H4) /4,5,8-11,16/. The fifth histone, H1, which is present at least in higher eukaryotes is probably associated with the region linking one repeating unit to another /16-18,20-23/.

In the last few years methods have been developed for the isolation of the bacterial chromosomes. The bacterial DNA has been isolated in a folded and supercoiled conformation, attached to membrane fragments, by gentle lysis of E.coli spheroplasts followed by sucrose gradient centrifugation /24-26/. Although a detailed study has been published on the nature of proteins at the DNA-membrane attachment site in the folded chromosomes /27/, there is practically no direct data on the possible existence of specific histone-like proteins in association with the folded E.coli DNA. On the other hand, a variety of low-molecular-weight DNA-binding basic proteins of apparently nonribosomal origin have been isolated from E.coli cells /1,28,29/. The isolation of these proteins generally started from DNase-treated extracts of E.coli cells and therefore cannot permit unambiguous identification of a particular basic protein as a chromosome-bound protein in vivo. Nevertheless, some of these proteins have properties sufficiently in common with eukaryotic histones to suggest similar function in E.coli cells /1,28/. These data, taken together with the electron microscopic evidence for the compact state of DNA in E.coli chromosomal fibers /30/ and with the evidence for supercoiled DNA in the folded E.coli chromosomes /24-26/ have led us to a search for histone-like proteins in the isolated E.coli chromosome.

MATERIALS AND METHODS

Isolation of folded E.coli chromosomes. Folded chromosomes were prepared by a modification of the method of Kornberg et al./26/. The modification included two major changes. Firstly, a 100-fold lower concentration of lysozyme was used for lysing the cells since this basic protein interacts with DNA and is preferentially accumulated in the folded chromosomes during their isolation (see below). Secondly, the ionic and "detergent" conditions of the medium were made milder and in particular similar to those used during isolation of the eukaryotic chromatin.

E.coli 802 /31/ was grown in a shaking bath at 37°C in the M9 medium /32/ supplemented with 0.5 per cent Casa-

mino acids (Difco). In most of the experiments, exponentially growing cells were double-labelled by adding /Me-^{3}H/thymidine (6 Ci/mmole) and a hydrolysate of Chlorella ^{14}C-proteins (50 mCi/mmole) to final concentrations of 1 µCi/ml and 10 µCi/ml, respectively. The exponentially growing cells were harvested by centrifugation at 2°C after 60 min of labelling and thereafter washed with 0.14 M NaCl, 1 mM triethanolamine (TEA)-HCl, pH 7.6 (pH of all buffers was measured at 20°C). The pellet (approximately $5x10^{10}$ cells) was resuspended at 0°C in 2 ml of 20% sucrose, 0.1 M NaCl, 10 mM TEA-HCl, pH 7.6. One-quarter volume of a freshly made lysozyme solution (50 µg egg-white lysozyme/ml (Worthington) in 50 mM Na-EDTA, 50 mM TEA-HCl, pH 7.6) was then added followed by addition of 0.5 M phenylmethylsulphonyl fluoride (PMSF, proteinase inhibitor; Sigma) in ethanol to a final concentration of 1 mM. After careful mixing, the suspension was incubated at 10°C for 40 min followed by addition of one volume of a solution containing 1% Nonidet P40 (Shell), 2 mM spermidine-HCl, 10 mM Na-EDTA, pH 7.6 and freshly added 1 mM PMSF. Cells were lysed at 10°C for 15-20 min. The suspension of lysed cells (~5 ml) was subjected to centrifugation at 4°C in the SW25.2 rotor (Beckman) for 30 min at 7,000 rpm through a 15-50% sucrose gradient (50 ml) which contained 3 mM $MgCl_2$ and 10 mM TEA-HCl, pH 7.6. The white, opalescent band at the center of the tube (see Fig.1) was carefully removed with a wide-bore pipette and thereafter was used for the next experimental stage.

Nuclease digestion of isolated E.coli chromosomes. Isolated chromosomes (100-300 µg of DNA per ml) were digested with staphylococcal nuclease (Worthington; 1-10 µg/ml) directly after isolation of the folded chromosomes or after additional purification of the chromosomes by gel chromatography (see below). The digestion was carried out at 37°C in 0.1 mM $CaCl_2$, 1 mM TEA-HCl, pH 7.6 (in some experiments 1 mM $MgCl_2$ was also present in the digestion buffer). The reaction was terminated by addition of 50 mM Na-EDTA, pH 7.6 to a final concentration of 2 mM followed by chilling of the sample in an ice bath and centrifugation at 10,000 g for 10 min to pellet the insoluble material. The extent of DNP solubilization

and the acid-soluble fraction of the digests were determined as described previously for nuclease digests of radioactively labelled eukaryotic chromatin /9,16-18/.

Gel chromatography of E.coli chromosomes. We have found at early stages of this work that our preparation of folded E.coli chromosomes contains nucleases which slowly cleave chromosomal DNA and RNA. For example, after an overnight dialysis of the suspension of isolated folded chromosomes against 1 mM TEA-HCl, pH 7.6 at ~4°C all DNA in the sample becomes fragmented to pieces ~5×10^4 base pairs in length (see below). At the same time little if any degradation of DNA-bound proteins occured during this and subsequent handling of the PMSF-treated E.coli DNP (see below). We used such a mildly fragmented E.coli DNP preparation to purify the DNP particles. Isolated folded E.coli chromosomes (see above) were concentrated by low-speed centrifugation (15,000 g for 20 min), thereafter suspended in 1 mM TEA-HCl, pH 7.6 to a final concentration of about 100 µg of DNA per ml followed by an overnight dialysis of the sample against the same buffer. The dialysed sample was centrifuged at 15,000 g for 20 min. The pellet which contained mainly membraneous material but no DNA was discarded and the supernatant (10 ml) which contained virtually all initial DNA was applied to a 400-ml Sepharose 2B column which had been equilibrated with 0.2 mM Na-EDTA, 10 mM TEA-HCl, pH 7.6. All the DNP was eluted in the void volume and was used for the next experimental stage.

Isolation of low-molecular weight basic proteins from extracts of E.coli cells. The method used was based on that of Rouviere-Yaniv and Gross /1,28/. Our purpose was to compare proteins from purified E.coli deoxyribonucleoprotein with DNA-binding proteins which were isolated previously /1, 28,29,33/ from extracts of E.coli cells. Briefly, the strain MRE 600 cells were sonicated in the presence of DNase I and the lysate was centrifuged at 100,000 g for 2 hr. The supernatant was made 50% saturated with $(NH_4)_2SO_4$. The supernatant after the first ammonium sulfate precipitation was then made 90% saturated with (NH4)2S04. The ammonium sulfate protein pellets were dissolved in 1 mM dithiotreitol, 5 mM Na-EDTA,

20 mM Tris-HCl, pH 7.6 and heated at 100°C for 15 min. The samples were centrifuged at 10,000 g for 15 min and the supernatants were then treated with 0.25 N HCl to extract acid-soluble, heat-stable proteins /1,28/.

