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On the Structure of Cellular and Viral Chromatin

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On the structure of cellular and viral chromatin

By A. J. Varshavsky†, V. V. Bakayev, T. G. Bakayeva, P. M. Chumackov, V. V. Shmatchenko and G. P. Georgiev
Institute of Molecular Biology, Academy of Sciences of the U.S.S.R., Moscow B-312, U.S.S.R.

Some of the recent experimental data obtained in our laboratory are briefly reviewed.

1. A mild staphylococcal nuclease digestion of either chromatin or nuclei from mouse Ehrlich tumour cells results in chromatin subunits (mononucleosomes) of three discrete kinds. The smallest mononucleosome (MN_1) contains all histones except H1 and a DNA fragment 140 base pairs long. The intermediate mononucleosome (MN_2) contains all five histones and a DNA fragment 170 base pairs long. The third mononucleosome (MN_3) also contains all five histones, but its associated DNA is longer and somewhat heterogeneous in size (180–200 base pairs). Most of the MN_3 particles are rapidly converted to mononucleosomes MN_2 and MN_1 by nuclease digestion. However, there exists a relatively nuclease-resistant subpopulation of the MN_3 mononucleosomes. *These* 200 base-pair MN_3 particles contain not only histones but also non-histone proteins and are significantly more resistant to nuclease then even the smaller mononucleosomes MN_1 and MN_2.

2. Nuclease digestion of hen erythrocyte nuclei or chromatin, in which histone H1 is partially replaced by histone H5 produces the mononucleosomes MN_1 and two electrophoretically resolvable kinds of MN_2 mononucleosomes, one containing histone H1 and the other one histone H5. A relatively nuclease-resistant subset of the mononucleosomes MN_3 is preferentially accumulated at later stages of the digestion.

3. Although pancreatic DNase (DNase I) and spleen acid DNase (DNase II) attack the DNA in chromatin in a manner different from that of staphylococcal nuclease, the deoxyribonucleoprotein (DNP) products of digestion are similar for all three enzymes under identical solvent conditions, as revealed by gel electrophoresis of the DNP at low ionic strength.

4. There are eight major kinds of staphylococcal nuclease-produced soluble subnucleosomes (i.e. particles smaller than the mononucleosomes). In particular, the subnucleosome SN_1 is a set of naked double-stranded DNA fragments *ca.* 20 base pairs long. Subnucleosome SN_2 is a complex of a specific highly basic non-histone protein and a DNA fragment *ca.* 27 base pairs long. Subnucleosomes SN_7 and SN_8 each contain all of the histones except H1 and DNA fragments *ca.* 100 and 120 base pairs long, respectively.

5. Nuclease digestion of isolated mono- and dinucleosomes does not produce all of the subnucleosomes. These and related findings indicate that the cleavages required to generate these subnucleosomes result from some aspect of chromatin structure which is lost upon digestion to mono- or dinucleosomes. Nuclease digestion of isolated minichromosomes of Simian virus 40 (SV40) (which contain all five histones including H1) produces mononucleosomes MN_1 and MN_2 but does not produce some of the subnucleosomes or the relatively nuclease-resistant subset of the MN_3 mononucleosomes.

6. The rate of sedimentation of the SV40 minichromosomes (*ca.* 60*S*) under 'physiological' ionic conditions ($\mu \approx 0.15$) is about two times higher than that in a low ionic strength buffer ($\mu \approx 0.005$). Occurrence of the compact state of the minichromosome critically depends upon the presence of histone H1 and can be irreversibly fixed by treatment with formaldehyde.

† Present address: Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, U.S.A.

1. Introduction

Subunit organization of isolated eukaryotic chromatin now appears to be well established (Hewish & Burgoyne 1973; Rill & Van Holde 1973; Olins & Olins 1974; Kornberg 1974; Noll 1974; Axel, Melchoir, Sollner-Webb & Felsenfeld 1974; Oudet, Gross-Bellard & Chambon 1975; Weintraub 1975; Bakayev, Melnickov, Osicka & Varshavsky 1975; Varshavsky, Bakayev & Georgiev 1976*a*; Hörz, Igo-Kemenez & Zachau 1976). Mild treatment of chromatin with deoxyribonucleases, in particular with staphylococcal nuclease, permits one to isolate single chromatin subunits (mononucleosomes) and their oligomers, which can be separated from each other by sucrose gradient centrifugation (figure 1; see also Noll 1974; Bakayev *et al.* 1975).

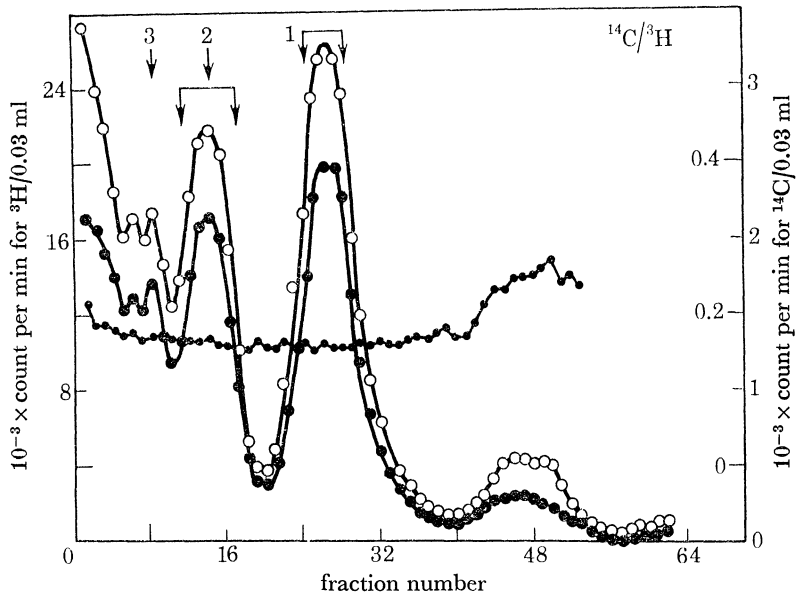

FIGURE 1. Sucrose gradient centrifugation of nuclease digest of mouse Ehrlich tumour chromatin in a Ti14 zonal rotor (Varshavsky *et al.* 1976*a*). ●, ^{3}H (DNA); ○, ^{14}C (protein); •, $^{14}C/^{3}H$. Arrows indicate peaks of mono-, di- and trinucleosomes.

We have shown previously that mononucleosomes and their oligomers in a mild staphylococcal nuclease digest of chromatin are heterogeneous with regard to their content of histone H1 (Varshavsky *et al.* 1976*a*). These and related findings (Shaw *et al.* 1976; Simpson & Whitlock 1976) strongly suggest the internucleosomal location of histone H1.† Further analysis has led to a more detailed understanding of the structural aspect of the situation (Bakayev, Bakayeva & Varshavsky 1977) and is briefly reviewed below.

In another line of investigation we have found histone H1 in the isolated minichromosomes of Simian virus 40 (SV40), previously believed to contain four histones (H2A, H2B, H3 and H4) but to be completely devoid of histone H1 (Varshavsky, Bakayev, Chumackov & Georgiev 1976*b*). Some new data bearing on the presence and rôle of histone H1 in the isolated SV40 minichromosomes are also considered below.

† The term 'internucleosomal' implies that only the H1-depleted 140 base-pair 'core' mononucleosome should be considered as a 'true' nucleosome. According to another terminology which we use in this paper to simplify presentation of the data, all DNP particles which contain one octamer of histone (with or without histone H1) are considered to be nucleosomes, irrespective of the length of the DNA fragment, presence or absence of histone H1, or non-histone proteins etc.

