Proc. Natl. Acad. Sci. USA
Vol. 77, No. 6, pp. 3244–3248, June 1980
Biochemistry

High-resolution fractionation of nucleosomes: Minor particles, "whiskers," and separation of mononucleosomes containing and lacking A24 semihistone

(nuclease digestion/nucleoprotein gel electrophoresis/two-dimensional fractionation)

LOUIS LEVINGER AND ALEXANDER VARSHAVSKY

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Communicated by Gary Felsenfeld, March 13, 1980

ABSTRACT **Staphylococcal nuclease digests of HeLa chromatin fractionated on low ionic strength nucleoprotein gels have been further analyzed by second-dimension DNA and protein gel electrophoresis. *In vivo* radioactive labeling of chromatin components and use of longer gels allowed a higher sensitivity and resolution than has been previously reported for this approach. A number of nonhistone protein spots and about 20 DNA spots can be detected in the mononucleosomal region of the second-dimension gel. In particular, there are three DNA spots identical in DNA size that correspond to three discrete kinds of core mononucleosomes resolved on the first-dimension nucleoprotein gel. Analysis of protein composition shows that the most rapidly migrating particle contains all four core histones but no A24 semihistone (A24 is a covalent conjugate of histone H2A and a specific nonhistone protein, ubiquitin), whereas the other two core mononucleosomes contain A24 semihistone. Thus, one can now quantitatively separate the A24-lacking core mononucleosomes from those containing A24, making it possible to directly address the question of whether A24 is associated with nucleosomes containing a specific subset of DNA sequences. Additional features of two-dimensional nucleoprotein-DNA patterns are "whiskers," which run slower than core mononucleosomes in the nucleoprotein dimension and both faster and slower than core-length DNA in the DNA dimension. In more extensive digests, "secondary whiskers" are observed, which run faster than core mononucleosomes in both dimensions and appear to coincide with previously described subnucleosomal particles SN7 and SN8 [Bakayev, V., Bakayeva, T. & Varshavsky, A. (1977) *Cell* 11, 619–629]. Possible mechanisms of whisker formation are discussed.**

The problem of nucleosome heterogeneity has been approached previously in a number of ways (for reviews on the nucleosomal organization of chromatin, see refs. 1–3). One of the most promising methods for fractionation of highly heterogeneous mixtures of mono- and oligonucleosomes in nuclease digests of chromatin is low ionic strength gel electrophoresis of deoxyribonucleoproteins (DNPs) (4–10). Under low ionic strength conditions ($\mu \lesssim 0.01$), DNP particles are stable, and they neither aggregate at the top of the gel nor display any significant affinity for the gel matrix; therefore, resolution comparable to that of DNA or protein electrophoresis can be readily achieved (4–10).

We describe below the results of a systematic attempt to increase the resolving power and sensitivity of this approach by using longer gels for both first-dimension nucleoprotein and second-dimension DNA or protein gel electrophoresis, and fluorographic detection of separated, radioactively labeled DNP components.

From a large number of both DNA and protein spots on second-dimension DNA and protein gels, we will concentrate on those corresponding to mononucleosomes containing A24 semihistone, a Y-shaped covalent conjugate of histone H2A and a specific nonhistone protein, ubiquitin (11–13). About 10% of all H2A molecules in chromatin are modified into A24 by connection through the ε-NH_2 group of lysine-119 to ubiquitin (13). A24 is associated with isolated mononucleosomes as an integral component of the nucleosome histone core, where it replaces histone H2A (14, 15). The relative content of A24 is higher in chromatin fractions enriched *in vitro* for transcriptionally inactive chromatin (16); the nucleolar A24 content decreases upon increased rRNA synthesis (17). Thus A24 may be associated with a specific functionally important subset of nucleosomes. The demonstration below that A24-lacking core mononucleosomes can be separated from A24-containing ones by nucleoprotein gel electrophoresis opens the way to directly address the question of DNA sequence-specific location of A24 in chromatin.

Several other features of two-dimensional DNP-DNA patterns, in particular, specific sets of DNA spots ("whiskers") around major mononucleosomal DNA spots, will be described.