Isopycnic banding of DNP in CsCl gradients. A ^{14}C,^{3}H-DNP sample in 2 mM Na-EDTA, 1 mM TEA-HCl, pH 7.6 was fixed with 1% HCHO, pH 7.6 for 20-80 hr at 4°C (the results did not depend on the time of fixation within this interval) followed by dialysis against 1 mM Na-EDTA to remove free HCHO. The samples were centrifuged in Sarcosyl-containing CsCl gradients in the SW50.1 rotor (Beckman) at 43,000 rpm for 65-70 hr at 15°C as previously described /36/. Sarcosyl did not change the density of the DNP in CsCl as compared with pure CsCl gradients and insured a 90-100% recovery of the ^{14}C and ^{3}H after centrifugation /36/.

Polyacrylamide gel electrophoresis of nucleoproteins. Nuclease digests of the unfractionated E.coli chromosomes or of the purified E.coli DNP were electrophoresed in six per cent slab polyacrylamide gels with an acrylamide:methylene-bisacrylamide ratio of 30:1. The buffer in the electrode vessels and in the gels was 2 mM Na-EDTA, 10 mM TEA-HCl, pH 7.6 /16-18/. The gels were stained with ethidium bromide or (in some experiments with chromosomes labelled with /Me-^{3}H/thymidine only) impregnated with a scintillator followed by detection of the ^{3}H-labelled DNA in the dried gels by fluorography /37/.

SDS-gel electrophoresis of proteins. Electrophoresis was carried out in stacking slab polyacrylamide gels as previously described /17/ except that 2 mM Na-EDTA was added to all buffers.

Acetic acid-urea gel electrophoresis of proteins. Electrophoresis was carried out in the system of Panyim and Chalkley /38/ using slab polyacrylamide gels in the presence of 2.5 M urea.

Two-dimensional gel electrophoresis. The DNP particles were fractionated by the low-ionic-strength gel electrophoresis (first dimension; see above and refs. 16-18/ followed by SDS-gel electrophoresis of their DNA and protein components

in a slab gel (second dimension). After removal of SDS by repeated washings of the slab gel with water the gel was stained with ethidium bromide. Alternatively, the slab gel was soaked in 20% CCl_3COOH and thereafter stained for proteins with Coomassie /17,18/. The technique of two-dimensional electrophoresis was essentially that of O'Farell /39/.

Agarose gel electrophoresis of DNA. Electrophoresis was carried out in a vertical slab gel apparatus for 0.8-1% agarose gels and in a horizontal gel apparatus /40/ for 0.2% agarose gels. High-molecular-weight DNA samples were appropriately diluted in a sample buffer (10% sucrose, 1 mM Na-EDTA, pH 7.6) and thereafter heated at 45°C for 15 min before electrophoresis in 0.2% agarose gels at a low voltage gradient (~ 0.5 V/cm) /40/.

RESULTS AND DISCUSSION

Isolation and purification of E.coli deoxyribonucleoprotein. Figure 1 shows a sedimentation pattern of the folded E.coli chromosomes isolated by a modification of previously developed methods /26,27/. The modification included in particular, a 100-fold lowering of the lysozyme concentration during lysis of E.coli cells since this basic protein interacts with DNA and was found to be preferentially accumulated in the isolated E.coli chromosomes (Fig. 2k,n,o; cf. 2a,b,h, i). Furthermore, we made ionic and "detergent" conditions of the medium milder and in particular more close to the conditions used during isolation of the eukaryotic chromatin (see Methods).

Chromosomal DNA constitutes 10-15% of the total nucleic acid content in the chromosome preparation isolated from exponentially growing population of E.coli cells (see Fig.3). The major nucleic acid species in the sample is ribosomal RNA within ribosomal RNP particles which are attached to DNA via mRNA and RNA polymerase molecules /24-27,41/. Thus a great variety of proteins is present in the unfractionated E.coli chromosome preparations (Fig. 2h-j). To purify E.coli DNP we made use of the observation that the preparation of

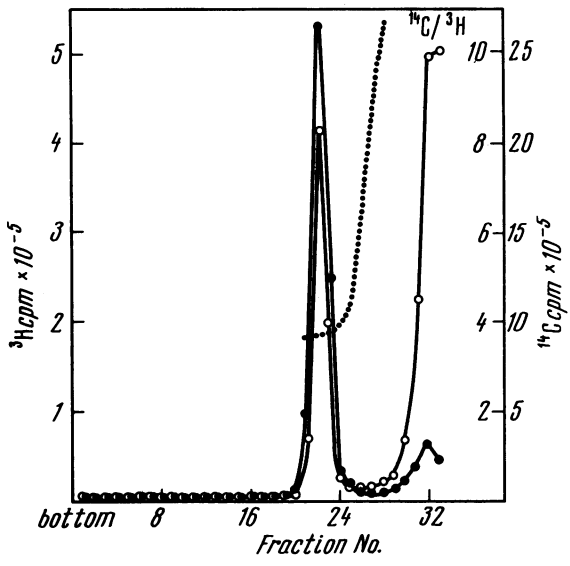

Fig. 1. Isolation of folded E. coli chromosomes. See Methods for detail. Analytical-scale pattern (SW50. 1 rotor, 8000 rpm for 15 min). —●—, ^{3}H (DNA); —○—, ^{14}C (protein); ·····, $^{14}C/^{3}H$.

E.coli chromosomes contained endogeneous nucleases which slowly cleaved both DNA and RNA in the sample. As a result in a few hours after isolation of the E.coli chromosomes all DNA in the sample becomes fragmented to pieces $\sim 5 \times 10^{4}$ base pairs in length (see Fig.4 and the legend to it for detail) and in addition most of the DNP becomes free from association with RNP particles (see Fig.3). Such a mildly fragmented chromosome preparation was used to purify the E.coli DNP by gel chromatography on Sepharose 2B (Fig.3; see also Fig.5). One can see that during this step a great majority of both RNP and free proteins is separated from the E.coli DNP (Fig.3). It should be noted that no detectable degradation of proteins occured during purification of the E.coli DNP which had been treated with a proteinase inhibitor PMSF at early stages of the DNP purification. In fact, the purified E.coli DNP which was prepared either in the absence of any proteinase inhibitor or in the presence of PMSF during all stages of the DNP purification displayed SDS-electrophoretic patterns indistinguishable from that shown in Fig.2 a,b. Thus it seems unlikely that the major kinds of proteins in the purified E.coli DNP (proteins 1 and 2; see below) are degradation products of higher molecular weight proteins.