2. Evidence for the existence of a third type of mononucleosome

Polyacrylamide gel electrophoresis at low ionic strength of either a total nuclease digest of the chromatin (figure 2) or isolated mono- and dinucleosomes (Varshavsky *et al.* 1976*a*) fractionates the mononucleosomes into the two discrete bands MN_1 and MN_2. Dinucleosomes are fractionated into three bands, DN_1, DN_2 and DN_3 (figure 2*a*). Mononucleosome MN_1 lacks histone H1 and contains a DNA fragment 140 base pairs long, whereas the MN_2 contains

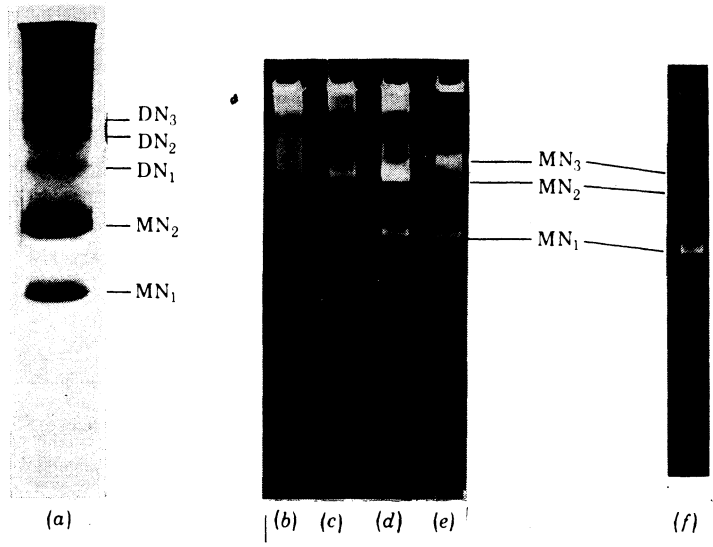

Figure 2. Polyacrylamide gel electrophoresis of deoxyribonucleoproteins (Varshavsky *et al.* 1976*a*; Bakayev *et al.* 1977). (*a*) Total 5% acid-soluble nuclease digest of the chromatin; electrophoresis in a 5% gel; stained with Coomassie; (*b*)–(*e*) total 2, 5, 7 and 30% acid-soluble digests, respectively; electrophoresis in a 7% gel; stained with ethidium bromide; (*f*) total 20% acid-soluble digest; electrophoresis in a 6% gel.

all five histones and a DNA fragment 170 base pairs long (figures 2, 3; see also Bakayev *et al.* 1977). More detailed analysis has revealed a third type of mononucleosome with unusual properties. At early stages of nuclease digestion of chromatin one can see a diffuse DNP band with a mobility slightly lower than that of mononucleosome MN_2 (figure 2*c*, *d*, *f*). Further digestion results in a complete disappearance of dinucleosomes, of higher nucleosomal oligomers and of MN_2 which are all digested to limit-digest insoluble products (figure 2*e*). At the same time, at later stages of digestion a band of a hitherto undescribed third mononucleosome (MN_3) can be seen just above the position of mononucleosome MN_2 (figure 2*e*, *f*). The mononucleosome MN_3 which is accumulated at later stages of the digestion is reproducibly more resistant to nuclease than the smaller mononucleosomes MN_1 and MN_2 (figure 2; see Bakayev *et al.* 1977 for detail). Finally, it should be stressed that the relatively nuclease-resistant subset of the mononucleosomes MN_3 was observed also in electrophoretic patterns of nuclease digests of whole, unwashed nuclei.

Figure 3 shows the results of analysis of protein composition of the mono-, di- and oligonucleosomes. The gels were impregnated with a scintillant and exposed to an X-ray film in order to detect ^{14}C-proteins (see the legend to figure 3 for details). Figure 3*a* shows that the great majority of the total non-histone protein of chromatin is not associated with mono- and dinucleosomes. At the same time an overexposed photograph of the X-ray film (figure 3*b*)

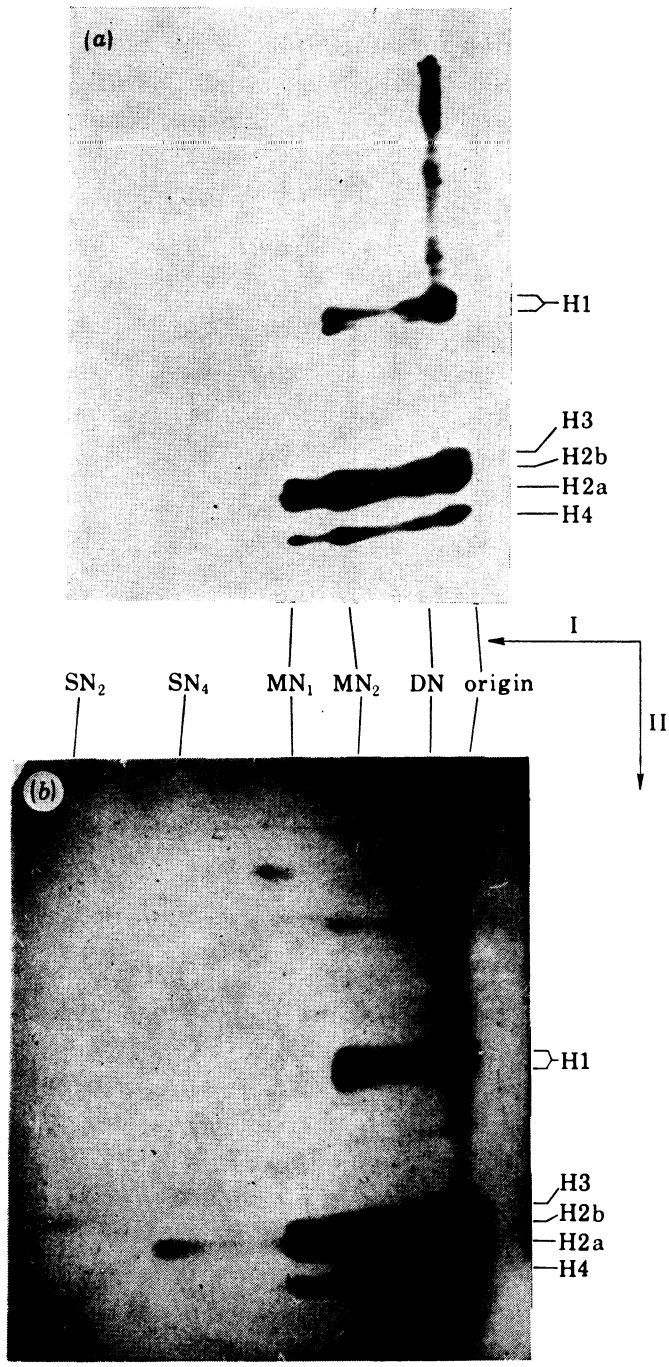

FIGURE 3. Two dimensional electrophoretic analysis of the protein composition of mono- and dinucleosomes (*a*) Total 6% acid-soluble nuclease digest of the chromatin which had been labelled *in vivo* with [^{14}C]amino acids was fractionated by gel electrophoresis at low ionic strength (first dimension; see figure 2) followed by SDS gel electrophoresis in the second dimension in a stacking slab gel. The gel was impregnated with a scintillant (Bonner & Laskey 1974) and the [^{14}C]proteins were detected by fluorography. (*b*) The same as (*a*) but the developed X-ray film was over-exposed upon photography to detect minor nonhistone protein spots.

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does show some minor non-histone protein spots which are apparently associated with at least some of the mono- and dinucleosomes. Work is in progress to characterize in detail these non-histone protein patterns.