MATERIALS AND METHODS

Cell Culture and *In Vivo* Labeling. Monolayer cultures of HeLa S-3 cells were propagated in Eagle's minimal essential medium with 10% fetal bovine serum (GIBCO). Cells were labeled with [*Me*-^{3}H]thymidine (New England Nuclear, 20 Ci/mmol); 1 Ci = 3.7×10^{10} becquerels) at 10 μCi/ml for 20 hours at about 50% confluency in fresh medium containing dialyzed serum. Specific radioactivities of 1–5 $\times$ 10^5 ^{3}H cpm/μg of DNA were obtained. For protein labeling with [^{3}H]lysine, monolayers at 50% confluency were washed with lysine-free minimal essential medium and then incubated in the lysine-free medium plus dialyzed serum and L-[^{3}H]lysine at 100 μCi/ml (Amersham, 60 Ci/mmol) for 20 hr. Specific radioactivities of 1–3 $\times$ 10^5 ^{3}H cpm/μg of chromatin protein were obtained. For protein labeling with [^{35}S]methionine, the same procedure was used, with methionine-free medium and L-[^{35}S]methionine (New England Nuclear, 400 Ci/mmol) at 200 μCi/ml. Specific radioactivities of 1–2 $\times$ 10^6 ^{35}S cpm/μg of chromatin protein were obtained.

Chromatin Preparation and Nuclease Digestion. Labeled cell monolayers were rinsed with 0.14 M NaCl/5 mM Tris·HCl, pH 7.5 at 4°C, followed by addition of 0.5% Nonidet P40/1 mM $MgCl_2$/5 mM sodium butyrate, 1 mM phenylmethylsulfonyl fluoride (proteinase inhibitor, freshly added from 0.5 M stock in absolute ethanol)/10 mM Tris·HCl, pH 8.0. The lysate was scraped with a rubber policeman and centrifuged at 1000 $\times$ *g* for 5 min. The pellet was resuspended and pelleted once more in the lysis buffer. Nuclei were then washed twice with 0.14 M NaCl/1 mM $MgCl_2$/5 mM sodium butyrate/0.1 mM phenylmethylsulfonyl fluoride/10 mM Tris·HCl, pH 8.0, for a total

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: DNP, deoxyribonucleoprotein; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetate; bp, base pair(s).

time of about 2 hr. The pellet obtained was washed twice with 0.1 mM phenylmethylsulfonyl fluoride/1 mM NaHepes, pH 7.5, and then the chromatin was gently resuspended in the same buffer, using a loosely fitted Dounce homogenizer, to about 400 μg of DNA per ml. Sodium butyrate was present in our buffers to inhibit artefactual histone deacetylation during chromatin isolation (18, 19). Digestions were carried out by sequential additions of staphylococcal nuclease (Sigma) and 50 mM $CaCl_2$ to the chromatin suspension at 0°C to final concentrations of 2 μg/ml and 0.2 mM, respectively. Digestions were carried out at 37°C for up to 30 min. Samples were taken at different times and added to 1/50 vol of 50 mM NaEDTA/25 mM sodium ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetate (EGTA), pH 7.5. Insoluble material was pelleted at 12,000 × *g* for 5 min. Supernatants were used for the next stage either immediately or after storage at −70°C in the presence of 10% (vol/vol) glycerol; freezing did not influence the electrophoretic patterns obtained.

Gel Electrophoresis and Fluorography. Low ionic strength gels for DNP (4–6) contained 5% polyacrylamide (acrylamide-to-bisacrylamide ratio of 30:1.12), 0.5 mM NaEGTA, 1 mM NaEDTA, 10 mM NaHepes at pH 7.5. Pre-electrophoresis was at 150 V for 1 hr. Slab gels 1.2 mm thick and 30 cm long were run at 4°C for about 24 hr at 150 V. Electrode buffer (the same as in the gel) was stirred in both upper and lower compartments and recirculated between the compartments. Second-dimension DNA runs were carried out at room temperature in 9% polyacrylamide gels (acrylamide/bisacrylamide ratio of 30:1.12) with 0.1% $NaDodSO_4$/10 mM sodium acetate/2 mM NaEDTA/80 mM Tris·HCl, pH 8.0, in both gel and electrode buffers. A strip of the first-dimension 5% gel was incubated for 1 hr at 50°C in half-strength electrophoresis buffer plus 1% $NaDodSO_4$ and 0.01% bromophenol (impregnation buffer) and then loaded onto a 9% gel (1.5 mm thick, 20 cm wide, 30 cm long) with an underlay of melted 0.3% agarose in the impregnation buffer without bromophenol. In some experiments, the DNA electrophoretic system described by Derynck and Fiers (20) was used, without a separate stacking gel.

Second-dimension protein runs were carried out either in 15% $NaDodSO_4$/polyacrylamide Laemmli gels (21) as modified by Weintraub *et al.* (22) or in Chalkley's acetic acid/urea/polyacrylamide gels (23) with protamine displacement of DNP components (24) as described by Shaw and Richards (25), with the omission of Triton X-100. Gel dimensions were the same as for DNA electrophoresis. Fluorography of the dried gels was carried out with presensitized Kodak X-Omat R films at −70°C after impregnation of the gels with 2,5-diphenyloxazole dissolved in dimethyl sulfoxide (26).

