Isolation and characterization of minichromosome particles that contain a glucocorticoid-modulated promoter

Michael C.Ostrowski

Department of Microbiology and Immunology, Duke University, Durham, NC 27710, USA

Received April 28, 1987; Revised July 13, 1987; Accepted July 31, 1987

ABSTRACT

A procedure for the enrichment of minichromosomes, composed of bovine papilloma virus and the long terminal repeat element of the mouse mammary tumor virus (MTV), from isolated nuclei is described. Up to 60% of the minichromosomes were extracted as nucleoprotein particles. These particles sediment in sucrose gradients as 160S complexes. Hormone-labeled glucocorticoid receptor co-purifies with these complexes in a specific fashion. Between four and six molecules of receptor are bound per minichromosome molecule. Analysis of DNase I hypersensitivity demonstrates that hypersensitive sites are preserved through the purification procedure in a manner that reflects the hormone-dependent *in vivo* pattern of digestion. These purified minichromosomes will allow features of chromatin structure that may be important for steroid hormone modulation of transcription to be studied *in vitro* without resorting to destructive nuclease digestion procedures.

INTRODUCTION

The part that chromatin structure plays in the regulation of transcription in eukaryotic cells remains an important question. It is generally accepted that alterations in chromatin structure as assayed by nuclease hypersensitivity are correlated with the potential for transcriptional activity (1, 2). However, whether such changes reflect an active or passive involvement of chromatin structure remains unknown.

Glucocorticoid hormones regulate the transcription of the mouse mammary tumor virus (MTV, 3). It has been shown that molecularly cloned MTV sequences derived from the viral long terminal repeat (LTR) contain high affinity binding sites for the purified glucocorticoid receptor *in vitro* (4, 5). Gene transfer experiments have indicated that the LTR sequences harboring high affinity receptor sites may be functionally defined as glucocorticoid-responsive elements (GRE): that is, transcriptional enhancer elements that function only when the receptor-hormone complex is bound to them (6).

The MTV GRE exhibits hormone-inducible DNase I hypersensitivity; the change in chromatin structure closely follows the kinetics of transcriptional

induction (7). Therefore, this system provides a good model to explore the relationship between alterations in chromatin structure and the modulation of transcription. Three important advantages to this system are: a) DNA synthesis is not required in order to observe changes in chromatin structure; b) the formation of hormone-inducible hypersensitive sites is rapid and reversible; c) such changes are initiated by the binding of a single protein, the receptor.

Our lab's approach to this problem has involved introducing the GRE into cultured murine cells as amplified, extrachromosomal elements (8). In this way, MTV minichromosomes may be purified from cells as chromatin. Such a system would allow us to study chromatin structure in vitro without relying on reconstitution techniques and the uncertainties inherent in these procedures. The present work details the recent success at purifying MTV minichromosomes. This work demonstrates that structures that contain the glucocorticoid receptor can be purified, and that nuclease hypersensitive sites are also preserved through the purification procedure in a manner consistent with the hormone-dependent in vivo pattern of nuclease hypersensitivity.

EXPERIMENTAL

Cell culture conditions

Cells were grown in Dulbecco's modified Eagles medium (high glucose formulation) containing 10% fetal bovine serum, 20 U/ml penicillin and 20 ug/ml streptomycin. Experiments were conducted when the cells had just reached confluency. For hormone treatments, dexamethasone (Sigma) was added to the culture medium to a final concentration of 10^{-7} M. The length of hormone treatment for the various experiments was two hours unless otherwise noted.

Isolation of MTV-minichromosomes

For isolation of minichromosomes, all manipulations were carried out at 0°C, using salt-ice baths where needed. For low speed centrifugations, a DuPont HS4 rotor and RC-5B superspeed centrifuge were used. Buffer A contained 0.01 M Tris-Cl (pH 7.5 at 25°C), 0.07 M KCl, 0.015 M NaCl, 0.004 M $MgCl_2$. Buffer B was the same as Buffer A except that it also contained 0.4% NP-40 and 0.5 mM PMSF, and Buffer C was composed of 0.01 M Hepes (pH 7.9), 0.001 M EDTA, 0.5 mM PMSF. All buffers were chilled in salt-ice baths prior to use.

