Organizational changes in chromatin at different malignant stages of Friend erythroleukemia

Kathleen E. Leonardson and Stuart B. Levy

Cancer Research Center and Department of Medicine, Tufts University School of Medicine and
Tufts-New England Medical Center, Boston, MA 02111, USA

Received 11 August 1980

ABSTRACT

The chromatin structure of morphologically-similar, but increasingly-malignant erythroleukemia cells was investigated using mild micrococcal nuclease digestion of isolated nuclei. The maximum solubilization of chromatin was unique for each of the three cell types: the least malignant (our Stage II) released 61% of its chromatin DNA, the most malignant (Stage IV), 46%, and the intermediate (Stage III) released 36%. An analysis of the nucleosome oligomers liberated by digestion also demonstrated differences. After 15 minutes of digestion when release was reaching its maximum, a greater proportion of large nucleosomal oligomers (sizes > trinucleosome) was released from Stage II nuclei than from Stage III or IV nuclei. The cell types also differed in the relative amount of H1-depleted mononucleosomes released.

Analysis of the size of the double-stranded DNA associated with mononucleosomal particles showed that Stage III mononucleosomes were smaller (148 bp) than Stage IV (167 bp) or Stage II (190 bp). In addition, while the DNA of mononucleosomes depleted in H1 was smaller than that in the H1-containing species, relative size differences among the different cell types were retained. These data suggested that the difference in the mononucleosome particle size resistant to nuclease digestion was independent of histone H1. Differences in nucleosome repeat length were also noted among the cell types. These studies have demonstrated dramatic differences in chromatin structure associated with malignant potential of an otherwise morphologically identical cell type. These findings may reflect changes in the relative amounts of H2a variants which we have previously described among the different malignant cell types.

INTRODUCTION

Chromatin in eukaryotic organisms is organized into discrete units, termed nucleosomes, comprised of approximately 200 base pairs of DNA coiled around an octomeric complex of histones H2a, H2b, H3 and H4, each occurring in pairs (1). Each unit nucleosome consists of a 145 base pair (bp) (2) DNA "core" particle which is protected against nuclease digestion by its association with core histones, and a "linker" region of 10-80 bp associated with histone H1 or H1-like histones (3), which is highly susceptible to endonuclease attack (4,5).

The DNA size of the core particle among different species appears to be invariant; however, the size of the complete nucleosome (core plus linker) varies widely among organisms, tissue within the same organism, developmental state, and cellular activity (6-15). Furthermore, both active and inactive gene regions are organized in the repeating nucleosomal structure of chromatin, although the physical conformation may be different (16-18). It appears, therefore, that changes in nucleosome repeat length which, by definition, reflect alterations in chromatin structure, may relate to changes in gene activity.

We sought to determine if malignant change in a single cell type was reflected in chromatin structural changes. Our model was the Friend virus-induced erythroleukemic cell. We have previously characterized four distinct stages of Friend leukemia which evolve from early, non-tumorigenic leukemia cells in the spleen to later, sequentially emerging, tumorigenic cell types with increased malignant potential (19,20). This report will describe changes in chromatin structure associated with malignant changes in three erythroleukemic cell types which are morphologically similar, but different in malignant properties.

MATERIALS AND METHODS

Growth and Harvesting of Tumor Cells. Tumors were passaged in six-week old DBA/2 female mice at 2-3 week intervals by subcutaneous implantation of cells.

Growth and Maintenance of Cultured Cell Lines. Cloned Friend leukemia A6 (Stage III) and C7D (Stage IV) cells were grown in vitro in Eagle's basal media with Earle's salts and glutamine (Gibco), supplemented with 10% fetal bovine serum and gentamicin sulfate (Schering Corporation).