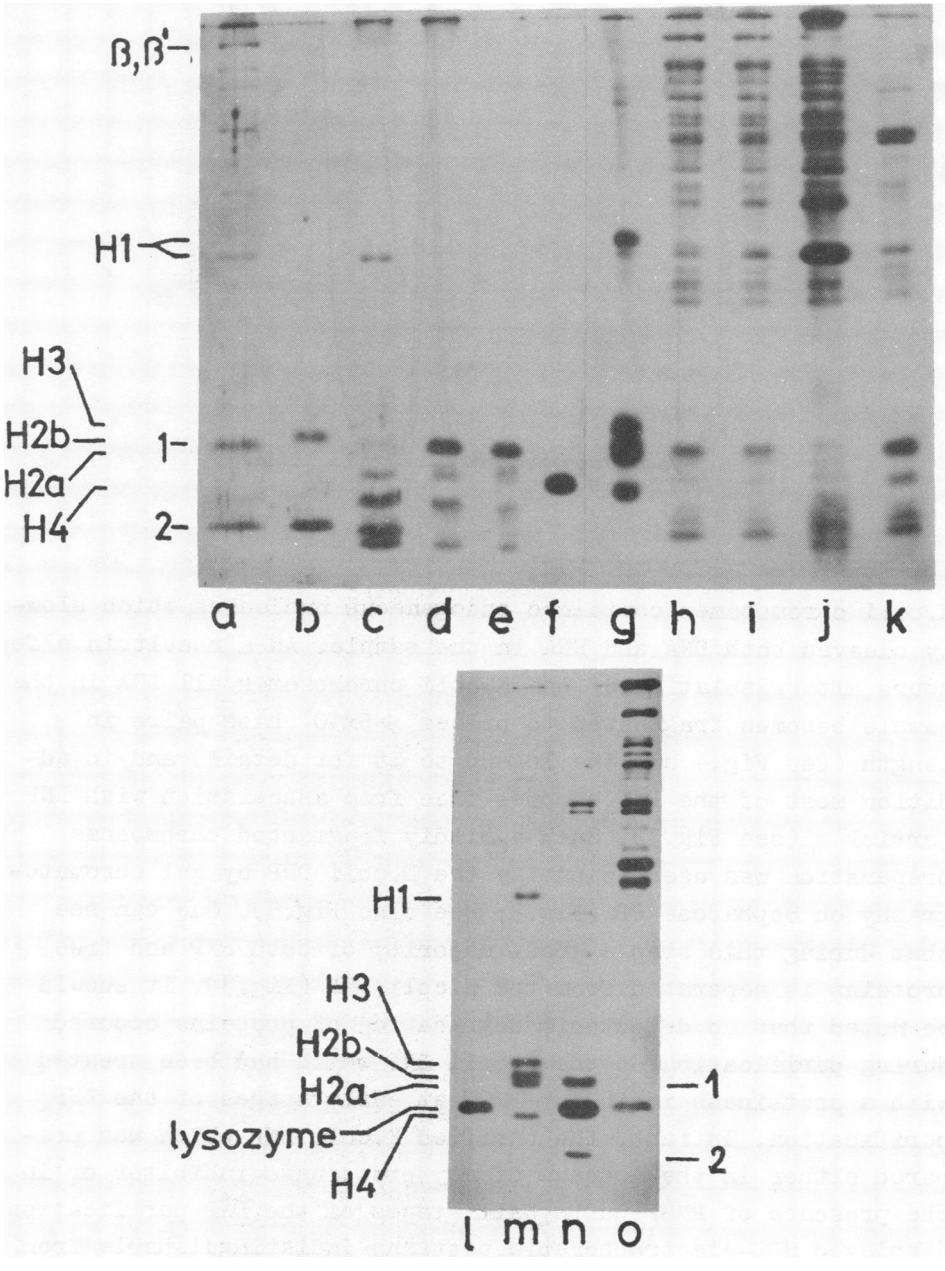

Fig. 2. Polyacrylamide SDS-gel electrophoresis of E. coli chromosomal proteins.
a - proteins of the purified E. coli DNP (see Fig. 3, fractions 8-10); b - proteins of the 0.25 N HCl -extract of the purified E. coli DNP; c - heat-stable proteins from extract of E. coli cells precipitated with 90% saturated $(NH_4)_2SO$ (see Methods); d - the same as c but proteins were precipitated with 50% saturated $(NH_4)_2SO_4$ (protein preparation enriched for DNA-binding protein H_1 /33/; see Methods); e - 0.25 N HCl-extract of proteins shown in d; f - egg white lysozyme; g - total protein from the mouse Ehrlich ascites tumor chromatin /9, 18/; h - proteins of the mildly DNase-fragmented E. coli chromosomes (clarified by low-speed centrifugation) before gel chromatography (see Methods and Fig. 3); i - the same as h but from another experiment; j - proteins of the initial unfractionated E. coli chromosome preparation (see Fig. 1); k - proteins of the 0.25 N HCl-extract of the clarified, unfractionated E. coli chromosome preparation which was obtained in the presence of 200 μg of lysozyme per ml (see Methods); l - the same as f; m - the same as g; n - the same as k but chromosomes were isolated in the presence of 1 mg of lysozyme per ml; o - the same as j but chromosomes were isolated in the presence of 1 mg of lysozyme per ml/26, 27/.

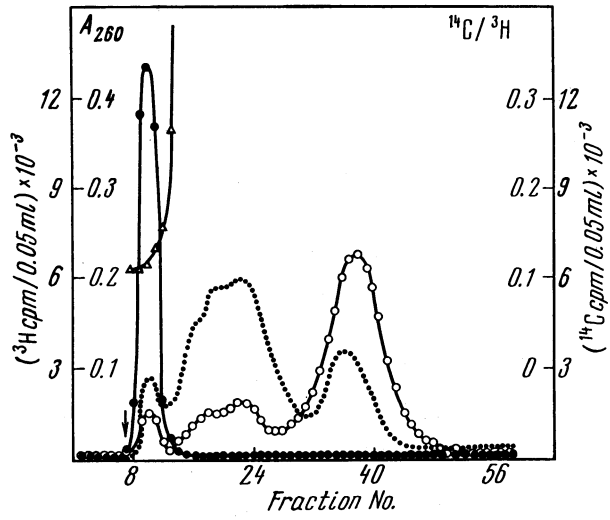

Fig. 3. Purification of E. coli DNP by gel chromatography on Sepharose 2B.
See Methods for detail. —●— , ^{3}H(DNA); —○— , ^{14}C(protein); -Δ-Δ-Δ-Δ- , $^{14}C/^{3}H$; ••••, A_{260}. Arrow indicates the void volume.
The $^{14}C/^{3}H$ ratio for the total chromosome preparation which was clarified by low-speed centrifugation before gel chromatography (see Methods) equals 2, whereas the $^{14}C/^{3}H$ ratio for the initial total chromosome preparation (see Fig. 1) is tw· times higher (see text for detail).