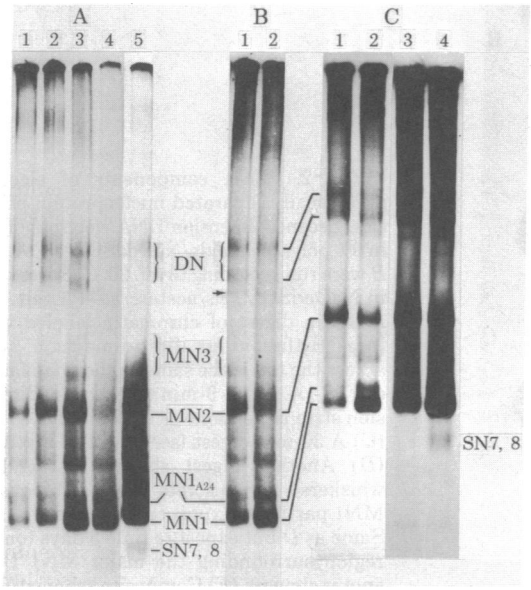

FIG. 1. Low ionic strength gel electrophoresis of nucleoproteins from staphylococcal nuclease digests of HeLa chromatin. Electrophoresis in 30-cm-long 5% polyacrylamide gel. (*A*) [*Me*-^{3}H]Thymidine label; digests were sampled at 1, 3, 9, and 30 min (lanes 1–4); lane 5 is a longer fluorographic exposure of lane 4. The digest shown in lane 4 contained approximately 30% trichloroacetic acid-soluble ^{3}H label. (*B*) [^{3}H]Lysine label; digests were sampled at 3 and 9 min (lanes 1 and 2). (*C*) [^{35}S]Methionine label; digests were sampled at 3 and 9 min (lanes 1 and 2); lanes 3 and 4 are longer fluorographic exposures of lanes 1 and 2, respectively. SN7, 8 indicates specific subnucleosomal particles unresolved in a 5% gel (see refs. 5 and 6); MN1, core mononucleosomes containing 146-base pair (bp) DNA fragment and a full set of core histones but neither histone H1 nor A24 semihistone; $MN1_{A24}$, core mononucleosomes containing A24 semihistone; MN2, discrete mononucleosomal intermediates containing DNA fragments ≈170 bp long; MN3, most slowly migrating species of mononucleosomes (see refs. 5 and 6); DN, dinucleosomes. Arrow points to a protein-containing band (*B, C*) that apparently does not contain DNA, as shown by comparison with [^{3}H]DNA-containing slots (*A*).

RESULTS

Resolution of DNP Particles by Gel Electrophoresis. Low ionic strength polyacrylamide gel electrophoresis resolves mononucleosome-sized DNP particles into several discrete DNP bands (Fig. 1), some of which were identified previously as histone H1-lacking, 146-bp core mononucleosomes (MN1) and histone H1-containing, ≈170-bp mononucleosomes (MN2), (5, 6) (see also refs. 7–10). In addition, longer fluorographic exposures of either DNA-labeled (Fig. 1*A*) or protein-labeled (Fig. 1 *B* and *C*) DNP patterns reveal a smear of minor unresolved DNP particles in staphylococcal nuclease digests of HeLa chromatin. Use of longer gels (about 30 cm) and of fluorographic methods of detection allowed us to clearly resolve some additional discrete bands in the first-dimension nucleoprotein gels. One of these bands migrates just behind the major MN1 band and, as will be shown below, corresponds to a discrete subspecies of the MN1 core mononucleosome that contains A24 semihistone. On the other hand, the band migrating just ahead of dinucleosomes in Fig. 1 *B* and *C* does not have a DNA "counterpart" in the DNA-labeled DNP patterns, even in strongly overexposed fluorograms (Fig. 1*A*). Thus, most, but not all, of the bands seen in the protein-labeled DNP patterns correspond to DNP particles.

A band appears strongly in the first-dimension (nucleoprotein) gels halfway between MN1 and MN2 (Fig. 1). This band is also seen on highly exposed fluorograms (not shown) of second-dimension protein gels as a buildup in intensity of the pattern in the corresponding region of the gel. However, no specific proteins have been detected so far in association with this band; its origin remains unclear (see also below). Although a majority of DNP bands are present in both lightly and extensively nuclease-digested HeLa chromatin, their relative intensities depend on the extent of digestion. Discrete species of mononucleosomes larger than MN2, which were observed in mouse chromatin and called mononucleosomes MN3 (5, 6), are also observed in nuclease digests of HeLa chromatin (e.g., Fig. 1*A*, lane 3).