Isolation of Nuclei. The procedure essentially followed that already described (21) with minor modifications. The buffers contained the following proteolytic inhibitors made fresh the day of the isolation: 0.2 mM PMSF (phenylmethylsulfonylfluoride, Sigma), 0.1 mM TLC (N- α -Tosyl-L-lysyl chloromethane hydrochloride, Calbiochem), 0.1 mM TPC (Tosyl-L-phenylalanyl chloromethane, Calbiochem) (19). Two volumes (twice the initial tissue weight) of Homogenization Buffer [$\overline{0.25\text{ M sucrose in TKMC (0.05 M Tris - 2.5 mM KCl - 5 mM MgCl}_2\text{ - 1 mM CaCl}_2\text{, pH 7.5)}}$] was added to finely minced tissue and homogenized with a Dounce homogenizer fitted with a "B" pestle. The homogenate was filtered through sterile gauze, then mixed with 2 volumes of 2.3 M sucrose - 0.75% Triton X100-TKMC. Purified nuclei were sedimented

through 2.3 M sucrose-TKMC at 4°C for 70 min in the Beckman SW 27 rotor at 25,000 rpm. Nuclei from tissue culture cells (>90% viable) were similarly prepared. Nuclear pellets were resuspended in Digestion Buffer (0.25 M sucrose - 15 mM Tris - 60 mM KCl - 10 mM MgCl₂ - 1 mM CaCl₂, pH 6.5) and washed twice. Nuclei were brought to a final concentration of 100-130 A₂₆₀/ml.

Micrococcal Nuclease Digestion of Nuclei. The procedure used was adapted from that of Rill *et al.* (21). Micrococcal nuclease (Worthington; 6,000-18,000 units/mg) was used at a ratio of 1 enzyme unit/ A₂₆₀ of nuclei. To initiate digestion, enzyme was added to nuclei with mixing. Samples were removed at desired intervals and added to tubes on ice containing 1/10th-volume of 0.15 M EDTA, pH 7.5. After sedimentation of digested nuclei, the supernatant (Su-1) was carefully removed. The pelleted nuclei were lysed by addition of 10 mM Tris - 1 mM EDTA, pH 7.5, equal in volume to the initial digestion sample. Lysis was allowed to proceed a minimum of 1 hr at 4°C with mixing. After centrifugation (12,000 x g) the supernatant, Su-2, containing nuclease-released buffer-soluble oligomers was removed. Digestion was followed by the percent of the initial A₂₆₀ of the nuclear preparation released as Su-2 soluble material.

Nucleoprotein (NP) Gel Electrophoresis. Nucleosome oligomers were resolved by electrophoresis of Su-2 material on 5% acrylamide (25:1 ratio of acrylamide to bisacrylamide) tube gels (20 cm x 0.5 cm). Running Buffer was 6.4 mM Tris - 3.2 mM sodium acetate - 0.32 mM EDTA, pH 8.0 (22). Samples containing 1-10 A₂₆₀ units were loaded in 0.1 ml NP Sample Buffer (10% glycerol - 2 mM EDTA, pH 7.5 with 0.025% Bromophenol Blue as marker dye) and electrophoresis performed at room temperature, 40 V, until the dye had migrated off the gels. Gels were stained in 0.4% Amido Black and scanned at 620 nm. Relative amounts of oligomers were determined from gel scans by estimation of the area under each oligomer peak from the weight of the peak cut out of photocopy paper (19). Weights were equalized to an initial sample of six A₂₆₀ units (300 µg DNA).

DNA Extraction. Double-stranded (ds) DNA was isolated from Su-1 and Su-2 by the method of Rill *et al.* (21).

Gel Electrophoresis of DNA. Mononucleosome sizing of dsDNA was performed in running buffer (TAE) containing 0.04 M Tris - 0.02 M sodium acetate - 2 mM EDTA, pH 7.2 (23). 1-10 µg of DNA was applied in DNA Sample Buffer (0.1 x TAE - 10% sucrose - 0.025% Bromophenol Blue) to a 6% acrylamide (20:1 ratio acrylamide to bisacrylamide) slab gel (25 cm x 15 cm x 0.2

cm). Electrophoresis was performed at room temperature at 40-80 V until the marker dye neared the bottom. The gel was soaked in ethidium bromide to visualize DNA. Gels were photographed under short UV illumination with an orange filter using Polaroid Type 667 film. Photographic negatives were scanned on a Gilford spectrophotometer. Size determinations were made by comparison of the distance of migration of the band peaks with a standard curve generated from the migration of ϕ X174 RF, Hae III digestion fragments (New England Biolabs). A plot of the log of the number of base pairs versus the distance of migration was linear in the mononucleosome range.