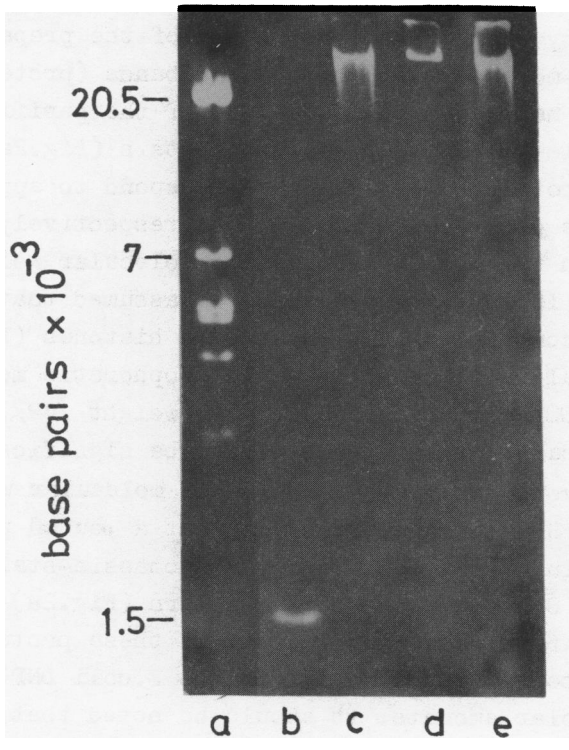

Fig. 4. Agarose gel electrophoresis of DNA from isolated E. coli chromosomes.
a - EcoRI restrictase digest of λ phage DNA/44/; b - Hae III restrictase digest of SV40 viral DNA/45/; c - DNA from isolated E. coli chromosome preparation which was dialysed against 1 mM TEA-HCl, pH 7.6 (see Methods); d - λ phage DNA; e - the same as c but from another experiment. Electrophoresis was carried out in 0.8% agarose at 0.5 V/cm/40/.
This system provides the minimal estimate for the length of isolated E. coli DNA but fails to determine its true molecular weight distribution /40/. Electrophoresis of DNA (see c and e) in horizontal 0.2% agarose gels /40/ and sucrose gradient centrifugation of DNA (see Methods) reveal a distribution of DNA lengths between 30,000 and 60,000 base pairs (unpublished data).

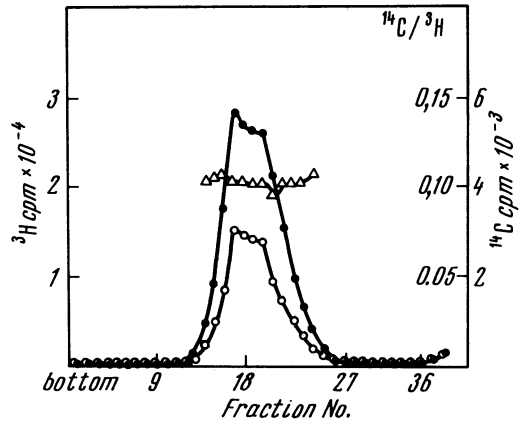

Fig. 5. Sucrose gradient centrifugation of purified E. coli DNP.
Purified E. coli DNP (see Fig. 3, fractions 8-10) was centrifuged in the SW60 Ti rotor at 45,000 rpm for 2 hr (see Methods). The same designations as in Fig. 3.

Purified E.coli deoxyribonucleoprotein contains two specific histone-like proteins. Figure 2a shows that the purification of the E.coli DNP by gel chromatography greatly reduces the complexity of the protein pattern of the preparation. Two specific low-molecular-weight protein bands (proteins 1 and 2) constitute a major protein component of the purified E.coli DNP as revealed by SDS-gel electrophoresis (Fig.2a; cf. Fig. 2h-j). The protein bands 1 and 2 correspond to apparent molecular weights of ~17,000 and ~9,000, respectively, as was determined with the use of appropriate molecular weight standards /42/ . In these measurements we assumed that the proteins 1 and 2, in contrast to the eukaryotic histones (Fig.2 g,m) reveal a usual relation between electrophoretic mobility of a protein in SDS-gels and its molecular weight /42/. The above-stated molecular weight values would be significantly lower if the eukaryotic histones are used as molecular weight standards in the SDS-gel electrophoresis at a neutral pH /42/. Densitomer tracings (not shown) of the Coomassie-stained bands 1 and 2 in the SDS-electrophoretic pattern (Fig.2a) combined with the apparent molecular weights of these proteins indicate that the proteins 1 and 2 occur in the E.coli DNP in approximately equal molar amounts. It should be noted that uncertainties inherent in the densitometric determination of the relative abundance of proteins as well as uncertainties about the exact values of molecular weight of proteins 1 and 2 do not

permit us at the present time to rigorously exclude the possibility for the protein 2 being in a slight molar excess over the protein 1. Further analysis is required to clarify this point.

The SDS-gel electrophoretic pattern of the total protein complement of the purified E.coli DNP contains in addition to the major bands of proteins 1 and 2 the bands corresponding to subunits of RNA polymerase (in particular the bands of β and β' subunits identified by running purified RNA polymerase in the same gel) and also a few other minor protein bands (Fig. 2a). However, a 0.25 N HCl-extract of the E.coli DNP contains almost exclusively the proteins 1 and 2 (Fig.2b), thus indicating both the basic nature of these proteins and the absence of other acid-soluble proteins in the purified E.coli DNP.

Figure 6 shows the results of analysis of the proteins 1 and 2 in the acetic acid-urea gel electrophoretic system which in contrast to the SDS-system separates proteins not only on the basis of their size but also on the basis of their charge properties /38/. One can see that the protein 1 which migrates between the eukaryotic histones H2a and H2b in the SDS-gels (Fig.2a,g,m,n) in urea-acetic acid gels migrates between eukaryotic histones H1 and H3 (Fig.6 a,b,f). The protein 2 which runs far ahead of histone H4 in SDS-gels (Fig.2a,g,m,n) is almost coelectrophoresed with H4 in the acetic acid-urea gels (Fig. 6a,b,f). Thus although future sequence studies may reveal significant similarities between primary structures of the E.coli proteins 1 and 2 and the eukaryotic histones, it is already clear that neither protein 1 nor protein 2 are strictly identical to any of the five eukaryotic histones.

It should be noted that while the protein 2 is readily and quantitatively extracted from the E.coli DNP by 0.25 N HCl, the protein 1 is extracted much less efficiently and is therefore underrepresented in the electrophoregrams of the acid extracts (Fig.2b and 6f; cf. Fig.2a). Furthermore, one can see that the proteins 1 and 2 constitute a very minor proportion of the total protein in the unfractionated E.coli chromosome preparation and in fact are barely detectable over