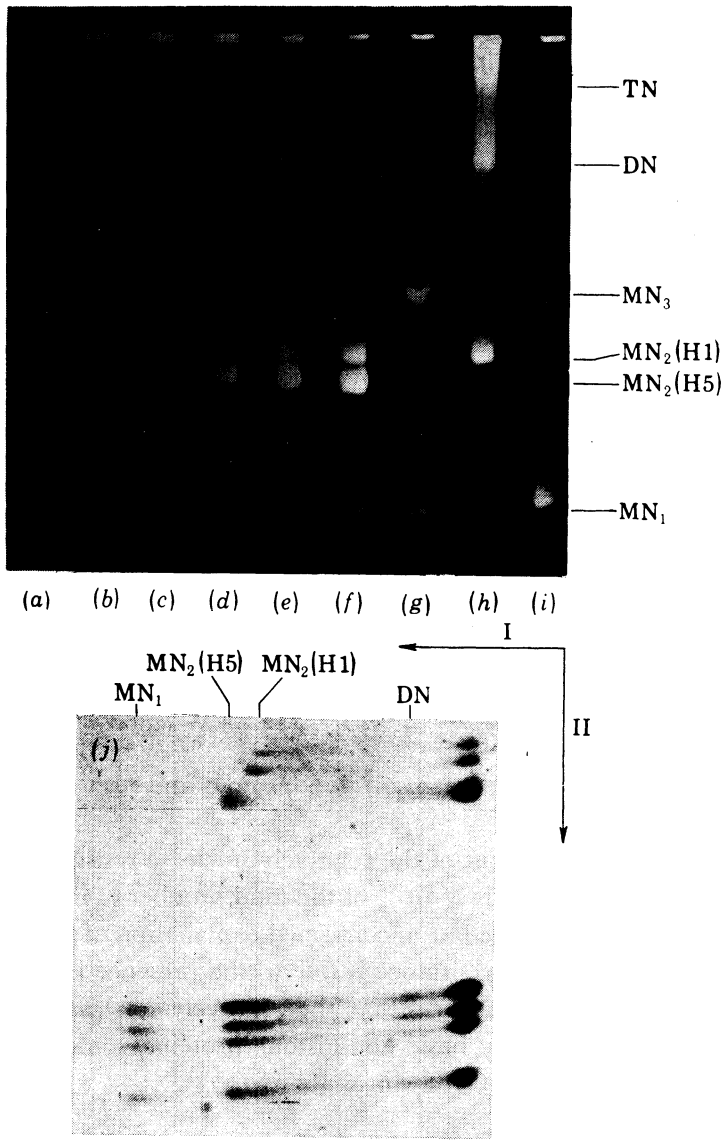

FIGURE 4. Nucleosomal patterns of histone H5-containing hen erythrocyte chromatin. (*a*)–(*g*) Total 1, 2, 3, 4, 6, 9 and 30% acid-soluble staphylococcal nuclease digests of the hen erythrocyte chromatin; electrophoresis of DNP in a 5% gel. (*h*), (*i*) Total 4 and 32% acid-soluble nuclease digests of the mouse Ehrlich tumour chromatin. (*j*) Two dimensional analysis: a total 6% acid-soluble digest (see (*e*)) was fractionated in the first and second dimensions as described in the legend to figure 3 except that the SDS gel was stained with Coomassie. Only the histone-containing part of the gel is shown.

3. MONONUCLEOSOMAL PATTERNS OF HISTONE H5-CONTAINING CHROMATIN

To see whether the nuclease-resistant subset of the MN_3 mononucleosomes is a universal feature of the chromatin structure, we extended the analysis to the transcriptionally inactive hen erythrocyte chromatin in which histone H1 is partially replaced by histone H5 (Shaw

et al. 1976). As can be seen from figure 4, the nucleosomal pattern of the erythrocyte chromatin is similar to that of the mouse Ehrlich tumour chromatin except that the mononucleosomes MN_2 migrate in two bands with close but non-identical mobilities (compare figure 4*a–g* with figure 4*h, i*). Analysis of the histone composition of MN_2 by two dimensional gel electrophoresis (figure 4*j*) shows that the fastest MN_2 band contains histone H5 plus all other histones except H1, whereas the second MN_2 band contains all five histones but no histone H5. Figure 4 clearly shows that the relatively nuclease-resistant subset of the MN_3 mononucleosomes does exist in the nuclease digests of the erythrocyte chromatin (compare figure 4*g* with figure 4*i*). Further descriptions of the composition and properties of the mono- and subnucleosomes will be concerned mainly with the mouse Ehrlich tumour chromatin.

4. Major features of MN_3 mononucleosomes

Summarizing the data presented above about the mononucleosome MN_3 and its relation to mononucleosomes of the two other types, we conclude that:

(i) Nuclease digestion of the chromatin proceeds in such a way that at relatively early stages of digestion the mononucleosome MN_3 is a quite unstable intermediate and is rapidly converted into the 170 base-pair, H1-containing, mononucleosome MN_2. After pausing at the MN_2, the digestion proceeds further to the 140 base-pair mononucleosome MN_1 (Varshavsky *et al.* 1976*a*; Shaw *et al.* 1976; Bakayev *et al.* 1977).

(ii) At the same time a relatively nuclease-resistant subset of the 200 base-pair mononucleosome MN_3 is preferentially accumulated in the course of digestion. At later stages of the digestion the nuclease-resistant subset of MN_3 becomes a major soluble mononucleosome in the digest.

(iii) These MN_3 particles contain all five histones and also non-histone proteins (Bakayev *et al.* 1977).

We estimate that the content of the relatively nuclease-resistant subset of the mononucleosomes MN_3 in the chromatin is 5–10 % of the total number of mononucleosomes (see Bakayev *et al.* 1977 for detail). A simple but unconfirmed explanation of the nuclease resistance of these MN_3 particles is that such a resistance is due to the presence in them of specific non-histone proteins. Preliminary data indicate that the relatively nuclease-resistant MN_3 particles are associated with specific highly basic non-histone proteins, some of which have been isolated by Johns and his colleagues (Goodwin & Johns 1973).

5. Different deoxyribonucleases produce similar DNP particles

Figure 5 shows that the enzymes staphylococcal nuclease, DNase I (pancreatic) and DNase II (spleen) all produce approximately the same mono- and dinucleosomal deoxyribonucleoprotein patterns as revealed by gel electrophoresis at low ionic strength. The differences are confined mainly to the relative quantities of the different DNP particles produced. For example, DNases I and II produce relatively more dinucleosomes DN_1 than does staphylococcal nuclease (figure 5). Furthermore, while the staphylococcal nuclease-produced mononucleosomes MN_1 appear to be relatively stable in the presence of the nuclease (figures 5*a–c*), the DNase II-produced MN_1 are rapidly converted into a subnucleosome-like material (figure 5*f–i*). Finally,

it should be noted that specific cleavages of DNA at multiples of 10 bases that are characteristic for these DNases (Camerini-Otero, Sollner-Webb & Felsenfeld 1976) are also observed under our relatively low ionic strength conditions of digestion (data not shown).