The resolution of oligomeric dsDNA for DNA repeat length determination was accomplished by electrophoresis on 2.5% polyacrylamide - 0.5% agarose slab gels in TAE running buffer (see above). 1-5 μ g DNA samples in 0.1 x TAE - 10% sucrose - 0.025% Bromophenol Blue were applied. Electrophoresis was at 100 V, room temperature for 5-6 hours. Oligomer repeat length was estimated by two methods. The number of base pairs associated with each oligomer (established by comparison with a ϕ X174 standard curve) was divided by the oligomer number. The mean of these values for oligomers larger than the dinucleosome provided an estimate of the repeat length size (24). Linear regression analysis of the oligomer number (larger than dinucleosome) and the corresponding number of base pairs resulted in a line with a slope that also estimated the nucleosome repeat length independent of exonuclease trimming (25).

Cell Cycle Studies using the Fluorescence Activated Cell Sorter (FACS). Tissue culture and tumor cells (2×10^6) were analyzed following the protocol of Vindelov (26).

RESULTS

Our laboratory has reported a shift in the histone H2a variant ratio which paralleled the changing malignant potential of Friend leukemia cells (19,20,27). This present study was designed to determine if the histone change and malignant differences reflected a change in chromatin structure.

Friend virus-induced leukemia is characterized by proliferation of an erythropoietin-independent proerythroid stem cell (cell Stage I), resulting in splenomegaly and death within 3-5 months (28,29). From among animals surviving 1 month, tumorigenic cells (cell Stage II) can be isolated and propagated as tumors by subcutaneous inoculation into normal mice (30) or peritoneally-inoculated into irradiated mice (31, unpublished). Stage III cells arise from among cells in these tumors and are characterized by their

ability to grow in vitro. Neither the Stage II nor the Stage III cell is able to differentiate. A more malignant cell (Stage IV) evolves in vitro from Stage III cells; this cell will differentiate in response to chemical inducers such as dimethylsulfoxide (DMSO) (32-34). Cells representing Stage II, III and IV show increased malignancy with each increased stage designation (20). This system allows examination of an evolving malignant progression of cells, each of which requires the preceding stage.

Kinetics of Digestion by Micrococcal Nuclease. Micrococcal nuclease digestion of chromatin or nuclei solubilizes portions of chromatin which differ in number of nucleosomes. This effect reflects the multinucleosomal organization of chromatin, the accessibility of different sites to the enzyme, and the length of time of contact with the enzyme (4,35). We analyzed chromatin by mild micrococcal nuclease digestion of intact nuclei isolated from cells at each of the different stages using a constant ratio of enzyme to DNA.

The cells representing Stage I are recovered from the spleen where they undergo differentiation and from where it is not yet possible to obtain sufficient homogeneous amounts for extensive studies. Moreover, we have discovered an endogenous nuclease in these cells which interfered with controlled micrococcal nuclease digestion (36,37). We, therefore, focused our studies on a comparison of the more homogeneous cell population of tumorigenic Stages II, III and IV. Micrococcal nuclease digestion solubilized different amounts of chromatin among these three cell types. A greater amount of chromatin was released into the Su-2 from Stage II nuclei than from Stages III or IV. Digestion profiles of cells propagated either subcutaneously as tumors or in vitro were identical (Fig. 1). These findings indicated that the nuclease digestion pattern of these cells was a stable characteristic representative of a similar chromatin structure in vivo and in vitro.

Distribution of Nucleosomal Oligomers Released by Micrococcal Nuclease Digestion. To characterize the products associated with the kinetic differences, we analyzed the material released by micrococcal nuclease on polyacrylamide gels under nondenaturing electrophoretic conditions. This system resolves mono-, di- and trinucleosomal subunits, as well as H1-containing and H1-depleted forms of the mono- and dinucleosomal species. Su-1 contained only H1-depleted mononucleosomes (data not shown). Su-2 material contained the oligomeric nucleosomal species. The relative amount of nucleosome oligomers generated by 15 and 30 min micrococcal nuclease digestion of

Figure 1: Kinetics of solubilization of chromatin from nuclei treated with micrococcal nuclease.

Chromatin released into supernatant (Su-2) after nuclear lysis was measured by absorption at 260 nm and the % solubilization calculated based on the starting concentration of chromatin in the nuclei. Bars indicate ± 1 Standard Deviation (S.D.).

- A: Nuclei from Stage II, III and IV cells grown as subcutaneous tumors.
 B: Nuclei isolated from Stages III and IV cells grown in tissue culture.