In some experiments, cells were labeled overnight with 50 uCi of tritiated-thymidine (NEN, 100 Ci/mmol) or for one hour with 2×10^{-7} M

tritiated-triamcinolone (NEN, 32.8 Ci/mmol) prior to extraction of minichromosomes.

Monolayers of confluent cells were washed with Hanks buffered saline solution, and collected by scraping with a rubber policeman and centrifugation (500 x g, 5 min). The pellet was resuspended in Buffer A at a concentration of 2×10^7 cells per ml and centrifuged (500 x g, 5 min). The pellet was resuspended in Buffer B at the same cell density and left at 0°C for 5 min. Following centrifugation (500 x g, 5 min), the crude nuclear pellet was resuspended in Buffer C (2×10^7 nuclei/ml) and incubated at 0°C for 60-120 min. Incubation of nuclei for longer periods of time did not substantially increase the final yield of extracted episomes. Following centrifugation (2000 x g, 10 min), the supernatant was loaded onto 28 ml linear sucrose gradients (15-45%) that had a 4 ml cushion of 70% sucrose. Gradients were prepared in Buffer C. These gradients were centrifuged in a Beckman VTi-50 rotor for 15 min at 50,000 rpm. Gradients were taken down without the brake and collected.

For some experiments, one-half of the samples was used for liquid scintillation counting, while DNA was extracted from the other half (see below). In some experiments, the refractive index of a portion of each fraction was determined; this information was used in the calculation of the Svedberg constant (9). Efficiency of counting was determined by the external standard method using commercially purchased quenched standards (Beckman).

Minichromosome concentrations following sucrose gradients were determined as follows. First, a portion of thymidine-labeled cells were TCA precipitated and counted. DNA content was determined by the Burton method (10). The specific activity of total cell DNA thus measured was used to calculate the amount of labeled DNA in the minichromosome peak of the gradient. Next, restriction enzyme digested DNA obtained from the minichromosome gradient peak was fractionated on agarose gels and Southern blotted. The hybridization of this material was compared to that of known amounts of plasmid DNA by densitometry in order to estimate the amount (in grams) of minichromosome DNA present. 80-90% of the DNA present in the minichromosome-specific peak is determined to be MTV-BPV episomal DNA by this procedure (see Table I).

Protein concentrations across the minichromosome peak were determined by a modification to the Bradford method (11). Commercial reagent was used in these assays (Biorad). However the final volume of the reaction was reduced to 0.2 ml. Material from sucrose gradients was precipitated with 20% TCA and resuspended in water. Histones that were acid extracted (12) from mouse C127

cells were used as standards. The concentration of the histone standards was determined by absorbance at 230 nm. The linear range of this assay was 0.4-2.0 ug of protein.

RNA in the minichromosome peak fractions was measured by labeling cells with ^3H -uridine. A fraction of the labeled cells were extracted with guanidine hydrochloride, and RNA was recovered by repetitive ethanol precipitation (8). The concentration of resulting RNA samples was determined by absorbance at 260 nm, and radioactivity was determined by scintillation counting. The specific activity of total cellular RNA determined in this way was used to estimate the amount of RNA in the minichromosome peak following extraction and gradient purification of material from ^3H -uridine labeled cells. The position of the minichromosome peak was determined by Southern blotting in these experiments.

For calculations of number of receptor molecules per minichromosome, molecules of MTV GRE were determined from the amount of DNA present, as calculated above. The number of receptor molecules present was determined by first subtracting the non-saturable background of labeled hormone from the value obtained for the labeled minichromosome peak (i.e., in Figure 3 the peak indicated with closed squares was subtracted from the peak depicted by open squares). This value, along with the specific activity of the radioactive hormone preparation (see above) was used to calculate receptor molecules. In these calculations, it was assumed that one receptor molecule binds one hormone molecule (13).

For all these analyses "peak" fractions indicate those fractions from gradients that contain the majority of MTV episomal DNA, as determined by Southern blotting and, where appropriate, the maximum tritiated thymidine counts. For example, in the experiment represented in Figure 3, the minichromosome peak was located between fractions 9 and 18.

Nuclease digestions

Procedures used for DNase I studies have been previously described (14). Briefly, 5×10^6 isolated nuclei per ml were digested with RNase-free DNase I (Worthington) for 2 min at room temperature. The amounts of enzyme used are indicated in the figure legend. Digestion was terminated by addition of SDS to 1% and EDTA to 0.01 M. DNA was extracted, digested with PstI, and analyzed by indirect end-labeling (15), using the probes indicated in Figure 6C.