Discrete Size Heterogeneity in Mononucleosomal DNA: Presence of "Whiskers." Second-dimension DNA gel electrophoresis shows that mononucleosomes are a considerably more heterogeneous set of particles than can be revealed by first-dimension DNP electrophoresis or by two-dimensional analysis of unlabeled mononucleosomes (6, 9) (Fig. 2; cf. Fig. 1). Because of large differences between intensities of different DNA spots present in any given fluorogram (Fig. 2), a series of different fluorographic exposures of two-dimensional patterns

shown in Fig. 2 *A–E* was used to make the schematic display shown in Fig. 2*F*. It should be emphasized that although each particular spot depicted in Fig. 2*F* was seen in fluorographic exposures of the two-dimensional DNP-DNA patterns (Fig. 2 and data not shown), the relative intensities of some of the spots (in particular, of MN1) in Fig. 2*F* were greatly reduced to make the diagrammatic representation possible. Thus, at least 20 discrete DNA spots with widely different intensities in addition to a relatively weak unresolved DNA smear are present in the mononucleosomal regions of two-dimensional DNP-DNA electrophoretograms. Running in the neighborhood of the MN1 spot are the following three distinct DNA patterns:

(*i*) A DNA spot as sharp as MN1, comigrating with MN1 in the DNA dimension but running slightly behind MN1 in the first (DNP) dimension (Fig. 2 *A–C*, *E*, and *F*), which corresponds to a subspecies of MN1 containing A24 semihistone ($MN1_{A24}$; see below).

(*ii*) Directly below MN1 are two DNA spots (I and II in Fig. 2*F*) about 90 and 55 bp long, respectively (Fig. 2 *D* and *F* and Table 1). Their sizes add up to that of MN1, and they comigrate with MN1 in the first (DNP) dimension. Thus they probably correspond to a subfraction of MN1 core mononucleosomes with a specific internal cut that is not revealed at the level of DNP structure before addition of $NaDodSO_4$ and second-dimension DNA electrophoresis (cf. ref. 28).

(*iii*) A pattern of DNA spots we call "whiskers," which run slower than MN1 in the DNP dimension and both slower and faster than MN1 DNA in the second (DNA) dimension (Fig. 2*F*; see Fig. 2 *A–E* for different views of whiskers). There are four distinguishable DNA spots (2, 2^-, 1, and 1^-; Fig. 2*F*), which form a V-shaped pattern with the MN1 DNA spot at the apex. Another whisker pattern ("secondary whiskers") shows up in more extensive digestions (Fig. 2*D*); it runs ahead of MN1 in both dimensions (spots 2′, $2'^-$, 1′, $1'^-$, and MN1′ in Fig. 2*F*). The DNP particles corresponding to DNA secondary whiskers migrate as a diffuse DNP band in the first-dimension (DNP) gel just below MN1 (Fig. 1*A*, lane 5, and Fig. 1*C*, lane 4). This subnucleosomal DNP band can be resolved into at least two bands in 7% polyacrylamide gels (5, 6) and was previously designated SN7, 8. The SN7 and SN8 particles contain core histones, as shown by second-dimension $NaDodSO_4$ gel electrophoresis of DNP proteins (Fig. 3). A third whisker-type DNA pattern is associated with $MN1_{A24}$ DNA spots (Fig. 2*F*), as revealed by overexposures of two-dimensional DNP-DNA fluorograms (Fig. 2*B*), suggesting that the ubiquitin moiety of A24 is located distinct from the ends of mononucleosome DNA (see ref. 29).

Features of DNP Species That Run Slower Than MN1. The central band between MN1 and MN2 in Fig. 1 is not resolved into a discrete single band on second-dimension DNA gels; rather, it appears in various cases as the crossover point of intensity between a line of core length DNA (146 bp) and mini-