Stage II, III and IV nuclei was quantitated from the nucleoprotein gel scans (Fig. 2). The total relative amount of material entering the gel after 15 min of digestion was different for the three stages. Stage II cell nuclei showed the least amount of material able to enter the gel as trinucleosomes or smaller (Fig. 3). More nucleosomal subunits migrated into the gel from digestion of Stage III and IV nuclei. The distribution between mono-, di- and trinucleosomes was also different for each stage. At 15 min, only Stage IV demonstrated a prominent trinucleosome population (Fig. 3). After 30 min of digestion, these quantitative differences among the cell types were not maintained, although approximately 2-3 times more chromatin entered the gels (data not shown).

A comparison of the mono- to dinucleosome ratio among the three stages did not show significant differences. In all cases, an increase in the relative amount of mononucleosomes was noted as digestion progressed: 0.72 ± 0.05 at 15 min, 0.95 ± 0.10 after 30 min digestion. However, analysis of the histone H1 content of the mononucleosomes revealed distinctions (Table 1). At 15 min, Stage II contained the highest ratio of $H1^+/H1^-$ mononucleo-

Figure 2: Analysis of chromatin solubilized by micrococcal nuclease. The Su-2 material released was analyzed on NP gels. TN = tri-nucleosomes, DN = dinucleosomes, MN = mononucleosomes; subscripts indicate subclasses of each oligomer (38).

Figure 3: Analysis of nucleosome oligomers released into Su-2 after 15 min of micrococcal nuclease digestion of nuclei. The mean distribution of the released oligomers was compared among the different tumor cell types from NP gel scans corrected to 300 μ g DNA applied per sample. Total represents the sum of mono-, di-, and trinucleosomes migrating into the gel. The percent of each size class is given above the appropriate bar.

Table 1. Histone H1 composition of mononucleosomes released into Su-2 fraction at 15 and 30 min of micrococcal nuclease digestion.

	H1 ⁺ /H1 ⁻ RATIO	
	15 min	30 min
Stage II (2.5)*	6.50 +1.66	3.62 +0.93
Stage III (2.0)*	2.81 +0.44	2.51 +0.47
Stage IV (≤ 1)*	3.65 +0.95	3.25 +0.53

H1⁺/H1⁻ ratios were determined from NP gel scans. Peak MN₁ contained the core histones minus H1, as determined by protein gel electrophoresis in a second dimension (data not shown). Ratios represent the mean + S.D. of 3-6 experimental samples.

*Ratio of histone H2a.1 to H2a.2 present in chromatin (20).

somes, Stage III the lowest, and Stage IV was intermediate. At 30 min, the ratio in Stage II cells had greatly decreased; the ratio in Stage III and IV cells was only slightly decreased.

Sizing of Native DNA Associated with Released Oligonucleosomes. dsDNA was extracted from Su-1 and Su-2 fractions. Su-1 contained a subset of mononucleosomes depleted in histone H1, which was soluble in Digestion Buffer. The Su-2 fraction contained over 90% of the released nucleosome oligomers; it also contained some H1-depleted mononucleosomes. The different migration of mononucleosomal DNA from each of the 3 stages was apparent (Fig. 4), and corresponded to different sizes (Table 2B).

We next examined mononucleosome DNA size at early and later periods of digestion. The mononucleosome DNA size did not change detectably over the digestion periods (Fig. 5). The DNA extracted from Su-1 mononucleosomes was uniformly smaller than Su-2 mononucleosomal DNA. Because of the mild digestion conditions utilized in these studies, rapid degradation of released nucleosomal DNA to core particle size was not observed. However, after prolonged (60 min) digestion of nuclei, the core particle-sized DNA was predominant (see Stage IV, Fig. 5).

The estimated size of the nucleosome core plus linker was provided by analysis of oligomers larger than dinucleosomes. Two methods were employed yielding slightly different values, but with the relative size difference

Figure 4: Gel electrophoresis of DNA isolated from micrococcal nuclease digested nuclei.