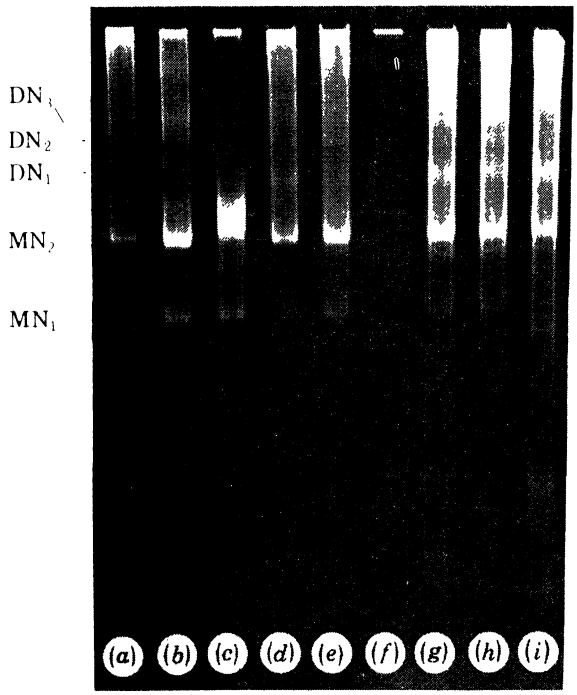

FIGURE 5. Different deoxyribonucleases produce similar DNP particles. Digestion was carried out in 0.1 mM $CaCl_2$, 1 mM triethanolamine–HCl, pH 7.6 (Bakayev *et al.* 1977). Electrophoresis of the DNP in a 5% gel: (*a*)–(*c*) 4, 6 and 10% acid-soluble staphylococcal nuclease digests of the mouse Ehrlich tumour chromatin; (*d*), (*e*) 4 and 7% acid-soluble DNase I digests of the chromatin; (*f*)–(*i*) 1, 3, 5 and 9% acid-soluble DNase II digests of the chromatin.

6. SUBNUCLEOSOMES: HETEROGENEITY AND COMPOSITION

It was found previously that a small but significant proportion of the DNP in a mild nuclease digest of the chromatin sedimented more slowly than mononucleosomes (Bakayev *et al.* 1975; Varshavsky *et al.* 1976*a*). These soluble DNP particles (subnucleosomes) contain undegraded proteins, in contrast to the trypsin-induced subnucleosomal DNP particles in Weintraub's experiments (Weintraub 1975). Figure 6 shows electrophoretic patterns of the subnucleosomes in a 7% polyacrylamide gel of low ionic strength at different extents of nuclease digestion of the chromatin. Subnucleosomes are numbered from the most rapidly migrating species (SN_1) to those migrating slightly faster than the mononucleosomes MN_1. At early stages of the digestion the major discrete subnucleosomes present in the pattern are SN_4 and SN_5 (figure 6*c*). Subnucleosomes SN_6 (apparently H1–DNA complexes; see Bakayev *et al.* 1977) are quite unstable in the presence of nuclease and could be detected only at early stages of the digestion (figure 6*h*). Further digestion leads to an enhancement of the SN_1, SN_4, SN_7 and SN_8 bands and to the appearance of the subnucleosomes SN_2 and SN_3 (figure 6*e*). Subnucleosomes are not resistant to nuclease. Extensive digestion leads at first to the disappearance of SN_6, then of SN_1, SN_4, SN_5, SN_7 and SN_8 and finally to the disappearance of the remaining subnucleosomes SN_2 and SN_3 (figure 6*f*, *g*).

Subnucleosome SN_1 is a set of naked double-stranded DNA fragments *ca.* 20 base pairs long (Bakayev *et al.* 1977). Subnucleosome SN_2 is a complex of a hitherto undescribed specific highly basic non-histone protein and a DNA fragment *ca.* 27 base pairs long (Bakayev *et al.* 1977, and unpublished data). This non-histone protein co-migrates with histone H3 in SDS-containing gels but runs ahead of histone H4 in urea–acetic acid gels (data not shown). Subnucleosome SN_3 contains the above-mentioned non-histone protein, a DNA fragment *ca.* 30 base pairs long and apparently also histone H4. Subnucleosomes SN_7 and SN_8 each contain all histones except H1 and DNA fragments *ca.* 100 and 120 base pairs long, respectively (see Bakayev *et al.* 1977) for additional detail).

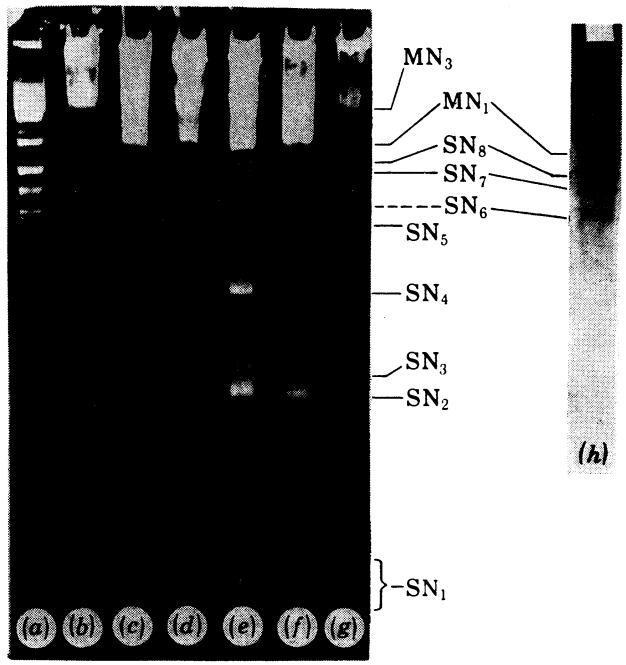

FIGURE 6. Electrophoretic patterns of subnucleosomes (Varshavsky *et al.* 1976*a*; Bakayev *et al.* 1977). A 7% gel was overexposed upon photography to reveal minor subnucleosomal bands. Therefore mono- and dinucleosomes are not seen here as discrete bands. (*a*) Eco RII digest of the SV40 DNA (for comparison of mobilities of defined fragments of naked DNA with mobilities of subnucleosomal DNP particles); (*b*)–(*g*) Total 2, 5, 6, 7, 14 and 30% acid-soluble staphylococcal nuclease digests of the chromatin, respectively; (*h*) Coomassie-stained pattern of the total 3% acid-soluble digest. A dashed line indicates the position of the SN_6 whose relative content in these ethidium-stained digests was too low to be detected at the loadings used.

We have found that the subnucleosomes SN_2–SN_5 are not produced upon nuclease digestion of isolated mono- and dinucleosomes or of isolated SV40 viral minichromosomes. A simple explanation of this result, but not the only possible one, is that the 'specific' origin of these subnucleosomes is due to the presence in them of specific non-histone proteins. Work is in progress to determine whether DNAs isolated from SN_2 and SN_3 constitute a subset of the total DNA nucleotide sequences.

7. Minichromosomes of Simian virus 40: compact and extended forms

We have previously found that isolated SV40 viral minichromosomes contain not only the four 'nucleosomal' histones but also histone H1 (Varshavsky *et al.* 1976*b*). Further analysis of such a 'model' chromatin showed that under 'physiological' salt conditions ($\mu \approx 0.15$) minichromosomes exist in a much more compact conformation than at low ionic strength ($\mu \approx 0.005$). As can be seen from figure 7 the SV40 minichromosomes sediment at *ca.* 60*S* in