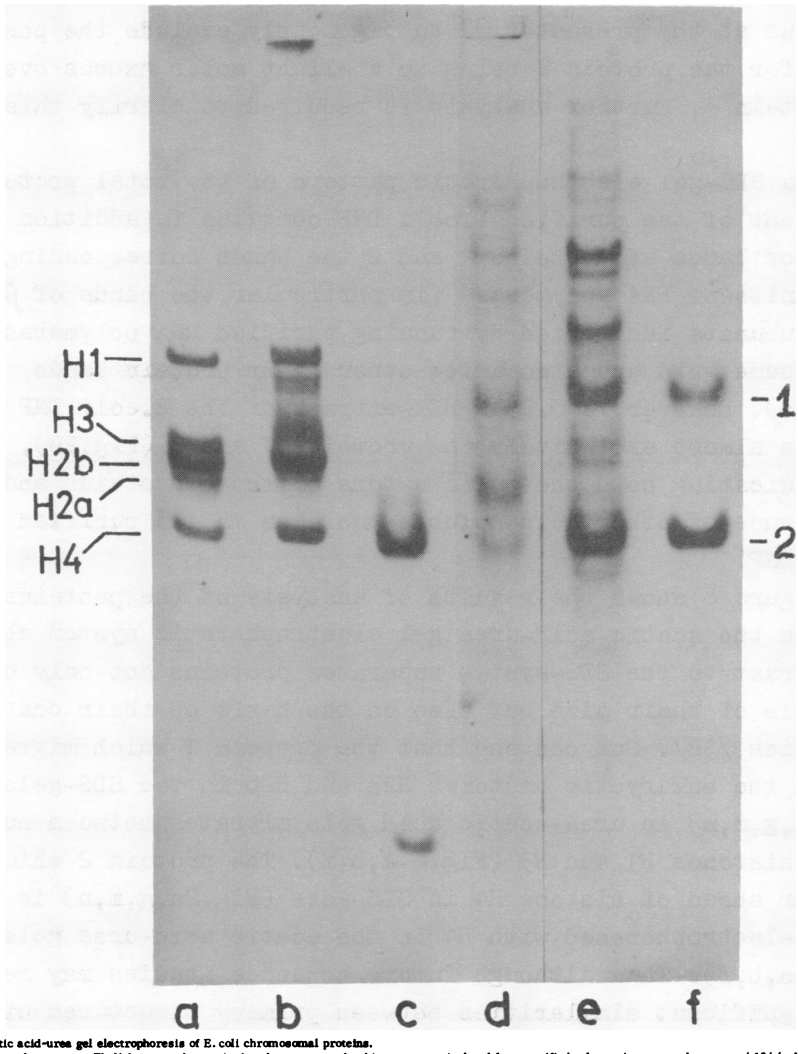

Fig. 6. Acetic acid-urea gel electrophoresis of E. coli chromosomal proteins.
a - total histone from mouse Ehrlich tumor chromatin; b - the same as a but histones were isolated from purified eukaryotic mononucleosomes / 18/ (a doublet of bands below the eukaryotic histone H1 are specific nonhistone proteins /46/; c - 0.25 N HCl - extract of the E.coli protein preparation enriched for DNA-binding protein HU / 1, 28/ (see also Fig. 2c); d - 0.25 N HCl-extract of the protein preparation showed in Fig. 2d; e - 0.25 N HCl-extract of the E. coli chromosome preparation (clarified by low-speed centrifugation) before gel chromatography (see Methods); f - 0.25 N HCl-extract of the purified E. coli DNP (see Methods and Fig. 3).

background of other proteins which are not found in the purified E.coli DNP (Fig. 2j; cf. Fig.2a). Clarification of the mildly DNase-fragmented DNP solution before Sepharose 2B gel chromatography (see Methods) leads to a considerable increase of the relative content of the proteins 1 and 2 (Fig.2h, i and Fig.6 e; cf. Fig.2j). Such an enrichment is a consequence of the removal of a major proportion of membraneous material from the mildly DNase-fragmented chromosome preparation by low-speed centrifugation (see Methods and Figs. 1 and 3; no-

tice also a two-fold decrease of the $^{14}C/^{3}H$ ratio corresponding to a protein/DNA ratio upon removal of membranes as indicated in the legend to Fig.3). The absence of both DNA and proteins 1 and 2 in the membraneous pellet (data not shown) suggests (but does not proove) that these two proteins are not membrane proteins which were shifted to DNA as a result of e.g., detergent treatment (see Methods).

The greatest increase of the relative content of the proteins 1 and 2 in the E.coli DNP preparation occurs upon the final purification of the DNP by gel chromatography (Figs. 2a and 6f; cf. Figs. 2h,i,j and 6e).

Comparison of protein 2 with DNA-binding basic protein HU /1/ isolated from extracts of E.coli cells. Several low--molecular-weight basic proteins in particular, a relatively abundant protein HU with apparent molecular weight close to 9,000 /1/ were isolated previously from extracts of E.coli cells /1,28,29,33/. To see whether the DNA-bound protein 2 (see above) is identical to the HU protein we compared proteins 1 and 2 in two different electrophoretic systems with protein preparations enriched in particular for the HU protein /1,28/ (see Methods and refs. 1,28,29 for detail). The data obtained strongly suggest that the protein 2 is identical to the HU protein (Figs. 2c and 6c; cf. Figs. 2a and 6 e,f). No detailed comparison of the protein 1 with other DNA-binding proteins from extracts of E.coli cells /2,28,29,33/ has been carried out so far.

What is the relative amount of proteins 1 and 2 in the isolated E.coli chromosome? Purified E.coli $^{14}C,^{3}H$-labelled DNP (see Fig.3 and 5) was fixed with formaldehyde and thereafter centrifuged to equilibrium in a CsCl density gradient (Fig.7). This technique fractionates DNP according to its protein/DNA ratio /34,36,43/; simultaneous presence of a protein (^{14}C) and DNA (^{3}H) label in the DNP permits one to detect a possible loss of proteins from the fixed DNP upon CsCl centrifugation /34,36/. Figure 7a shows that the HCHO-fixed E.coli DNP bands at a density of 1.62 g/cm^3 which corresponds to a protein/DNA weight/weight ratio of approximately 0.2 /34,36, 43/. Conservation of the $^{14}C/^{3}H$ ratio corresponding to the

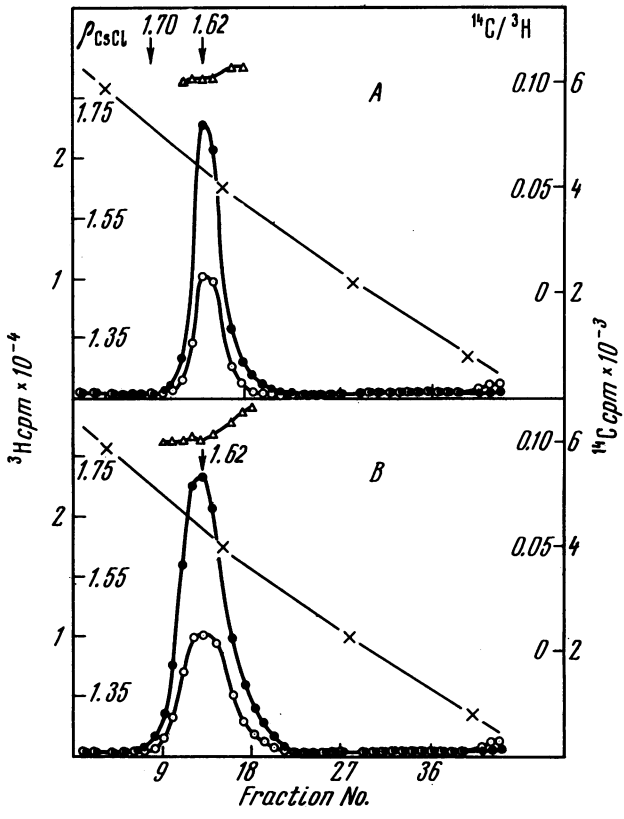

Figure 7a and 7b.

Fig. 7. Isopycnic banding of E. coli DNP in CsCl gradients.
a - purified HCHO-fixed E. coli DNP (see Methods and Fig. 3); b - the same as a but before centrifugation the fixed DNP was hydrodynamically sheared to an average DNA length of approximately 400 base pairs / 36/; c - the same as a but the E. coli ^{3}H- DNP was fixed with HCHO before gel chromatography (see Methods); d - purified HCHO-fixed eukaryotic mononucleosome preparation /9/.
The same designations as in Fig. 3 - x -, density (g/cm^3).

protein/DNA ratio (Fig. 7a; compare the $^{14}C/^{3}H$ ratio for the CsCl-banded DNP with the $^{14}C/^{3}H$ ratios for DNP peaks in Figs. 3 and 5) shows that no significant loss of proteins occured upon isopycnic banding of the HCHO-fixed E.coli DNP in CsCl.