(A) Standard (ϕ X174 RF, Hae III digest). The number of base pairs (bp) associated with each fragment is indicated. DNA isolated from Su-2 after 30 min of digestion was: (B) Stage II; (C) Stage III; (D) Stage IV. Slots E, F and G were Su-1 DNA samples from Stage III nuclei after digestion for (E) 8 min; (F) 15 min; (G) 24 hrs at 0°C, i.e., conditions to minimize exonuclease trimming (46); Stage III Su-1 material after 10 min (H) and 30 min (I); Stage IV Su-1 after 30 min of digestion (J).

among the cell types retained (Table 2A). Mononucleosomal size, reflecting in part characteristic linker lengths as well as stability of the linker regions to nuclease degradation, paralleled the oligomer repeat sizes (Table 2B). The largest repeat length was found in Stage II and the smallest in Stage III cells.

Relationship of Cell Cycle to Micrococcal Nuclease Digestion. The increased sensitivity of newly-replicated chromatin to nuclease is well documented (39,40). To determine if the differences we observed by micrococcal nuclease digestion were related to alterations in the cell cycle, a fluorescence-activated cell sorter was used to generate cell cycle profiles for each stage (data not shown). When Stage II and III tumor cells grown in vivo were analyzed, Stage II cells had a greater number of cells present in G1 (63%) as compared to Stage III cells (58%). When Stage III cells were grown in vitro, fewer cells were present in G1 (51%), a finding which suggested that growth conditions in vivo were more restrictive than those in

Table 2. Repeat length of nucleosome subunits at 5 and 15 min of digestion and mononucleosomal DNA analysis.

	STAGE II		STAGE III		STAGE IV	
	5 min	15 min	5 min	15 min	5 min	15 min
A. <u>Average Repeat of Oligomers</u>						
a ¹	204.0 +5.6	215.3 +6.8	194.3 +12.2	192.0 +4.4	184.3 +2.5	204.0 +1.0
b ²	197.1	207.6	178.6	199.2	179.0	198.1
B. <u>Analysis of Mononucleosomes</u> ³						
<u>Su-2</u>	190.2 +7.8 (6)		147.8 +5.7 (8)		167.2 +10.6 (13)	
<u>Su-1</u>	170.8 +4.3 (5)		131.8 +3.4 (8)		153.3 +6.5 (7)	

¹ Average repeat length $\pm$ S.D. was determined as the mean of the repeat length by analysis of oligomers 3 through 5.

² Average repeat length was calculated as the slope of the line after linear regression analysis of the DNA size of oligomers consisting of 3-9 nucleosomes.

³ Mononucleosome size $\pm$ S.D. was calculated from the migration of mononucleosome DNA samples in 6% polyacrylamide slab gels. The number of samples analyzed is given in parentheses.

vitro. Stage IV cells grown in vitro showed no detectable cell cycle differences from Stage III cells in vitro. This latter finding, as well as the similarity of micrococcal nuclease digestion kinetics of cells grown in vivo or in vitro (see Fig. 1) indicated that cell cycle differences were not responsible for the observed nuclease digestion differences.

DISCUSSION

The chromatin structure of three different tumorigenic Friend erythroleukemic cell types was dramatically different as revealed by micrococcal nuclease digestion studies. This finding is of great interest since the three cell types studied are derived from the same proerythroid stem cell and form a progression toward increased malignancy. They are morphologically indistinguishable by light and electron microscopy (41).

The kinetics of digestion (Fig. 1) provided the first indication of

Figure 5: DNA size of mononucleosomes released early and late by micrococcal nuclease digestion.

Polyacrylamide gel electrophoresis was performed on DNA samples purified from Su-1 (A) and Su-2 (B). Ethidium bromide-stained gels were photographed and the negatives scanned to determine the migration of the peaks relative to DNA standards (ϕ X174 RF) analyzed in the same gel. Each symbol represents a separate gel sample. Closed symbols indicate the abundant species; open symbols reflect minor species. Half-shaded symbols indicate co-dominant species. The dotted lines enclose the area encompassed by the mean $\pm$ 1 S.D. Bracketed point in Stage IV Su-2 shows the emergence of the core particle as the predominant species after 60 min of digestion.

differences among the three cell types. Solubilization was greatest in Stage II (61%), least in Stage III (36%), and intermediate in Stage IV (46%). A solubilization plateau, observed in each cell type between 15 and 30 min of digestion, did not reflect an inactivation of the nuclease since an increased portion of smaller nucleosome oligomers continued to be released throughout the digestion period. The kinetics of digestion of tissue culture cells of Stage III and IV were identical to those of the tumor cells (Fig. 1B), underscoring the stability of the characteristic solubilization profiles and suggesting that the kinetics reflect fundamental structural differences which were maintained in vivo and in vitro.