For digestion of isolated minichromosomes, the procedure was essentially the same, except that either sheared salmon sperm DNA (Sigma) or lineared pBR322 plasmid DNA were added in order to keep the DNA concentration the same

Figure 1. Map of recombinant pm23.

The dark line indicates sequences derived from BPV I. The identity of other sequences is given in the open boxes. Numbers indicate positions in nucleotides from the BPV HpaI site. Note that this is the form that replicates in mammalian cells. Bacterial plasmid sequences necessary for replication in *E. coli* hosts were removed by SalI digestion prior to transfection of murine cells (8). Figure 1A: Northern blot of RNA isolated from 1361.5 cells grown in the absence or presence of 10^{-7} M dexamethasone (lanes 1 and 2, respectively). 10 μ g of total RNA was applied to run in each gel lane. A MTV LTR probe was used for hybridization

as in the nuclear digestion experiments.

Micrococcal nuclease digestions were performed as indicated in the legend to Figure 5. Following digestion, EGTA (0.002 M) was added, DNA was purified and fractionated on 1.8% agarose gels. For purified minichromosomes, tRNA could be used to adjust the nucleic acid concentration prior to digestion.

Isolation and analysis of DNA and RNA samples

Procedures for the isolation and analysis of both RNA and DNA samples have been previously described (8).

RESULTS

Purification of MTV minichromosomes

For the experiments described in this work, a NIH 3T3 cell line transformed by pm23 was used. This cell line is termed 1361.5. 1361.5 cells contain 150 copies of pm23 per cell. The structure of pm23 is shown in Figure 1. This molecule is identical to MTV-BPV constructions previously described (8) except that a portion of the early region of SV 40 that includes the t-antigen intron and the poly A-addition site for T-antigen (16) have been inserted 3' to the v-ras gene.

Figure 2. Efficiency of the minichromosome extraction procedure.

Insoluble and soluble DNA fractions were analyzed by Southern blotting following hypotonic extraction of nuclei as described in the text. Panel A: Lanes 1-4 contained 4 ng, 3 ng, 2 ng and 1 ng, respectively, of pm23 plasmid DNA to serve as quantitative standards. Lane M contained lambda-HindIII digested size standards. Lane 5 and 6 contained 5% of the total DNA found in the insoluble (pellet) versus the soluble (supernatant) fraction, respectively, following the extraction of 2×10^7 nuclei. The DNA was restricted with EcoRI prior to electrophoresis. A MTV LTR probe was used for this experiment. Panel B: Lane 1 and 2 each contained 1% of the DNA remaining in the pellet following nuclear extraction; lane 1 sample was restricted with EcoRI, lane 2 sample was untreated. Lane 3 and 4 each contained 1% of the DNA found in the supernatant following extraction; lane 3 sample was EcoRI restricted, lane 4 sample was untreated. 10^8 nuclei and a BPV probe were used for this experiment.

Figure 1A contains data demonstrating that hormone treatment of 1361.5 cells results in a 20-fold stimulation of transcription initiated from the episomal MTV LTR (Figure 1A, compare lane 1 to lane 2). The major band detected by this analysis is 1900 bases in size, as predicted from the structure of pm23. S1-mapping indicates that these transcripts initiate at the authentic MTV cap-site (data not shown).

The same results for the 1361.5 minichromosome extraction procedure as described below have been obtained when C127 cells transformed with MTV-BPV constructs were used (data not shown).

The procedure for isolation of pm23 episomes involves isolation of nuclei from transformed cells using isotonic buffers containing the non-ionic detergent NP-40 and hypotonic extraction of nuclei (see **EXPERIMENTAL** for details). One way to measure the efficiency of this procedure is to compare

TABLE I. Determination of nucleic acid and protein composition of enriched minichromosomes.

Exp. # ^a	Tot. DNA ^b (ng)	pM23 DNA (ng)	Total Protein ^b (ng)	RNA ^c (ng)	Protein/DNA ratio
1	440	400	750	3.5	1.7
2	510	410	765	6.0	1.5

^a5x10⁸ cells were used for each experiment. See text for details.

^bDNA and protein were determined in same experiment: 1/6 of the peak gradient fractions were used for DNA analysis, and the rest for protein analysis.