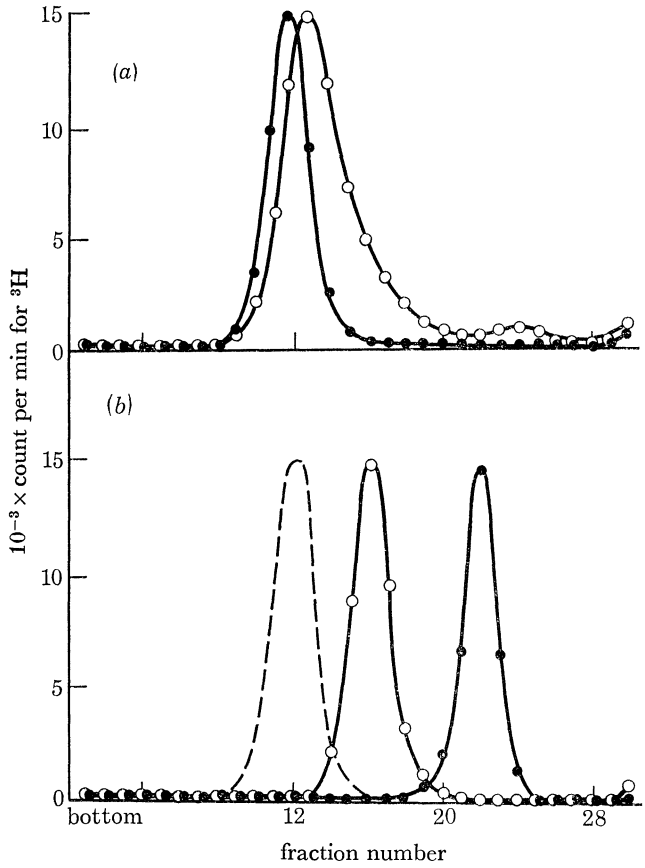

Figure 7. Sedimentational patterns of SV40 minichromosomes. (*a*) ●, Isolated and purified ^{3}H-labelled SV40 minichromosomes (Varshavsky *et al.* 1976*b*) were centrifuged in the SW40 rotor at 37000 rev/min for 2.7 h through a 5–25% sucrose gradient in 0.14 M NaCl, 2 mM Na-EDTA, pH 7.6. ○, The same, but before layering onto the gradient the minichromosome preparation was dialysed overnight against 1 mM Na-EDTA, pH 7.6, followed by addition of an equal volume of 0.28 M NaCl, 3 mM Na-EDTA, pH 7.6. (*b*) ●, Isolated SV40 minichromosomes were dialysed overnight against 1 mM Na-EDTA, pH 7.6, followed by centrifugation through a 5–25% sucrose gradient in 1 mM Na-EDTA, pH 7.6. ○, Isolated SV40 minichromosomes were treated overnight with 1% formaldehyde in the presence of 0.14 M NaCl, 2 mM Na-EDTA, pH 7.6 and were centrifuged through a sucrose gradient of low ionic strength. The dashed line shows a superimposed sedimentational pattern of the SV40 minichromosomes in 0.14 M NaCl.

a high ionic strength buffer ($\mu \approx 0.15$; figure 7*a*), whereas at low ionic strength ($\mu \approx 0.005$) they sediment at *ca.* 30*S* (figure 7*b*). 'Extended' histone H1-containing SV40 minichromosomes undergo condensation as the ionic strength of the solution is increased to *ca.* 0.15 (figure 7*a*). Thus a transition from the compact to the extended minichromosome is apparently a reversible one. However, the sedimentation criterion for condensation is clearly an approximate one

and therefore cannot be considered as evidence for the formation of a true initial compact minichromosomal structure from the extended minichromosome. It may also be noted that the peak of 'reconstructed' compact minichromosomes has a significant 'light' shoulder which is not present in the symmetrical sharp peak of the initial compact minichromosomes (figure 7*a*). We have found that the salt-induced transition from the extended to the compact form of the minichromosomes does not require the integrity of the covalently closed state of the SV40 DNA since the minichromosomes which contained nicked SV40 DNA II were indistinguishable from the intact minichromosomes upon such a transition (data not shown).

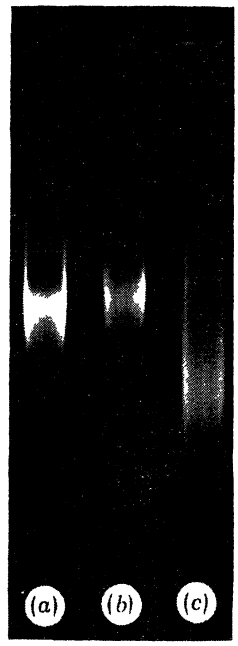

FIGURE 8. Agarose gel electrophoresis of SV40 minichromosomes at low ionic strength. (*a*) Unfixed SV40 minichromosomes dialysed against 1 mM Na-EDTA, pH 7.6. (*b*) The same as (*a*) but the minichromosomes were treated overnight at *ca.* 4 °C with 1% formaldehyde in 1 mM Na-EDTA, pH 7.6, followed by removal of formaldehyde and gel electrophoresis. (*c*) Isolated minichromosomes were treated overnight at *ca.* 4 °C with 1% formaldehyde in 0.14 M NaCl, 2 mM Na-EDTA, pH 7.6, followed by dialysis against 1 mM Na-EDTA, pH 7.6, and gel electrophoresis.

The compact state of the initial SV40 minichromosomes can be fixed with formaldehyde (figure 7*b*). Minichromosomes which were treated with formaldehyde (Varshavsky *et al.* 1976*c*) before lowering the ionic strength of the solution, sediment significantly faster than the extended minichromosomes (figure 7*b*). Similar results can be obtained by agarose gel electrophoresis of the minichromosomes at low ionic strength (figure 8). One can see that the minichromosomes which were treated with formaldehyde before lowering the ionic strength of the solution migrate in the gel much more rapidly than either unfixed minichromosomes or minichromosomes which were fixed at low ionic strength ($\mu \approx 0.005$) (figure 8). The origin of the faint slowly moving band in the preparation of the fixed 'compact' minichromosomes (figure 8*c*) remains unclear.

Our findings are in good agreement with earlier electron microscopic data of Griffith (1975) who observed different structural forms of the SV40 minichromosomes under conditions of low and high ionic strength. Finally, it should be noted that the above-mentioned structural

transitions critically depend upon the presence of histone H1 since the histone H1-depleted minichromosomes existed in the extended form in either high or low ionic strength buffers (data not shown). Work is in progress to determine the mechanism of 'higher order' DNA folding in the compact form of the SV40 minichromosome which appears to be a very useful model for the larger and apparently more complex 'supranucleosomal' chromatin structures.

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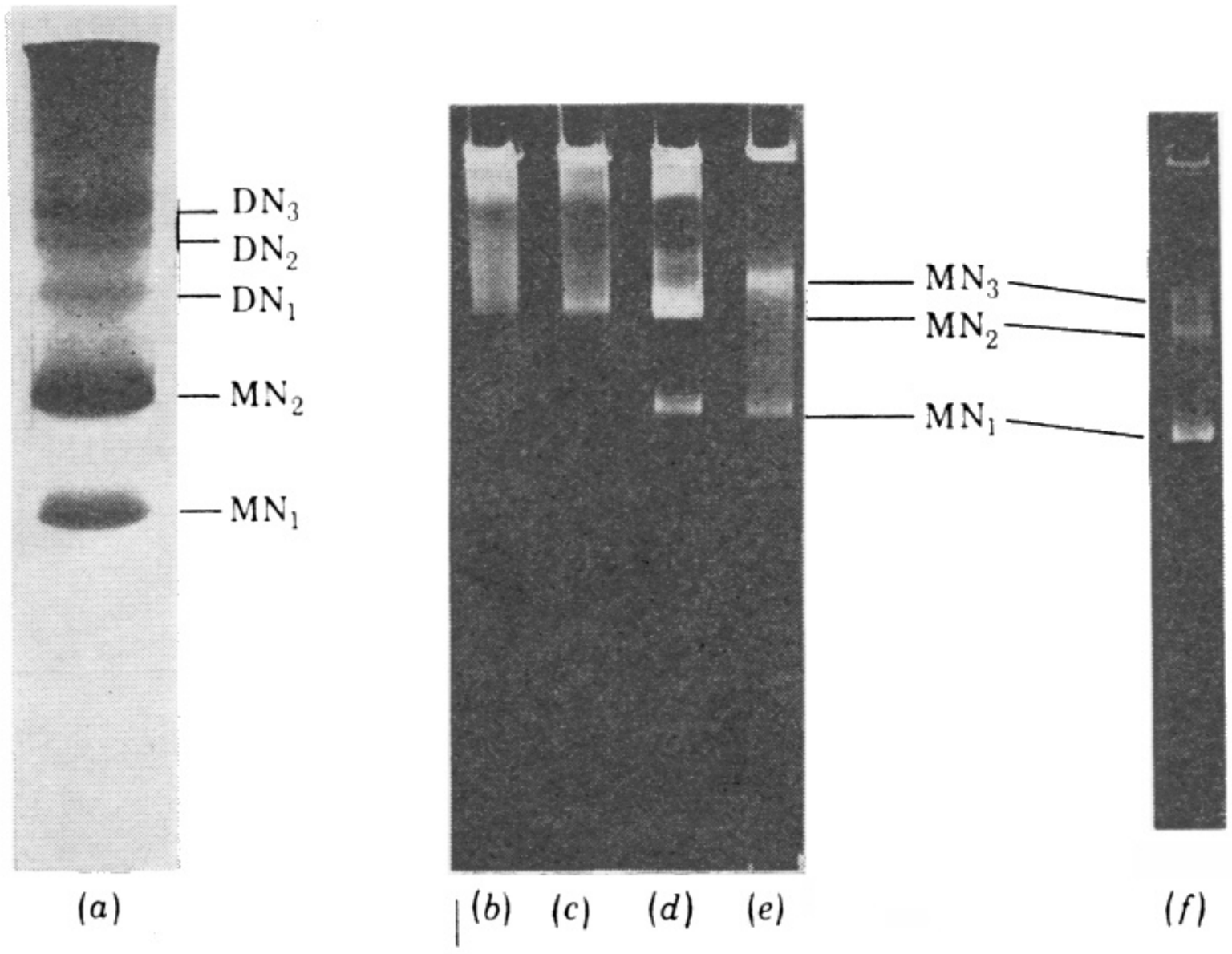