To characterize further the altered nuclease accessibilities, we analyzed the products of digestion. Clear distinction between Stage II, III

and IV digestion products was observed at 15 min (Fig. 3). Stage III and IV both released a greater amount of mono + di + trinucleosomal products than Stage II despite a greater total solubilization by Stage II. The inverse relationship between the amount solubilized and the fragment size is best explained by regional accessibility differences, with regionally concentrated enzyme activity resulting in the production of smaller nucleosome oligomers.

One means of comparing the extent of digestion is to analyze the relative number of mononucleosomes which have lost histone H1. When linker regions are degraded, more nucleosomes lose histone H1 (42) and the $H1^+/H1^-$ ratio should reflect this digestion. Stage II chromatin appeared less digested by this criterion (Table 2); the $H1^+/H1^-$ ratio was highest at both 15 and 30 min. In contrast, Stage III mononucleosomes at both 15 and 30 min digestion had the lowest $H1^+/H1^-$ ratio. These findings may reflect differences in the total amount of H1 or tightness of H1 binding in the three chromatins. The presence of H1, its variants or other proteins has been shown to affect nuclease activity in other systems (43-45), and such conditions could explain these observed differences.

The size of the dsDNA generated after nuclease digestion provided evidence for the more extensive digestion of Stage III solubilized chromatin. The predominant 148 bp mononucleosome class of Stage III nuclei approximated the 145 bp core particle size, while the major DNA fragments of Stage II (190 bp) and Stage IV (167 bp) nuclei were significantly larger (Fig. 5, Table 2B). These differences could reflect a greater susceptibility to nuclease "trimming" in Stage III than in Stage II or IV. However, it is noteworthy that the repeat length of higher oligomers of each of the cell types reflected, in general, the relative mononucleosome size. H1-depleted, Su-1 mononucleosome fractions also retained the respective size (Fig. 4, Table 2B). These data suggest that the discrete mononucleosome classes observed are stabilized due to components other than histone H1.

Our data show several coexistent mononucleosome size classes (Fig. 5). Although a precursor/product relationship is possible, it is equally plausible that the classes were derived from chromatin domains with different oligomer repeat sizes, as reported in other systems (47,48). The products of early digestion times (≤ 5 min) tended to have a smaller oligomer repeat size than longer incubations (Table 2A). This supports the domain interpretation, since nuclease processing alone would predict larger particles

released early, with subsequent size reduction.

The stability of the unique major mononucleosomal DNA size in each cell type emphasizes basic organizational differences between the different cell types. The significance in terms of gene activity is unclear, although nucleosomal repeat length has been shown to change during development (24,49,50). Correlations between increased repeat sizes and decreased transcriptional activity have been attempted but are not absolute (51).

Differential accessibility and stability of nucleosomal particles to micrococcal nuclease digestion has been related to the presence of a unique H1 variant in the linker region of Holothuria (52). Studies on the relationship of nucleosomal repeat sizes and altered amounts of H1 and H5 in erythroid cells failed to show a correlation (53). This was unexpected, since H1 and H5 both bind to linker regions and vary in charge and size (54). Yet, successful reconstitution of nucleosomes with native size repeat units has been found to require H1 (55). These findings can be reconciled if interaction of histone H1 with the nucleosome core is necessary for proper linker spacing. Histone H2a and H2b are believed to lie at the periphery of the nucleosome core, proximal to the linker region (56,57); interactions with lysine-rich histones have been reported (58,59). They may, therefore, affect linker size. Both H2a and H2b exist in several nonallelic variant forms (60,61) which provide variability to individual nucleosome core particles. The three stages of Friend erythroleukemia characterized in this communication differ in the ratio of two histone H2a variants (19). The chromatin structure differences among the three stages may directly relate to the histone H2a variant change. The role of H2a variants in chromatin structural alterations and the malignant process remain speculative. Our studies do provide evidence, however, that malignant change in a single cell type is reflected in the total chromatin structure of its DNA.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant GM25792 and Grant CA-24530 to Tufts Cancer Research Center for the use of the cell sorter. We wish to thank Carolyn Becker for performance of the cell sorter studies, and Dan Waters for his expert technical assistance in the use of the FACS IV.