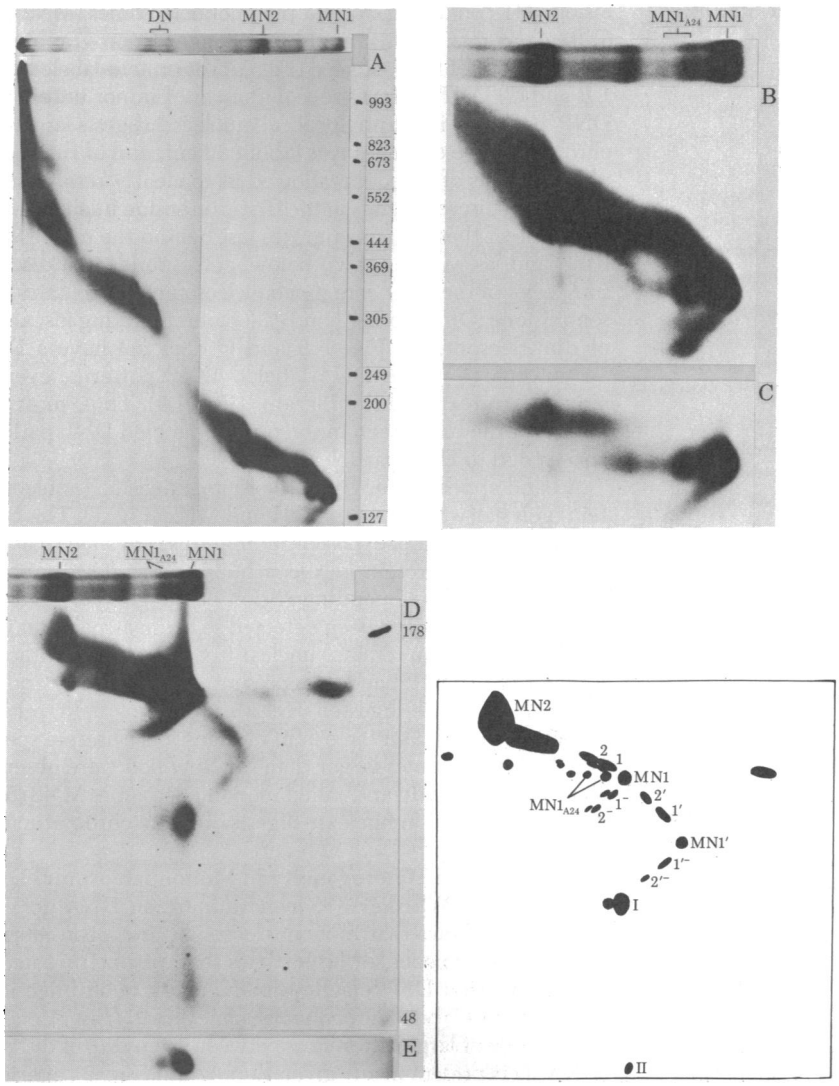

FIG. 2. DNA components of electrophoretically separated nucleoprotein particles. Second-dimension DNA electrophoresis in 9% polyacrylamide/$NaDodSO_4$ gels. *A* and *B* were run according to ref. 20; *C–E* were run in $NaDodSO_4$/Tris/acetate/EDTA gels. (*A*) A 3-min digest of chromatin labeled with [*Me*-^{3}H]thymidine; first-dimension strip across the top is the same as shown in lane 2 of Fig. 1*A*. (*B*) A 9-min digest; first-dimension strip is the same as in lane 3 of Fig. 1*A*. (*C*) A 30-min digest (see lane 4 of Fig. 1*A*). (*D*) Another digest showing "secondary whiskers" and discrete internal breaks in MN1 particles (exposure time 30 days). (*E*) Same as *D* but exposure time 3 days (only a region surrounding the major MN1 DNA spot is shown). (*F*) Composite schematic diagram of mononucleosomal DNA spots detected in *A*, *B–E*, and other DNP-DNA patterns not shown, at different times of fluorographic exposure. Relative intensities of spots are necessarily not to scale. In particular, the MN1 spot is strongly underrepresented. *Bst*NI and *Hae* III digests of simian virus 40 viral [^{3}H]DNA (27) are molecular weight markers shown on the right of *A* and *D*, expressed as bp. Spots in *F* designated 2, 2^- and 1, 1^- correspond to related pairs of DNA fragments forming primary "whiskers." Spots 2′, $2'^-$, 1′, $1'^-$, and MN1′ correspond to secondary whiskers. I and II are sub-MN1 fragments, which presumably result from an internal cleavage in the MN1 particles. Other designations are as in Fig. 1.

mum MN2-length DNA (166 bp). The appearance of two "shelves" of intensity is observed in extensive digestions (Fig. 2C), in which the top half of whiskers has disappeared. The MN2 DNA displays a genuine heterogeneity in size, from ≈186 to 166 bp. A sharp minor spot of slightly over core DNA length runs just ahead of MN2 in the first (DNP) dimension (Fig. 2 A–D and F). On long exposures this spot is seen to contain whiskers around it (not shown). MN3 is resolved into two bands in Fig. 1; the faster-running band has the same heterogeneity and range of DNA sizes as MN2, whereas the slower of the two bands is sharp, with a DNA size of 166 bp (not shown). The above verbal descriptions and the schematic diagram (Fig. 2F) are obvious oversimplifications of the fine detail of the patterns in Fig. 2.