FIGURE 2. Polyacrylamide gel electrophoresis of deoxyribonucleoproteins (Varshavsky *et al.* 1976*a*; Bakayev *et al.* 1977). (*a*) Total 5% acid-soluble nuclease digest of the chromatin; electrophoresis in a 5% gel; stained with Coomassie; (*b*)–(*e*) total 2, 5, 7 and 30% acid-soluble digests, respectively; electrophoresis in a 7% gel; stained with ethidium bromide; (*f*) total 20% acid-soluble digest; electrophoresis in a 6% gel.

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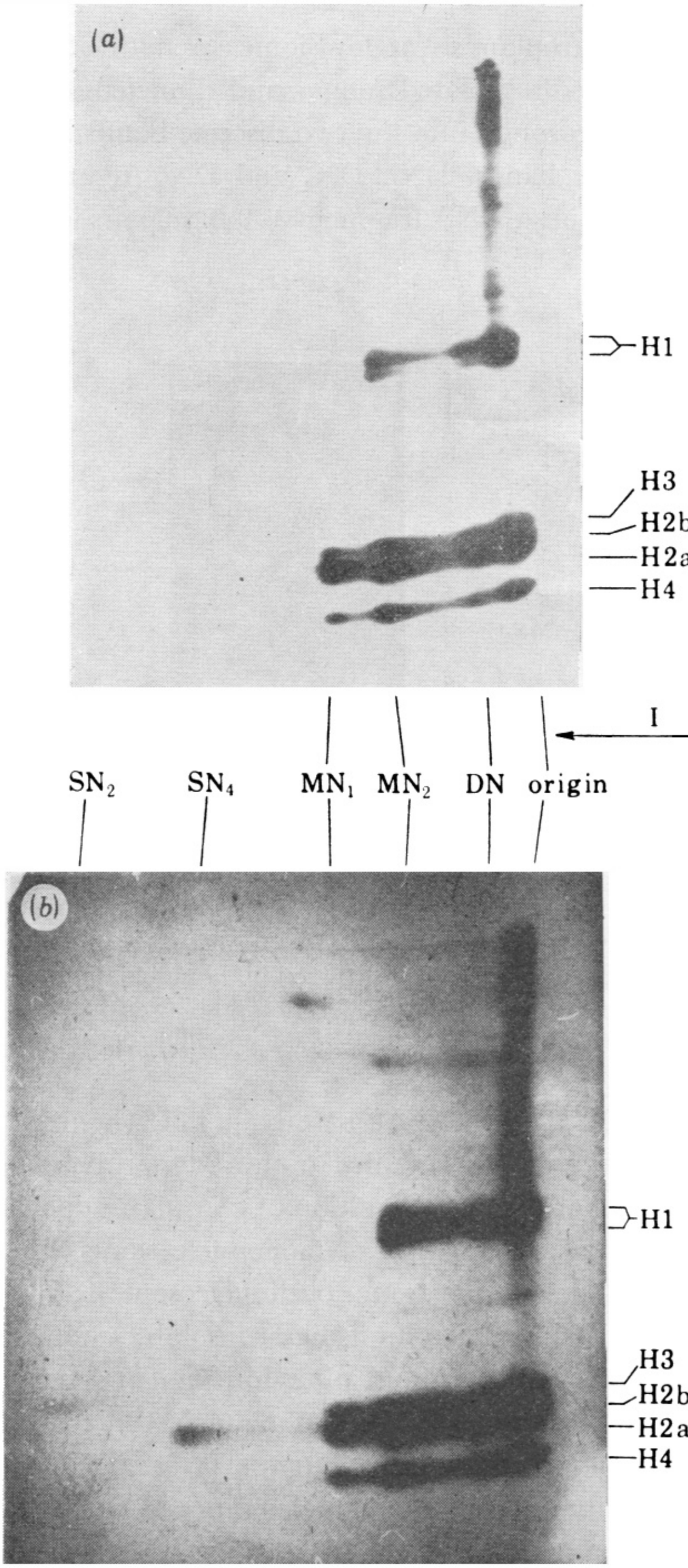

FIGURE 3. Two dimensional electrophoretic analysis of the protein composition of mono- and dinucleosomes (*a*) Total 6% acid-soluble nuclease digest of the chromatin which had been labelled *in vivo* with [^{14}C]amino acids was fractionated by gel electrophoresis at low ionic strength (first dimension; see figure 2) followed by SDS gel electrophoresis in the second dimension in a stacking slab gel. The gel was impregnated with a scintillant (Bonner & Laskey 1974) and the [^{14}C]proteins were detected by fluorography. (*b*) The same as (*a*) but the developed X-ray film was over-exposed upon photography to detect minor nonhistone protein spots.