^cRNA was determined in different experiments than protein and DNA.

the amount of minichromosomes present in the soluble fraction versus the insoluble fraction following hypotonic treatment of 1361.5 nuclei. This can be accomplished by Southern blotting of agarose gels containing proportional amounts of material from each fraction. Figure 2 contains results typical of such analyses.

Figure 2, panel A shows the results following extraction of 2 x 10⁷ cells (one T-150 flask). Lane 5 contained 5% of the material remaining in the pellet following extraction, and lane 6 contained 5% of the material in the supernatant. These samples were digested with EcoRI and the blot was hybridized to an LTR-specific probe. Figure 2, panel B shows a separate experiment in which 10 x 10⁷ cells were used. The first two lanes contained 1% of the material from the pellet, either digested with EcoRI or undigested, respectively, while lanes 3 and 4 contained 1% of the soluble material, again either digested or undigested. This data demonstrates the range of results obtained. Between 30% and 60% of MTV minichromosomes are routinely extracted by the procedures used. Higher percentage yields are obtained when more starting material is used.

When DNA was labeled with ³H-thymidine, in 3 separate experiments 0.1-0.25% of the total acid precipitable counts were recovered in the soluble fraction following hypotonic extraction of nuclei. Based on a copy number of 150 minichromosomes per cell, a diploid mouse genome size of 6 x 10⁹ bp and an efficiency of recovery for episomes of 60% (see above), the MTV minichromosomes represent 6-10% of the DNA present following hypotonic extraction. This represents a purification at the level of DNA of 200-400

fold. However, the minichromosomes are contaminated by large amounts of DNA and soluble protein at this stage.

The final step of the fractionation procedure is velocity sedimentation through sucrose gradients. The analysis of gradients containing thymidine-labeled samples indicates that this procedure yields MTV episomes that are greater than 80%-90% pure at the level of DNA (Table 1). This represents a final purification of 2000-fold at the level of DNA. These episomes are also highly enriched with respect to protein and RNA as well (Table I). The Svedberg value for the peak fraction of these MTV minichromosomes is approximately 160S. Re-sedimentation of the minichromosomes located on either the leading or following edges of this peak resulted in a distribution identical to that observed in the first gradient, including the same 160S peak value (data not shown).

Characterization of MTV minichromosomes

The results presented above indicate that MTV minichromosomes can be isolated from nuclei. They do not address whether the isolation procedure yields native structures. To study this point two criteria were tested. First, the co-purification of the glucocorticoid receptor with the MTV minichromosomes was assayed. Second, the digestion of isolated minichromosomes with micrococcal nuclease and DNase I was compared to the digestion of intact nuclei.

For glucocorticoid receptor studies, 1361.5 cells were incubated with tritium-labeled hormone for one hour prior to minichromosome extraction. In a parallel experiment, cells were labeled overnight with tritiated thymidine, in order to label DNA. The MTV minichromosomes were extracted and loaded onto linear sucrose gradients. After centrifugation, fractions were collected. The radioactivity in one-half of the individual fractions was determined by liquid scintillation counting, while DNA from the other half was extracted and analyzed by Southern blotting.

Figure 3 contains the data from one experiment. This shows that a peak of hormone radioactivity (Figure 3A, open squares) is found coincident with a radioactive peak of DNA (Figure 3A, closed circles). Southern blotting of the samples demonstrates that these peaks correspond to the position of MTV minichromosomes in the sucrose gradients (Figure 3B). The apparent difference in the DNA peak as determined by radioactivity versus the peak determined by Southern blotting in this experiment (gradient fraction 13 versus 15) is not reproducible and probably reflects sampling error during the preparation and analysis of the DNA samples. Since the soluble glucocorticoid receptor would

Figure 3. Co-purification of glucocorticoid receptor with MTV minichromosomes.

For the experiment shown in panel A, 10^8 1361.5 cells were treated with either ^3H -thymidine (closed circles), 10^{-7}M ^3H -triamcinolone (open squares) or 10^{-7}M ^3H -triamcinolone diluted with 10^{-5}M cold triamcinolone (closed squares). The times cells were labeled is given in the text. Following extraction, the entire soluble fraction was loaded on sucrose gradients and centrifuged as described in the text. Fractions (1 ml) were collected and one-half of each was analyzed by scintillation counting. The right is the top of the gradient. Thymidine counts through the gradient are indicated by the numbers on the right, while hormone counts are indicated by the numbers on the left. Panel B shows the position of DNA hybridizing to a BPV probe in the hormone-labeled gradient (open squares, panel A). The major form seen is closed circular supercoiled (Form I) DNA and is indicated by the arrow. In this analysis, DNA was not extracted from the first four gradient samples, as these fractions contained the 70% sucrose cushion (see text). Therefore, the first sample shown is gradient fraction 5.

sediment as a 3.6S particle in these gradients (13), these results indicate that the isolated episomes contain the glucocorticoid receptor.