REFERENCES

1. Kornberg, R.D. (1977) Ann. Rev. Biochem. 46, 931-954.
2. Tatchell, K., and VanHolde, K.E. (1978) Proc. Natl. Acad. Sci. USA 75, 3583-3587.

3. Whitock, J.P., Jr., and Simpson, R.T. (1976) *Biochemistry* 15, 3307-3314.
4. Sollner-Webb, B., and Felsenfeld, B. (1976) *Biochemistry* 14, 2915-2925.
5. Noll, M., and Kornberg, R.D. (1977) *J. Mol. Biol.* 109, 393-404.
6. Compton, J.L., Bellard, M., and Chambon, P. (1976) *Proc. Natl. Acad. Sci. USA* 73, 4382-4386.
7. Lohr, D., Corden, J., Tatchell, K., Kovacie, R.T., and VanHolde, K.E. (1977) *Proc. Natl. Acad. Sci. USA* 74, 79-83.
8. Lohr, D., Kovacie, R.T. and VanHolde, K.E. (1977) *Biochemistry* 16, 463-471.
9. Rill, R.L., Nelson, D.A., Oosterhof, D.K., and Hozier, J.C. (1977) *Nucleic Acids Res.* 4, 771-789.
10. Morris, N.R. (1976) *Cell* 8, 357-363.
11. Noll, M. (1976) *Cell* 8, 349-355.
12. Spadafora, C., Bellard, M., Compton, J.L., and Chambon, P. (1976) *FEBS Lett.* 69, 281-285.
13. Wilhelm, M.L., Mazen, A., and Wilhelm, F.X. (1977) *FEBS Lett.* 79, 404-408.
14. Thomas, J.O., and Thompson, R.J. (1977) *Cell* 10, 633-640.
15. Martin, D.Z., Todd, R.D., Lang, D., Pei, P.N. and Garrard, W.T. (1977) *J. Biol. Chem.* 252, 8269-8277.
16. Bellard, M., Gannon, F. and Chambon, P. (1978) *Cold Spring Harbor Symp. Quant. Biol.* 42, 779-781.
17. Weintraub, H., and Groudine, M. (1976) *Science* 193, 848-856.
18. Garel, A., and Axel, R. (1976) *Proc. Natl. Acad. Sci. USA* 73, 39966-3970.
19. Blankstein, L.A., and Levy, S.B. (1976) *Nature* 260, 638-640.
20. Levy, S.B., Blankstein, L.A., Vinton, E.C., and Chambers, T.J. (1979) in *Oncogenic Viruses and Host Cell Genes*, (Ikawa, Y., and Odaka, T., Eds.), pp. 409-428, Academic Press, New York.
21. Rill, R.L., Shaw, B.R., and VanHolde, K.E. (1978) in *Methods in Cell Biology*, (Stein, G., Stein, J., and Kleinsmith, L.J., Eds.), Vol. 18, pp. 69-103, Academic Press, New York.
22. Shaw, B.R., and Richards, R.G. (1979) in *Chromatin Structure and Function Part A*, NATO Advanced Study Institute Series 21A, pp. 125-136, Plenum Press, London and New York.
23. Loening, U.E. (1967) *Biochem. J.* 102, 251-257.
24. Ermini, M., and Kuenzle, C.C. (1978) *FEBS Lett.* 90, 167-172.
25. Johnson, E.M., Littau, V.C., Allfrey, V.G., Bradbury, E.M., and Matthews, H.R. (1976) *Nucleic Acids Res.* 3, 3313-3329.
26. Vindelov, L. (1977) *Virchows Arch. B. Cell. Path.* 24, 227-242.
27. Blankstein, L.A., Stollar, B.D., Franklin, S.G., Zweidler, A., and Levy, S.B. (1977) *Biochemistry* 16, 4557-4562.
28. Axelrad, A.A., and Steeves, R.A. (1964) *Virology* 24, 513-518.
29. Mirand, E.A., Steeves, R.A., Avila, L., and Grace, J.T. (1968) *Proc. Soc. Exp. Biol. Med.* 127, 900-904.
30. Friend, C., and Haddad, J.R. (1960) *J. Natl. Acad. Inst.* 25, 1279-1289.
31. Tambourin, P.E. (1977) in *Stem Cells and Tissue Homeostasis* (British Society for Cell Biology Symposium 2) pp. 259-316, Cambridge University Press, Cambridge, London, New York and Melbourne.
32. Friend, C., Scher, W., Holland, J.G., and Sato, T. (1971) *Proc. Natl. Acad. Sci. USA* 68, 378-382.
33. Ostertag, W., Melderis, H., Steinheider, G., Kluge, N., and Dube, S. (1972) *Nature New Biol.* 239, 231-234.
34. Reuben, R.C., Khanna, P.L., Gazitt, Y., Breslow, R., Rifkind, R.A., and Marks, P.A. (1978) *J. Biol. Chem.* 258, 4214-4218.
35. Simpson, R.T. (1978) *Biochemistry* 17, 5524-5531.