The proteins 1 and 2 with apparent molecular weights of 17,000 and 9,000 respectively (see above) constitute 70-80% of the total protein in the purified E.coli DNP (see Figs. 2a and 3). Thus one can calculate that the protein/DNA w/w ratio of 0.2 (see above) corresponds to one molecule of protein 1 plus one molecule of protein 2 per 180 (150-200) base pairs of DNA. The values in parentheses are shown to allow for a limited accuracy both of molecular weight measurements /42/ and of determinations of protein/DNA ratios from densities of the

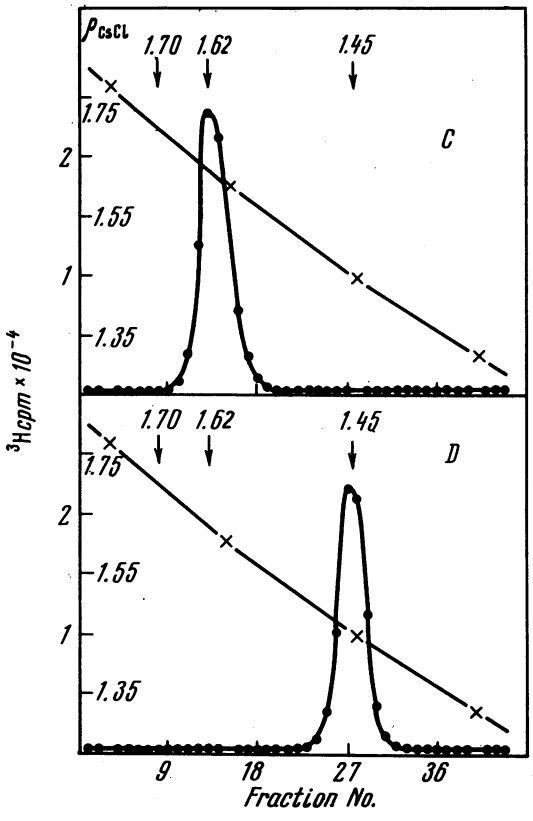

Figure 7c and 7d.

DNP in CsCl /43/. Thus the relative content of the proteins 1 and 2 in the isolated E.coli chromatin is 4–5 times lower that the relative content of the five eukaryotic histones in the eukaryotic chromatin (Fig.7d; cf. Fig.7a,c; see also Introduction).

The isopycnic data (Fig.7) tell nothing about the mutual arrangement of proteins 1 and 2 on the DNA. Nevertheless, the CsCl technique can be used to determine whether there is a relatively uniform distribution of proteins along DNA or whether long stretches of naked DNA exist in the isolated E.coli DNP. Figure 7b shows that shearing of the HCHO-fixed E.coli DNP to an average DNA length of approximately 400 base pairs leads to a slight increase of the width of the DNP band in

the CsCl gradient but does not result in appearance of any naked DNA fragments. Thus the distribution of the DNA-bound proteins (most of which are proteins 1 and 2) appears to be fairly uniform along the DNA.

Nuclease digestion of isolated E.coli chromosomes. Figure 8 shows the results of analysis of staphylococcal nuclease-digested E.coli and eukaryotic chromosomes by a low--ionic-strength polyacrylamide gel electrophoresis. This method permits one to fractionate eukaryotic chromatin subunits (mononucleosomes) and their oligomers into series of discrete DNP bands at a much higher resolution than can be achieved by e.g., sucrose gradient centrifugation of the DNP (Fig. 8g; see also refs. 16-18). Since the DNP is a minor component of the unfractionated E.coli chromosome preparation (see above) the major ethidium-stained material in the gel at early stages of digestion are RNA-containing particles (Fig. 8 a-f). Discrimination between RNA- and DNA-containing material was carried out by fluorography of the scintillator-impregnated gels /37/ containing ^{3}H-labelled DNA (see Methods, Fig.8 and the legend to it for detail). Preliminary experiments showed that under the conditions of labelling used the incorporation of /Me-^{3}H/thymidine into RNA and proteins is negligibly small (see e.g., Fig.3) and thus ^{3}H-fluorography of polyacrylamide gels is an adequate method for detection of DNA in the presence of RNA and proteins. Figure 8 k-m shows that staphylococcal nuclease digestion of the E.coli chromosome proceeds through formation of discrete DNA-containing intermediates (Fig. 8 k-m; cf. Fig, 8 n). In fact, these intermediates can be seen not only by fluorography (Fig. 8 k-m) but also as relatively faint bands migrating below the eukaryotic mononucleosomes MN_1 and MN_2 (Fig.8g) in ethidium-stained patterns shown in Fig. 8 h-j. At these later stages of nuclease digestion of the E.coli chromosome most of the RNA-containing material (bright white areas in Fig. 8 e,f,h,i) was degraded by staphylococcal nuclease (which has both DNase and RNase activity) to fragments migrating to the bottom of the gel.

What is the length of DNA fragments in the DNP intermediates seen in Fig. 8 k,l? To answer the question E.coli DNP