Two control experiments suggest that this receptor association with minichromosomes is a specific phenomenon. First, 100-fold isotopic dilution of the steroid used to label cells results in the depression of the

Figure 4. Isolation of BPV episomes from ID13 cells.

ID13 cells were treated with radioactive hormone and minichromosomes prepared as described in the text. This figure represents the profile of hormone counts through the gradient (top of gradient is to the right). The position of BPV DNA in these gradients was determined by Southern analysis, and was found in fractions 9-18. Closed squares: cells grown in the presence of radioactive hormone only. Open squares: cells grown in the presence of radioactive hormone plus 100-fold excess of cold hormone.

radioactivity bound to episomes (Figure 3A, closed squares). This result indicates that the hormone binding is saturable, as is expected for specific binding of steroid by receptor (13).

Second, cells containing genomic BPV minichromosomes (ID13 cells, 17) were labeled with the same concentration of radioactive hormone, and BPV episomes isolated as described above. The distribution of labeled hormone indicates that a substantially reduced amount of radioactivity co-sediments with the BPV minichromosomes compared to the MTV episomes analyzed above (Figure 4, closed squares). Isotopic dilution of hormone indicates that very little of this radioactivity can be attributed to saturable-binding (Figure 4, open squares). Thus, no significant binding of the glucocorticoid receptor to minichromosomes that lack the MTV GRE can be detected.

Using the data of Figure 3, a calculation of the number of hormone receptor molecules bound to each MTV minichromosome can be made (see **EXPERIMENTAL**). For the experiment shown in Figure 3, the ratio of receptor to minichromosome was 6 to 1. In four additional experiments (data not shown), this ratio varied between 4 and 6 receptors to each minichromosome.

Micrococcal nuclease digestion of MTV minichromosomes isolated from sucrose gradients shows the characteristic nucleosome digestion pattern also

Figure 5. Micrococcal nuclease digestion of enriched minichromosomes.

Minichromosomes isolated from sucrose gradients were digested with micrococcal nuclease as described in the text. Lanes 1-4: isolated minichromosomes digested with 10 U micrococcal nuclease (Boehringer-Mannheim) for 1, 2, 4 or 8 min, respectively. Lanes 5-8: Isolated nuclei from 1361.5 cells digested with micrococcal nuclease for 1, 2, 4 or 8 min, respectively. A MTV LTR probe (-107-+118) was used for hybridization. Arrows indicate positions of mono-, di-, and tri-nucleosomes.

evident in isolated nuclei (Figure 5). Therefore, minichromosomes isolated by this procedure retain a structure consistent with intact chromatin. As a final test of the integrity of isolated episomes, defined alterations in chromatin structure, as revealed by DNase I digestion, were compared to structures present in isolated nuclei. The results of this analysis are revealed in Figure 6A and 6B. Figure 6C gives a map of the Pst I fragment of pM23 that contains the MTV LTR and the Ha-v-ras gene, including the positions of DNase I hypersensitivity. For the data presented here, probe A, as designated in Figure 6C, was used. The positions of the sites detected with this probe were confirmed when probe B, also shown in Figure 6C, was used on the same nuclease digested samples (data not shown).

The most important aspects of this experiment were: a) The DNase I hypersensitivity detected for enriched episomes reflects the hormone-dependent patterns found in nuclei, i.e., GRE hypersensitivity is detected for minichromosomes isolated from cells grown in the presence of hormone, and not detected for those isolated from cells grown in the absence of hormone. b)

Figure 6. Nuclease hypersensitivity of enriched MTV minichromosomes.