36. Levy, S.B., Leonardson, K.E., Benezra, R., Stollar, B.D., and LaPierre, K. (1980) in In vivo and in vitro Erythropoiesis: the Friend System, (Rossi, G.B., Ed.), pp. 309-321, Elsevier/North Holland Biomedical Press, Amsterdam, New York, Oxford.
37. Leonardson, K.E., and Levy, S.B. (1980) J. Biol. Chem. (submitted for publication).
38. Bakayev, V.V., Bakayeva, T.G., and Varshavsky, A.J. (1977) Cell 11, 619-629.
39. Murphy, R.F., Wallace, R.B., Bonner, J. (1978) Proc. Natl. Acad. Sci. USA 75, 5903-5907.
40. Levy, A., and Jakob, K.M. (1978) Cell 14, 259-267.
41. Levy, S.B., Daoust, P.O., Vinton, E., Andre-Schwartz, J., Leonardson, K.E., and LaPierre, K. (1979) in UICC Workshop on Retroviruses and Differentiation (abst).
42. VanHolde, K., and Weischet, W.O. (1978) in The Cell Nucleus IV, Busch, H., Ed., pp. 75-104, Academic Press, New York.
43. Whitlock, J.P., Jr., and Simpson, R.T. (1976) Biochemistry 15, 3307-3314.
44. Allan, J., Staynov, D.Z., and Gould, H. (1980) Proc. Natl. Acad. Sci. USA 77, 885-889.
45. Muyldermans, S., Lasters, I., and Wyns, L. (1980) Nucleic Acids Res. 8, 731-739.
46. Jackson, J.B., Pollock, J.M., and Rill, R.L. (1979) Biochemistry 18, 3739-3748.
47. Prunell, A., and Kornberg, R.D. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 103-108.
48. Bafus, N.L., Albright, S.C., Todd, R.D. and Garrard, W.T. (1978) J. Biol. Chem. 253, 2568-2574.
49. Brown, I.R. (1978) Biochem. Biophys. Res. Comm. 84, 285-292.
50. Schelgel, R.A., Haye, K.R., Litwack, A.H., and Phelps, B.M. (1980) Biochem. Biophys. Acta 606, 316-330.
51. Chambon, P. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 1209-1234.
52. Cornudella, L., and Rocha, E. (1979) Biochemistry 18, 3724-3732.
53. Miki, B.L.A., and Neelin, J.M. (1980) Nucleic Acids Res. 8, 529-542.
54. Glotov, G.O., Nikolaev, L.G., Severin, E.S. (1978) Nucl. Acids. Res. 5, 2587-2605.
55. Yaneva, M., Taseva, B., and Dessev, G. (1976) FEBS Lett. 79, 67-70.
56. Carter, C.W., Jr. (1978) Proc. Natl. Acad. Sci. USA 75, 3657-3653.
57. Mirabekov, A.D., Shick, V.V., Belyavsky, A.V., Barykin, S.G. (1978) Proc. Natl. Acad. Sci. USA, 75, 4184-4188.
58. Bonner, W.M., and Stedman, J.D. (1979) Proc. Natl. Acad. Sci. USA 76, 2190-2194.
59. Chapman, G.E., Hartman, P.G., and Bradbury, E.M. (1976) Eur. J. Biochem. 61, 69-75.
60. Franklin, S.G., and Zweidler, A. (1977) Nature 266, 273-275.
61. West, M.H.P., and Bonner, W.M. (1980) Biochemistry 19, 3238-3245.