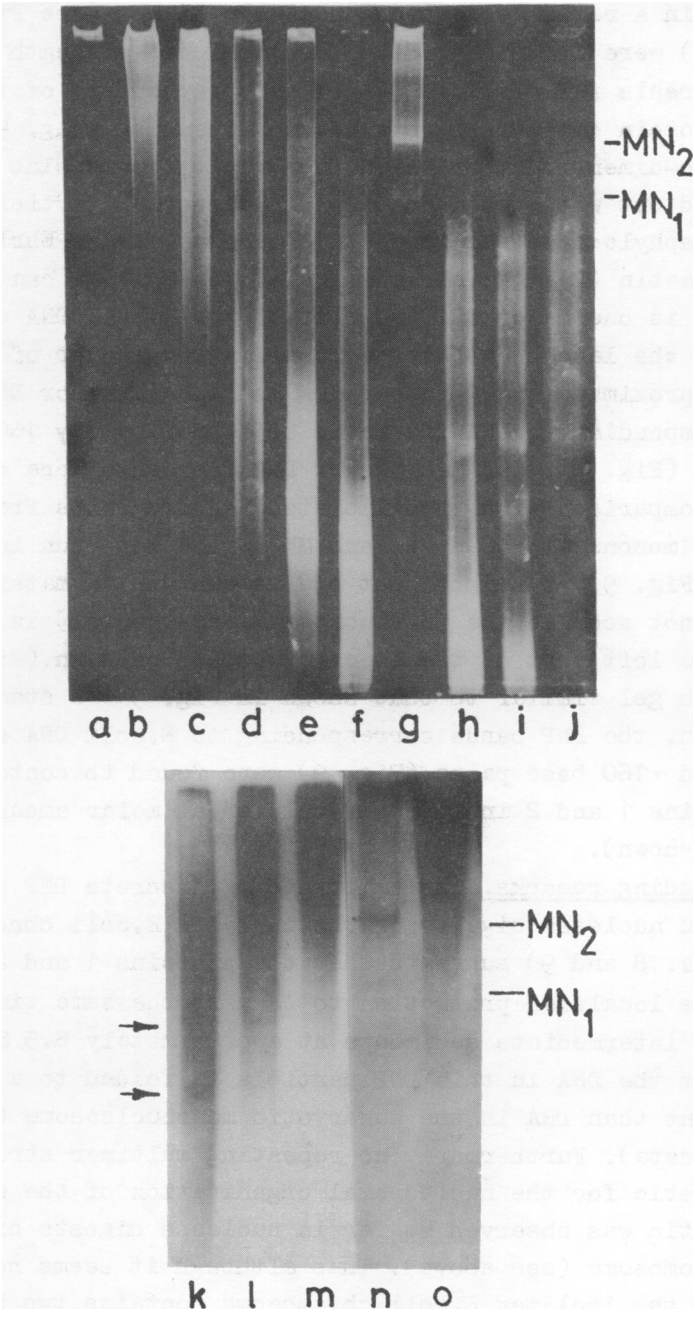

Fig. 8. Low-ionic-strength polyacrylamide gel electrophoresis of staphylococcal nuclease-digested E. coli chromosomes.
/Me-^{3}H/thymidine-labelled unfractionated E. coli chromosome preparation was digested with nuclease followed by gel electrophoresis of nucleoproteins (see Methods).
(a)-(f) Total 0, 1, 3, 6, 8, 12% ^{3}H-acid-soluble nuclease digests of the unfractionated E. coli chromosome preparation, respectively; (g) Total 5% acid-soluble digest of the mouse Ehrlich tumor chromatin /9, 16-18/; (h)-(j) The same as a-f but 17, 23 and 27% ^{3}H-acid-soluble digests, respectively.
Bright white areas in this ethidium-stained gel are RNA-containing particles which are not detected upon ^{3}H-fluorography (see below).
(k)-(n) ^{3}H-fluorography of the total 28, 20 and 5% ^{3}H-acid-soluble nuclease digests of the unfractionated E. coli chromosome preparation, respectively; (n) the same as (g) but ^{3}H-fluorography of DNA-labelled digest; (o) The same as k-m but 3% ^{3}H-acid-soluble digest.
MN_1 and MN_2 refer to the positions of the 140-base pair, H1-depleted eukaryotic mononucleosome(MN_1) and of the 170-base pair MN_2 mononucleosome containing all five eukaryotic histones (see g and refs. 16-18). Arrows in k indicate the positions of discrete E. coli DNP particles.

particles in a relatively strong nuclease digest (see Fig. 9 for detail) were fractionated by the low-ionic-strength gel electrophoresis followed by SDS-gel electrophoresis of their DNA and protein components in a second dimension (Fig. 9). Similar two-dimensional fractionation (in the same slab gel) was carried out with the mono- and dinucleosomal particles from a staphylococcal nuclease digest of the mouse Ehrlich tumor chromatin (Fig. 9; see also refs. 16-18). One can see that there is one major diffuse spot of the E.coli DNA corresponding to the length of DNA fragments (in the center of the spot) of approximately 120 base pairs and also a minor DNA spot corresponding to DNA fragments of approximately 160 base pairs long (Fig. 9). The lengths of DNA fragments were determined by comparison with precalibrated DNA fragments from the eukaryotic mononucleosomes MN_1 and MN_2 which were run in the same gel (Fig. 9). A bright spot of RNA-containing material (which is not seen in the ^3H-fluorograms of the gel) is present in the left part of the electrophoretic pattern (Fig. 9). When a slab gel similar to that shown in Fig. 9 was stained for protein, the DNP bands corresponding to E.coli DNA spots at ~120 and ~160 base pairs (Fig. 9) were found to contain only proteins 1 and 2 in approximately equal molar amounts (data not shown).

Concluding remarks. The existence of discrete DNP intermediates in nuclease digests of the isolated E.coli chromosomes (Figs. 8 and 9) suggests that the proteins 1 and 2 afford some localized protection to DNA. At the same time, the 120-bp DNP intermediate sediments at approximately 6.5 S indicating that the DNA in this DNP particle is folded to a much lower extent than DNA in the eukaryotic mononucleosome (unpublished data). Furthermore, no repeating multimer structure characteristic for the nucleosomal organization of the eukaryotic chromatin was observed so far in nuclease digests of the E.coli chromosome (see above). Thus although it seems now clear that the isolated E.coli chromosome contains two DNA-bound specific proteins structurally similar but not identical to the eukaryotic histones, many important questions remain unanswered. In particular, it is not known whether

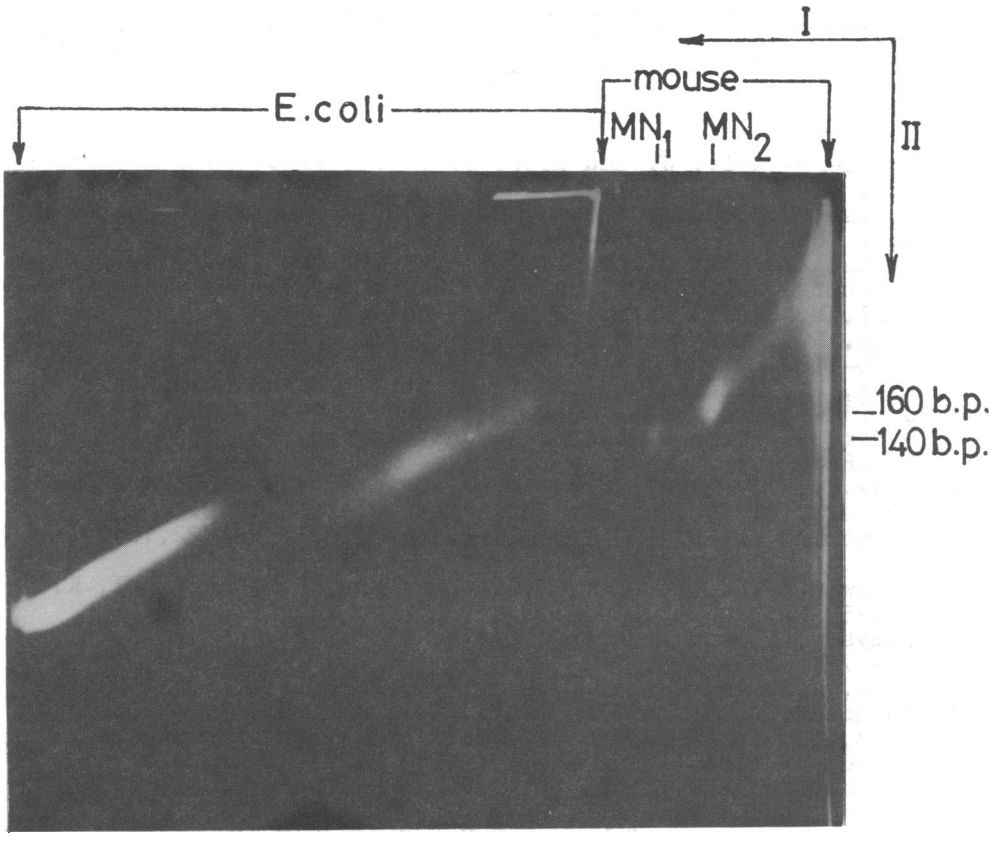

Fig. 9. Two-dimensional analysis of nuclease digests of E. coli and mouse chromatin.
A total unfractionated E. coli chromosome preparation was digested with staphylococcal nuclease to 29% ^{3}H-acid-solubility followed by a low-ionic-strength polyacrylamide gel electrophoresis of nucleoproteins (first dimension; see Methods). The gel was then immersed in an SDS-containing solution followed by SDS-gel electrophoresis in a 7.5% polyacrylamide gel (second dimension; see Methods). The gel was stained with ethidium bromide.
Staphylococcal nuclease digest of the eukaryotic (mouse Ehrlich tumor) chromatin was similarly analysed in the same gel (see Fig. 8g for the DNP pattern in the first dimension and for the designations of MN_1 and MN_2).
A bright white area in left part of the pattern corresponds to RNA-containing material which is not seen upon ^{3}H-fluorography of the gel (data not shown).