Panel A: lanes 1-3 contained DNA prepared from sucrose gradient purified minichromosomes isolated from 10^8 untreated 1361.5 cells. Minichromosome samples were digested with 0, 2, or 4 units of DNase I, respectively. Lanes 4-6 contained DNA prepared from purified minichromosomes isolated from 10^8 hormone-treated 1361.5 cells. Minichromosome samples were digested with 0, 2, or 4 units of DNase I, respectively. Panel B: Nuclease digestion was performed on isolated nuclei. Lanes 1-3 contained DNA samples prepared from untreated 1361.5 cells; 0, 2 or 4 units of DNase I was used, respectively. Lanes 4-6 contained DNA samples prepared from hormone treated 1361.5 cells; 2, 4 or 0 units of DNase I was used, respectively. Numbers to the left indicate position of lambda-EcoRI/HinDIII digested size markers (in nucleotides), while numbers to the right indicate the calculated size of major pm23 bands detected. All samples were digested with PstI before agarose gel fractionation. Panel C represents the map of the LTR-containing 2.6 kb PstI fragment of pm23. The open box indicates the MTV LTR; the hatched region is the GRE. The positions of characteristic restriction enzyme sites are given with respect to the RNA cap-site (designated +1). The positions of the broad hypersensitive regions detected are shown (CH1, IH1 and IH2); coordinates indicate the middle of these hypersensitive regions. Probes A and B are two subcloned regions used for the indirect end-labeling analysis. Probe A was the probe used for the experiments presented here.

The DNase I hypersensitivity detected is identical for MTV minichromosomes present in nuclei or in extracted, soluble form. c) Two types of sites are observed: constitutive (CH1 in Figure 6C) and hormone-inducible (IH1 and IH2 in Figure 6C). d) The position of two of these extrachromosomal hypersensitive regions, IH1 and CH1, are in good agreement with regions detected in integrated MTV chromatin (7).

DISCUSSION

In this work, a method to purify BPV minichromosomes containing the steroid-regulated MTV LTR is described. This method yields highly enriched particles (see Table I). It can be calculated that 2500-fold purification at the level of DNA of these minichromosomes is required to achieve a homogenous preparation. The two step procedure described here achieves approximately 2000-fold purification. In addition, analysis of protein and RNA indicates that the enriched minichromosomes are highly pure with respect to these potential contaminants. The ratio of protein to DNA in these minichromosomes (1.6) is in close agreement with published results for bulk mammalian chromatin (e.g., 1.9 in 18).

Pederson et al (19) have published a procedure for purification of minichromosomes from bakers yeast that is similar to the procedure described here. However, the larger size of the MTV minichromosomes versus the yeast minichromosomes (160S and 30S, respectively) allows for a simpler and more rapid purification procedure. In particular, the isopycnic gradient steps, probably required to remove ribosomal RNA from the yeast episomes (19), are not required for the larger MTV minichromosomes.

The work presented here demonstrates that the glucocorticoid receptor co-purifies with MTV minichromosomes (Figure 3). Control experiments demonstrate that this association involves specific interactions between the receptor and the MTV portion of the episomes. Quantitation of receptor binding indicates that 4-6 molecules of receptor are bound to each minichromosome. This number is in close agreement with the number of binding sites detected on naked DNA (4, 5). While the binding of steroid hormone receptors to naked DNA is well established, this provides the first demonstration of specific receptor association with a chromatin substrate.

The data in Figures 5 and 6 indicate that the chromatin organization of these MTV minichromosomes remains intact following the purification procedure. Micrococcal nuclease digestion of isolated plasmid chromatin results in a

pattern consistent with a nucleosome organization of the DNA. In addition, the DNase I digestion studies indicate that: a) the hormone-dependent changes in GRE chromatin structure are preserved through the procedure, that is, GRE hypersensitivity is detected only in minichromosomes isolated from cells grown in the presence of hormone (see Figure 6A); and b) the positions of hypersensitivity appear the same for nuclear or extracted chromatin.

In particular, the results concerning hypersensitive sites are of general interest. Hypersensitive sites have been reconstituted in vitro on genes from animal cells previously (20). In addition, nuclease hypersensitive sites have been purified along with yeast minichromosomes (19). However, to the best of my knowledge, this is the first demonstration of the enrichment of an intact enhancer-associated nuclease hypersensitive site from mammalian nuclei.

Previous work on chromatin structure has been largely dependent on the use of nucleases or other chemical agents that cleave chromatin. While a great deal of information has been gathered in this fashion, such experimental approaches destroy the features of chromatin structure that are of greatest interest, that is, the hypersensitive sites themselves. The purified MTV minichromosomes described in this report should allow these sites to be probed in vitro using techniques, such as cross-linking of protein and DNA, that are not destructive. Such an approach may give a better understanding of the nature of hypersensitive sites.