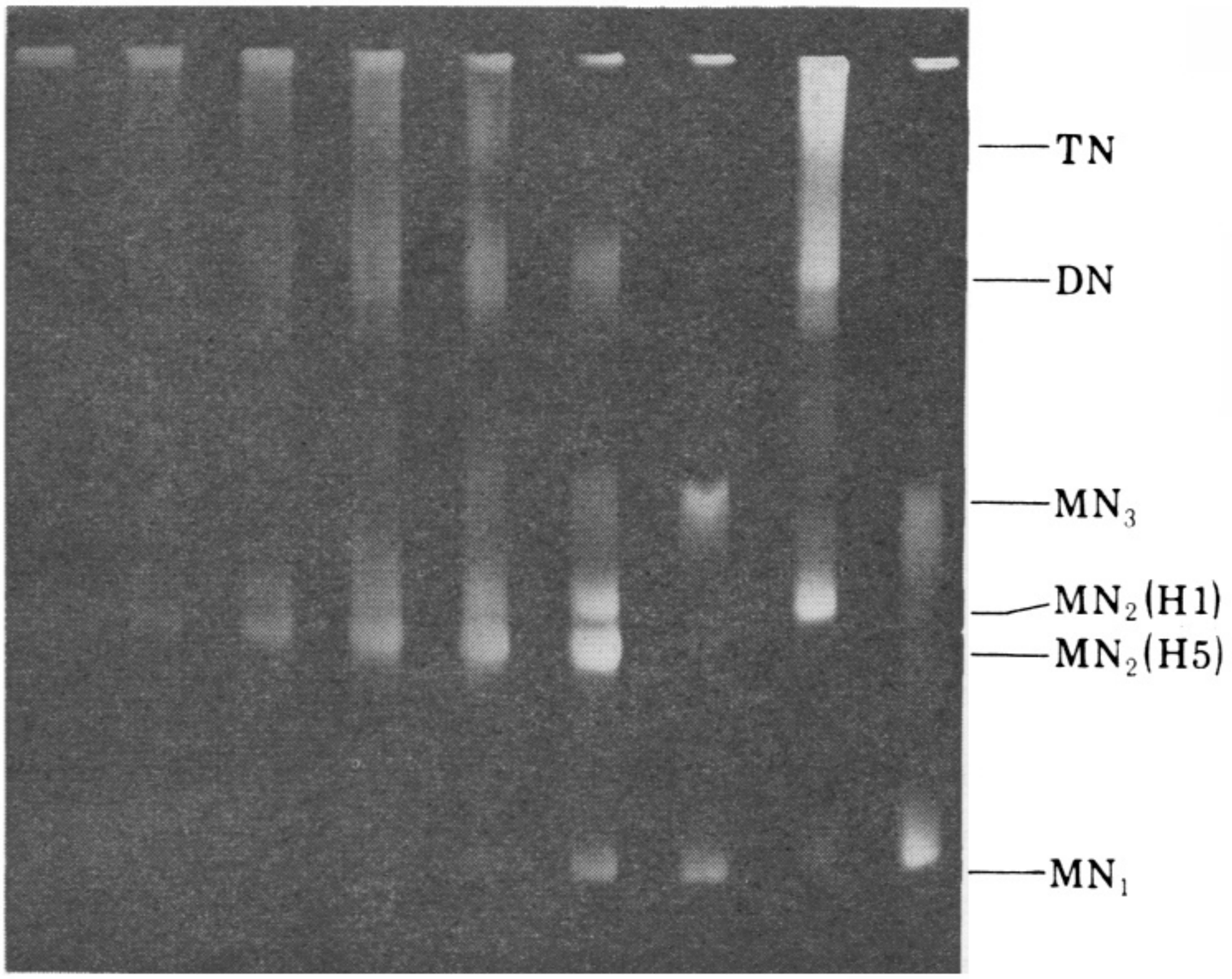

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FIGURE 4. Nucleosomal patterns of histone H5-containing hen erythrocyte chromatin. (*a*)–(*g*) Total 1, 2, 3, 4, 6, 9 and 30% acid-soluble staphylococcal nuclease digests of the hen erythrocyte chromatin; electrophoresis of DNP in a 5% gel. (*h*), (*i*) Total 4 and 32% acid-soluble nuclease digests of the mouse Ehrlich tumour chromatin. (*j*) Two dimensional analysis: a total 6% acid-soluble digest (see (*e*)) was fractionated in the first and second dimensions as described in the legend to figure 3 except that the SDS gel was stained with Coomassie. Only the histone-containing part of the gel is shown.

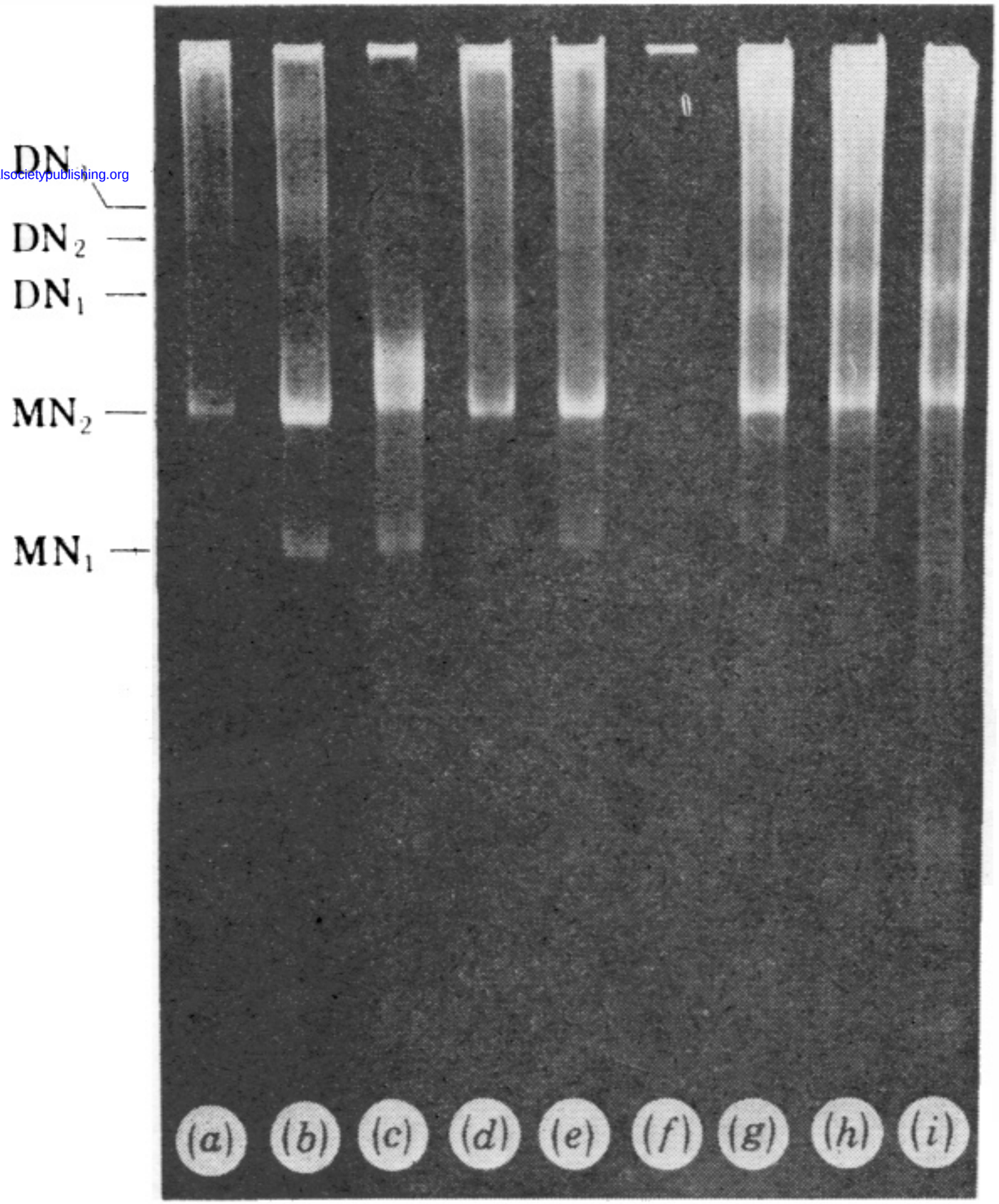

FIGURE 5. Different deoxyribonucleases produce similar DNP particles. Digestion was carried out in 0.1 mM $CaCl_2$, 1 mM triethanolamine–HCl, pH 7.6 (Bakayev *et al.* 1977). Electrophoresis of the DNP in a 5% gel: (*a*)–(*c*) 4, 6 and 10% acid-soluble staphylococcal nuclease digests of the mouse Ehrlich tumour chromatin; (*d*), (*e*) 4 and 7% acid-soluble DNase I digests of the chromatin; (*f*)–(*i*) 1, 3, 5 and 9% acid-soluble DNase II digests of the chromatin.