there is an interaction between DNA-bound proteins 1 and 2 in the E.coli chromosome and what is the exact role of these two proteins in a structural organization of the E.coli chromosomal fibers. Furthermore, although it seems probable that the proteins 1 and 2 are associated with both transcriptionally active and inactive regions of the E.coli genome (because there are virtually no long stretches of naked DNA in the isolated E.coli DNP (see above)), the question clearly requires further study.

ACKNOWLEDGEMENTS

We thank M.I.Shifman for his excellent assistance in some of the experiments, V.V.Shmatchenko for advice on acetic

acid-urea gel electrophoresis and Dr. R. Hoffman (Harvard Medical School) for a critical reading of the manuscript.

REFERENCES

1 Rouviere-Yaniv, J. and Gros, F. (1975) Proc. Nat. Acad. Sci. USA 72, 3428-3432.
2 Hewish, D.R. and Burgoyne, L.A. (1973) Biochem. Biophys. Res. Communs. 52, 504-510.
3 Olins, A.L. and Olins, D.E. (1974) Science 184, 330-333.
4 Kornberg, R.D. (1974) Science 184, 868-871.
5 Noll, M. (1974) Nature 251, 249-251.
6 Rill, R.L. and Van Holde, K.E. (1973) J.Biol.Chem. 248, 1080-1087.
7 Axel, R., Melchior, W., Sollner-Webb, B. and Felsenfeld, G. (1974) Proc. Nat. Acad. Sci. USA 71, 4101-4105.
8 Oudet, P., Gross-Bellard, A. and Chambon, P. (1975) Cell 4, 282-295.
9 Bakayev, V.V., Melnickov, A.A., Osicka, V.D. and Varshavsky, A.J. (1975) Nucleic Acids Res. 2, 1401-1419.
10 Weintraub, H. (1975) Proc. Nat. Acad. Sci. USA 72,1212-1216.
11 Baldwin, J.R., Boseley, P.G., Bradbury, E.M. and Ibel, K. (1975) Nature 253, 245-249.
12 Polysky, B. and McCarthy, B.J. (1975) Proc. Nat.Acad.Sci. USA 72, 2895-2899.
13 Hyde, J.E. and Walker, I.O. (1975) FEBS Lett. 50, 150-154.
14 Woodcock, C.L.F. (1976) Exptl. Cell Res. 97, 101-110.
15 Hörz, W., Igo-Kemenez, G., Pfeiffer, W. and Zachau, H.G. (1976) Nucleic Acids Res. 3, 3213-3227.
16 Varshavsky, A.J., Bakayev, V.V. and Georgiev, G.P. (1976) Nucleic Acids Res. 3, 477-492.
17 Varshavsky, A.J., Bakayev, V.V., Chumackov, P.M. and Georgiev, G.P. (1976) Nucleic Acids Res. 3, 2101-2114.
18 Bakayev, V.V., Bakayeva, T.G. and Varshavsky, A.J. (1977) Cell, in press.
19 Noll, M. (1976) Cell 8, 349-356.
20 Morris, N.R. (1976) Cell 8, 357-364.
21 Noll, M. and Kornberg, R.D.. (1977) J.Mol.Biol. 109, 393-404.
22 Shaw, B.R., Herman, T.M., Kovacic, R.T., Beadreau, G.S. and Van Holde, K.E. (1976) Proc. Nat. Acad. Sci. USA 73, 505-509.
23 Simpson, R.T. and Whitlock, J.P. (1976) 15, 4305-4314.
24 Stonington, G.O. and Pettijohn, D.E. (1971) Proc. Nat. Acad.Sci. USA 68, 6-10.
25 Worcel, A. and Burgi, E. (1972) J.Mol.Biol. 71, 127-142.
26 Kornberg, T., Lockwood, A. and Worcel, A. (1974) Proc. Nat.Acad.Sci. USA 71, 3189.
27 Portalier, R. and Worcel, A. (1976) Cell 8, 245-255.
28 Haselkorn, R. and Rouviere-Yaniv, J. (1976) Proc. Nat. Acad.Sci. USA 73, 1917-1920.
29 Berthold, V. and Geider, K. (1976) Eur. J. Biochem. 71,443-451.
30 Griffith, J.D. (1976) Proc. Nat. Acad.Sci. USA 73,563-567.
31 Wood, W.B. (1966) J.Mol.Biol. 16, 118-133.
32 Weigle, J., Meselson, M. and Paigen, K. (1959) J.Mol.Biol. 1,379-385.

33 Cukier-Kahn, R., Jackuet, M. and Gros, F. (1972) Proc. Nat. Acad.Sci. USA 69, 3643-3647.
34 Varshavsky, A.J. and Ilyin, Y.V. (1974) Biochim.Biophys. Acta 340, 207-213.
35 Petes, T.D. and Fangman, W.L. (1972) Proc. Nat.Acad. Sci. USA 69, 1188-1192.
36 Varshavsky, A.J., Bakayev, V.V., Ilyin, Y.V., Bayev, A.A. and Georgiev, G.P. (1976) Eur.J.Biochem.66, 211-223.
37 Bonner, W.M. and Laskey, R.A. (1974) Eur.J.Biochem. 46,83-88.
38 Panyim, S. and Chalkley, R. (1969) Biochemistry 8,3972-3979.
39 O'Farell, P. (1975) J.Biol.Chem. 250, 4007-4021.
40 McDonnel, M.W., Simon, M.N. and Studier, F.W. (1977) J. Mol.Biol. 110, 119-146.
41 Miller, O.L., Beatty, B.R., Hamkalo, B.A. and Thomas,C.A. (1970) Cold Spring Harb. Symp. Quant. Biol. 35, 505-520.
42 Weber, K. and Osborn, M. (1975) In: The Proteins (ed. by H.Neurath and R.L.Hill) pp.180-221, Acad.Press, N.Y.
43 Ilyin, Y.V., Varshavsky, A.J. and Georgiev, G.P. (1970) Mol. Biol. (USSR) 4, 821-830.
44 Thomas, M. and Davies, R.W. (1975) J.Mol.Biol. 91, 315-328.
45 Roberts, R.J., Breitmayer, A., Tabachnik, B. and Myers, P.A. (1975) J.Mol.Biol. 91, 121-129.
46 Goodwin, G. and Johns, E.W. (1973) Eur. J. Biochem. 40, 215-219.