Two of the hypersensitive sites found in the MTV minichromosomes, CH1 and IH1 in Figure 6B, appear to be identical to hypersensitive sites previously identified in integrated MTV LTRs (7). The rather broad regions of MTV LTR hypersensitivity detected here are between positions -626 to -706 and -70 to -180 from the RNA cap-site, respectively. These coordinates are in good agreement with those reported for integrated MTV sequences. Additionally, as in the case of integrated MTV sequences, the former is a constitutive site, while the latter is located within the GRE and is induced by hormone treatment. Besides these two sites, another hormone inducible site, termed IH2 in Figure 6C, is found. The area where this site is located (at +480 to +550) is derived from Harvey sarcoma virus, and is reported to contain both RNA pol II and RNA pol III promoters (21, 22). The significance of this hypersensitive site, and of the constitutive site, is unknown.

The purified minichromosomes described here should be useful in the reconstitution of hormone regulation of MTV in vitro. In particular, these isolated minichromosomes will make interesting in vitro transcription

templates, and may allow the association of purified receptor with chromatin to be analyzed in vitro.

ACKNOWLEDGEMENTS

The author would like to thank members of his laboratory for useful discussions through the course of this work. This work was supported by NIGMS grant # GM34615. MCO is a Scholar of the Leukemia Society of America.

REFERENCES

1. Elgin, S.C.R. (1981) *Cell* 27, 413-415.
2. Weisbrod, S. (1982) *Nature* 297, 289-295.
3. Hager, G.L., Richard-Foy, H., Kessel, M., Wheeler, D., Lichtler, A. and Ostrowski, M.C. (1984) *Rec. Prog. Hor. Res.* 40, 121-139.
4. Payvar, F., Firestone, G.L., Ross, S.R., Chandler, V., Wrange, O., Okret, S., Carlstedt-Duke, J., Gustafsson, J.-A. and Yamamoto, K.R. (1983) *Cell* 35, 381-392.
5. Scheidereit, C., Geisse, S., Westphal, H. and Beato, M. (1983) *Nature* 304, 749-752.
6. Chandler, V. L., Maler, B. and Yamamoto, K. R. (1983) *Cell* 33, 489-499.
7. Zaret, K. and Yamamoto, K.R. (1984) *Cell* 38, 29-38.
8. Ostrowski, M.C., Richard-Foy, H., Berard, D., Wolford, R. and Hager, G.L. (1983) *Mol. Cell. Biol.* 3:2045-2057.
9. McEwen, C.R. (1967) *Anal. Biochem.* 20, 114-149.
10. Burton, K. (1968) *Methods Enzymol.* 128, 163-166.
11. Bradford, M. (1976) *Anal. Biochem* 72, 248-262.
12. Hoffmann, P. and Chalkley R. (1978) *Methods Cell Biol.* 17, 1-12.
13. Wrange, O., Carlstedt-Duke, J. and Gustafsson, J.-A. (1984) *J.Biol. Chem.* 254, 9284-9290.
14. Siebenlist, U., Hennighausen, L., Battey, J. and Leder, P. (1984) *Cell* 37, 381-391.
15. Wu, C. (1980) *Nature* 286, 854-860.
16. Gorman, C. M., Moffat, L. F. and Howard, B. H. (1982) *Mol. Cell. Biol.* 2, 1044-1051.
17. Law, M.-F., Lowy, D., Dvoretzky, I. and Howley, P. (1981) *PNAS USA* 78, 2727-2731.
18. Stein, G.S. and Stein, J.L. (1978) *Methods Cell Biol.* 19, 379-385.
19. Pederson, D.S., Venkatesan, M., Thoma, F. and Simpson, R.T. (1986) *PNAS USA* 83, 7206-7210.
20. Emerson, B. and Felsenfeld, G. (1984) *PNAS USA* 81, 95-99.
21. Gruss, P., Ellis, R.W., Shih, T.Y., Konig, M., Scolnick, E.M. and Khoury, G. (1981) *Nature*, 293, 486-488.
22. Ostrowski, M.C., Berard, D. and Hager, G. (1981) *PNAS USA* 78, 4485-4489.