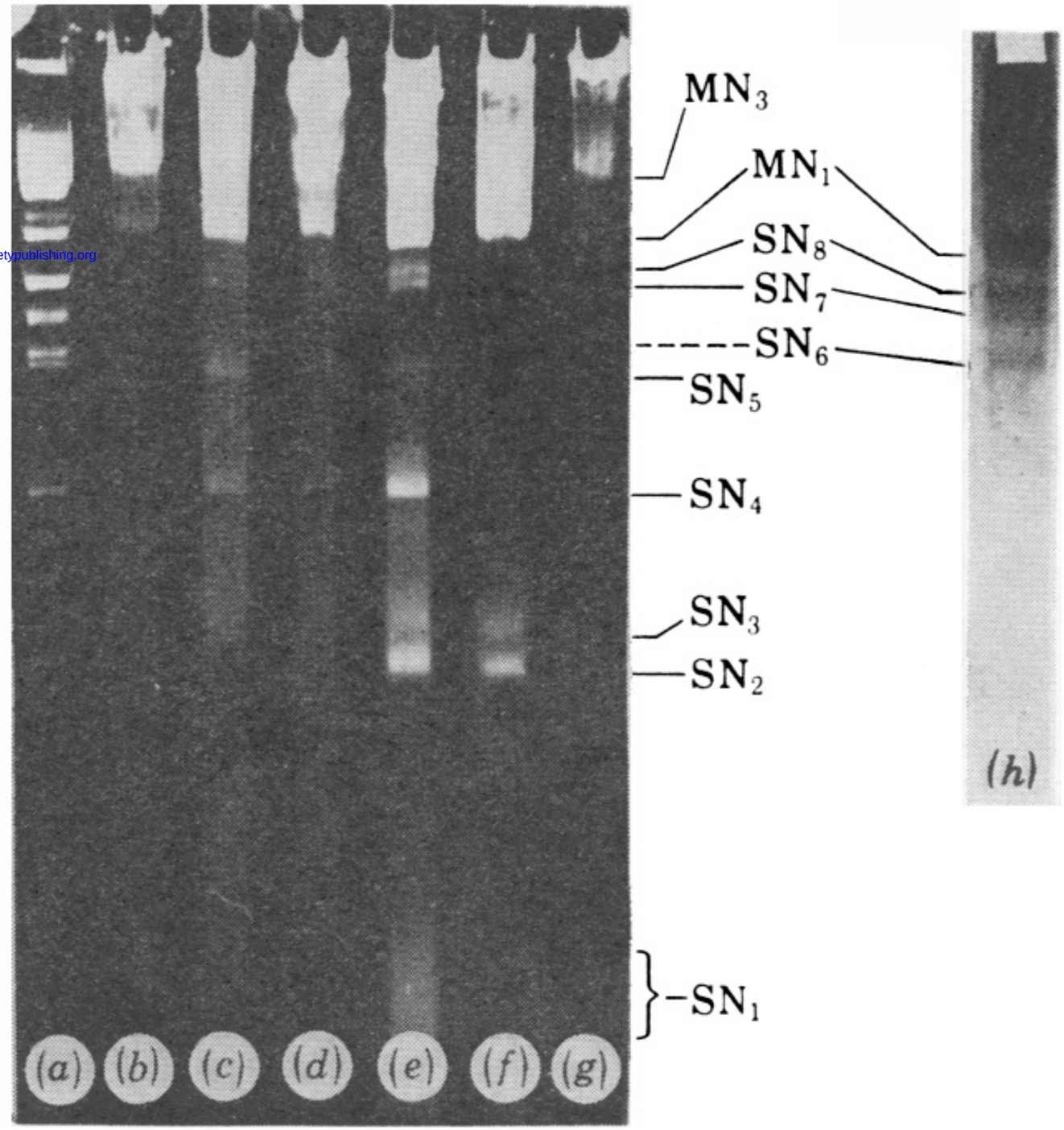

FIGURE 6. Electrophoretic patterns of subnucleosomes (Varshavsky *et al.* 1976*a*; Bakayev *et al.* 1977). A 7% gel was overexposed upon photography to reveal minor subnucleosomal bands. Therefore mono- and dinucleosomes are not seen here as discrete bands. (*a*) Eco RII digest of the SV40 DNA (for comparison of mobilities of defined fragments of naked DNA with mobilities of subnucleosomal DNP particles); (*b*)–(*g*) Total 2, 5, 6, 7, 14 and 30% acid-soluble staphylococcal nuclease digests of the chromatin, respectively; (*h*) Coomassie-stained pattern of the total 3% acid-soluble digest. A dashed line indicates the position of the SN_6 whose relative content in these ethidium-stained digests was too low to be detected at the loadings used.

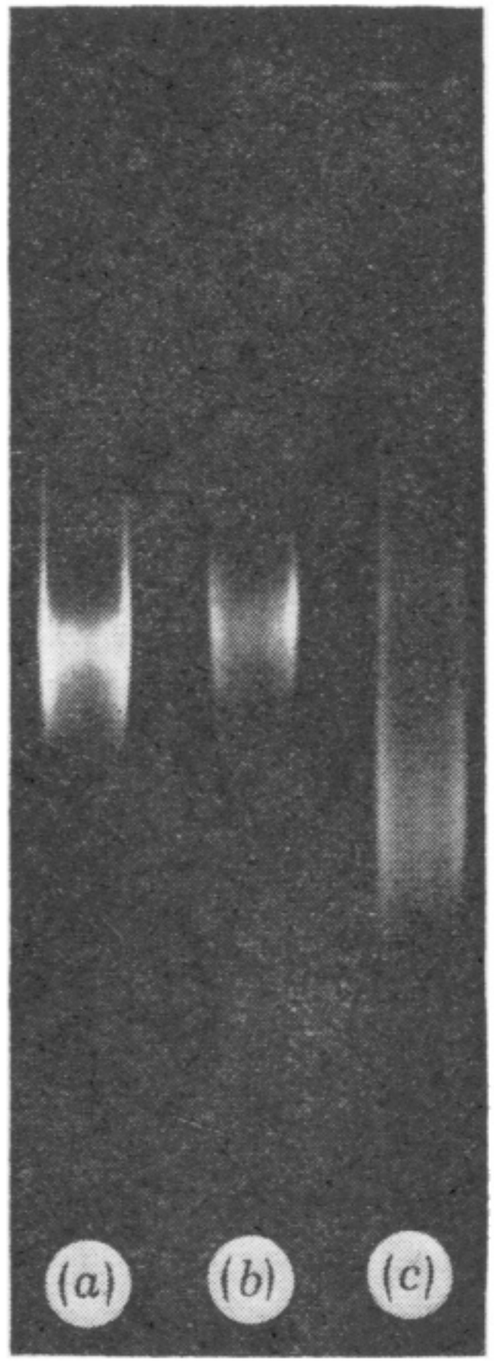

FIGURE 8. Agarose gel electrophoresis of SV40 minichromosomes at low ionic strength. (*a*) Unfixed SV40 minichromosomes dialysed against 1 mM Na-EDTA, pH 7.6. (*b*) The same as (*a*) but the minichromosomes were treated overnight at *ca.* 4 °C with 1% formaldehyde in 1 mM Na-EDTA, pH 7.6, followed by removal of formaldehyde and gel electrophoresis. (*c*) Isolated minichromosomes were treated overnight at *ca.* 4 °C with 1% formaldehyde in 0.14 M NaCl, 2 mM Na-EDTA, pH 7.6, followed by dialysis against 1 mM Na-EDTA, pH 7.6, and gel electrophoresis.