Mononucleosomes Containing A24 Semihistone Can Be Separated from A24-Lacking Mononucleosomes. Analysis of protein components of electrophoretically separated DNP particles by second-dimension $NaDodSO_4$ or acetic acid/urea gel electrophoresis (Fig. 3) clearly shows that the minor 146-bp DNA spot ($MN1_{A24}$) migrating just behind the major 146-bp DNA spot (MN1) in the two-dimensional DNP-DNA displays (Fig. 2) corresponds to a subset of the core MN1 monocleosomes that contain A24 semihistone. The identity of A24 was confirmed by comparison of its relative mobilities in two different electrophoretic systems (Fig. 3) as described in detail elsewhere (13–15, 29).

Is A24 substituting for one or both H2A molecules in $MN1_{A24}$? Relative intensities of protein spots falling on the $MN1_{A24}$ and MN1 vertical lines in Fig. 3 were estimated by densitometry of appropriately exposed fluorograms, using presensitized X-ray films (26). Any quantitation of the patterns shown in Fig. 3 should give an underestimate of the content of A24 because of the presence of whiskers (see above). Whisker DNA running above and below $MN1_{A24}$ DNA presumably corresponds to particles containing no A24 and falls on the same vertical line as $MN1_{A24}$ in two-dimensional DNP-DNA patterns (Fig. 2). However, $MN1_{A24}$ core mononucleosomes may be more than 90% pure after the *first-dimension* DNP run, providing they are products of a relatively extensive digestion (see Fig. 2C), because whiskers are digested away more rapidly than core mononucleosomes (Fig. 2). Densitometric measurements show that the amount of H2A relative to H4 is 30–33% lower for $MN1_{A24}$ than for MN1 in a lighter exposure of the fluorogram presented in Fig. 3B. The less than 50% reduction in the apparent content of H2A in $MN1_{A24}$ is consistent with the presence of whisker DNP particles in the same region of the gel (see above). The fact that the H2A-to-H4 ratio in $MN1_{A24}$ comes down significantly toward 50% of the ratio in MN1 suggests that the principal $MN1_{A24}$ spot (Fig. 3) corresponds to MN1 particles monosubstituted with A24. The same conclusion was reached when relative intensities of A24 and H4 spots in $MN1_{A24}$ were compared. H4 and A24 each contain a single methionine residue (3, 13). When the contribution of methionine in an H2A variant (30) to the total methionine content of A24 is taken into account, the estimated molar ratio of A24 to H4 in $MN1_{A24}$ falls into the range 0.3–0.5, compatible with monosubstitution. A faint spot in the second-dimension DNA gels is observed (Fig. 2F), which corresponds to a very faint A24 spot in the second-dimension protein gels (Fig. 3 and unpublished data). The second $MN1_{A24}$ DNA spot has migrated slower in the first (DNP) dimension than the major $MN1_{A24}$ and thus may correspond to a minor proportion of MN1 core mononucleosomes disubstituted with A24 in HeLa chromatin.

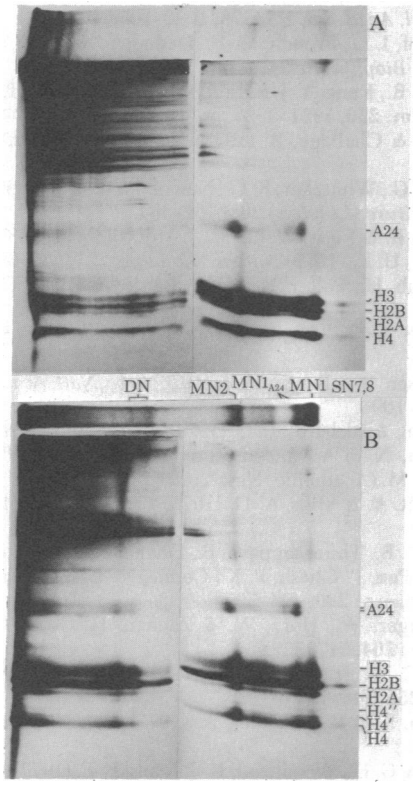

FIG. 3. Protein components of electrophoretically separated nucleoprotein particles. Second-dimension polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled nucleoproteins in $NaDodSO_4$ gels (*A*) or in acetic acid/urea gels (*B*). Each first-dimension strip (shown in the middle) was cut into two and put onto two separate second-dimension gels. Fluorographic exposure was 15 days. H4′ and H4″ correspond to mono- and diacetylated forms of histone H4, respectively.

A24 is also associated with a specific subset of the MN2 mononucleosomes (apparently precursors to $MN1_{A24}$) that migrate just behind the bulk MN2 mononucleosome band in the first (DNP) dimension (Fig. 3). We could not detect A24 in association with any of the subnucleosomal DNP spots, in particular, secondary whiskers (see above). Finally, Fig. 3B shows that $MN1_{A24}$ contains acetylated species of histone H4 in approximately the same proportions as does A24-lacking MN1. Thus A24 substitution in the nucleosomes does not seem to correlate with histone H4 acetylation, at least at the present level of resolution of DNP fractionation techniques.

Histone H1 is completely absent from the [^{35}S]methionine fluorograms (Fig. 3), and histone H2A is strongly underrepresented, which correlates with the known absence of methionine from human H1 and with the presence of methionine in only a minor subfraction of human H2A histone (3, 30). The continuous lines of proteins with apparent molecular weight of about 40,000 end abruptly in the MN3 region of the second-dimension protein gels (Fig. 3), suggesting that these proteins are components of a specific subset of the MN3 mononucleosomes. Finally, because methionine is strongly underrepresented in high mobility group (HMG) nonhistone proteins (3, 31, 32), their detection in the MN3-type mononucleosomes, in particular, (refs. 5, 6, and 10 and unpublished data) requires labeling with [^{3}H]lysine (Fig. 1B) or mixtures of amino acids.

DISCUSSION

Are A24-Containing Nucleosomes Associated with a DNA Sequence-Specific Subset of the Genome? The demonstration that the A24-lacking core mononucleosomes ($MN1_{A24}$) can be separated from the A24-containing core mononucleosomes (see Figs. 2 and 3) opens the way to directly address the question stated above. For example, one can now transfer DNA from the gel shown in Fig. 2C to a DNA-binding paper (33) by either the

Table 1. Sizes of DNA fragments in two-dimensional DNP-DNA displays

DNA spot*	DNA fragment size, bp†
MN2	186–166
Discrete spot under MN2	151
Primary whiskers: 2	165
1	159
1^-	133
2^-	128
MN1	146
Secondary whiskers: 2′	137
1′	128
$1'^-$	107
$2'^-$	100
MN1′	117
Spots I	94
Spot II	51

* See Fig. 2F for designations of DNA spots.

† DNA sizing was carried out by using patterns shown in Fig. 2 *A* and *D* that include *Hae* III-digested simian virus 40 DNA markers down to 48 bp. The diameter of spots, nature of the markers, and potentially staggered DNA ends limited the absolute accuracy to approximately ±3 bp.

method of Reiser *et al.* (34) or by electrophoretic transfer (unpublished data) and then hybridize the transferred DNA with a variety of specific radioactive DNA probes (35).

On the Mechanism of Whisker Formation. Whiskers (see above) are observed around both MN1 and $MN1_{A24}$ DNA spots (Fig. 2*F*) and also around the 117-bp DNA spot corresponding to the subnucleosomes SN7 and SN8 (see Figs. 1*A* and 2*F* and Table 1). The first two sets of whiskers are similar in size, shape, and segmentation into five spots forming V-shaped patterns, with MN1 and $MN1_{A24}$ DNA spots at the apexes (see *Results*). An interesting feature of the whisker patterns is that DNP particles containing presumably one and the same protein sets but DNA fragments of different lengths comigrate in the first-dimension (nucleoprotein) gel. One possible phenomenological explanation of these patterns is that each lower member of a pair of whisker DNA spots (Fig. 2*F*) is derived from an upper member by a specific DNA cut that remains hidden at the DNP level and is revealed only upon second-dimension DNA electrophoresis. The lengths of DNA fragments composing whisker sets show that the "external" (2, 1) and "internal" (2^-, 1^-) cuts are indeed symmetrical about 146 bp (Table 1). This argument, however, is indirect and further analysis is required to verify the above explanation. Although it is likely that the primary whisker set (2, 1, 2^-, 1^-, and MN1) is a precursor to the secondary set of whiskers (2′, 1′, $2'^-$, $1'^-$, and MN1′) (see Fig. 2*F*), at the present time there is no direct evidence to support this suggestion. Therefore, the designation MN1′ in the set of secondary whiskers (Fig. 2*F*), implying a precursor–product relationship between MN1 and MN1′, remains an arbitrary one. Finally, the above phenomenological interpretation does not explain the striking V-shaped pattern of whisker DNA spots, that is, a progressive decrease of DNA sizes in the upper branch of the V accompanied by a matching increase of DNA sizes in the lower branch of the V (Fig. 2*F* and Table 1). So far we have been unable to arrive at any simple explanation of the V-shaped whisker patterns compatible with our present understanding of nucleosome structure.

It is unknown whether whiskers contain a specific subset of genomic DNA sequences or whether they constitute an alternative pathway of nuclease-induced conversion to core mononucleosomes or subnucleosomes for almost any oligonucleosomal precursor structure. The question can be answered by a hybridization approach.

We thank Frederick Boyce and Bruce Yazejian for their collaboration at early stages of this work and Jim Barsoum for helpful discussions. These studies were supported by grants to A.V. from the National Institutes of Health (GM26086) and the Whittaker Health Sciences Fund. L.L. is supported by a National Institutes of Health postdoctoral fellowship (AI 5937).

1. Kornberg, R. D. (1977) *Annu. Rev. Biochem.* **46,** 931–955.
2. Felsenfeld, G. (1978) *Nature (London)* **271,** 115–121.
3. Isenberg, I. (1979) *Annu. Rev. Biochem.* **48,** 159–191.
4. Varshavsky, A., Bakayev, V. V. & Georgiev, G. P. (1976) *Nucleic Acids Res.* **3,** 477–489.
5. Bakayev, V. V., Bakayeva, T. G. & Varshavsky, A. (1977) *Cell* **11,** 619–629.
6. Varshavsky, A., Bakayev, V. V., Nedospasov, S. A. & Georgiev, G. P. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 457–472.
7. Todd, R. D. & Garrard, W. T. (1977) *J. Biol. Chem.* **252,** 4729–4738.
8. Bafus, N. L., Albright, S. C., Todd, R. D. & Garrard, W. T. (1978) *J. Biol. Chem.* **253,** 2568–2574.
9. Todd, R. D. & Garrard, W. T. (1979) *J. Biol. Chem.* **254,** 3074–3083.
10. Bakayev, V. V., Bakayeva, T. G., Shmatchenko, V. V. & Georgiev, G. P. (1978) *Eur. J. Biochem.* **91,** 291–301.
11. Goldstein, G., Scheid, M. S., Kammerling, V., Boyse, E. A., Schlesinger, D. H. & Niall, H. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 11–15.
12. Olson, M. O. J., Goldknopf, I. A., Gnetzow, R. A., James, G. T., Hawkins, T. C., May-Rottberg, G. J. & Busch, H. (1976) *J. Biol. Chem.* **251,** 5901–5903.
13. Goldknopf, I. A., French, M. F., Musso, R. & Busch, H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5492–5495.
14. Bonner, W. M. & Stedman, J. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2190–2194.
15. Martinson, H. G., True, R., Burch, J. B. E. & Kunkel, G. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1030–1034.
16. Goldknopf, I. L., French, M. F., Daskal, Y. & Busch, H. (1978) *Biochem. Biophys. Res. Commun.* **84,** 786–793.
17. Ballal, N. R., Kang, Y. J., Olson, M. O. J. & Busch, H. (1975) *J. Biol. Chem.* **250,** 5921–5925.
18. Sealy, L. & Chalkley, R. (1978) *Nucleic Acids Res.* **5,** 1863–1876.
19. Riggs, M. G., Whittaker, R. G., Newmann, J. R. & Ingram, V. M. (1977) *Nature (London)* **268,** 462–464.
20. Derynck, R. & Fiers, W. (1977) *J. Mol. Biol.* **110,** 387–404.
21. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
22. Weintraub, H., Palter, K. & Van Lente, F. (1975) *Cell* **6,** 85–110.
23. Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130,** 337–346.
24. Schafhausen, B. & Benjamin, T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1092–1096.
25. Shaw, B. R. & Richards, R. G. (1979) in *Chromatin Structure and Function,* Nato Advanced Study Institute Series, Part A, ed. Nicolini, M. (Academic, New York), pp. 125–135.
26. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
27. Reddy, V. B., Thimmappaya, B., Dhar, R., Subramanian, K. N., Zain, S., Pan, J., Ghosh, P. K., Celma, M. L. & Weisman, S. M. (1978) *Science* **200,** 494–502.
28. Atlenburger, W., Hörz, W. & Zachau, H. G. (1976) *Nature (London)* **264,** 517–522.
29. Carter, C. W., Jr., Levinger, L. F. & Birinyi, F. (1980) *J. Biol. Chem.* **255,** 748–754.
30. Franklin, S. G. & Zweidler, A. (1977) *Nature (London)* **266,** 273–275.
31. Goodwin, G. H., Woodhead, L. & Johns, E. F. (1977) *FEBS Lett.* **73,** 85–89.
32. Watson, D. C., Levy W., B. & Dixon, G. H. (1978) *Nature (London)* **276,** 196–198.
33. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5350–5354.
34. Reiser, J., Renart, J. & Stark, G. R. (1978) *Biochem. Biophys. Res. Commun.* **85,** 1104–1112.
35. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.