Rapid and reversible changes in nucleosome structure accompany the activation, repression, and superinduction of murine fibroblast protooncogenes *c-fos* and *c-myc*

[chromatin/transcription/histones/mercury(II)-affinity chromatography]

THELMA A. CHEN AND VINCENT G. ALLFREY*

Laboratory of Cell Biology, Rockefeller University, New York, NY 10021

Communicated by Igor Tamm, April 13, 1987 (received for review February 10, 1987)

ABSTRACT A procedure for the isolation of transcriptionally active nucleosomes was used to monitor changes in chromatin structure during the activation, repression, and superinduction of the protooncogenes *c-fos* and *c-myc*. Nuclei were isolated from murine fibroblasts at successive times after stimulation of quiescent cell cultures with serum or platelet-derived growth factor. The nucleosomes released by a brief micrococcal nuclease digestion were fractionated by Hg^{II}-affinity chromatography to separate the unfolded nucleosomes of transcriptionally active genes (in which the sulfhydryl groups of histone H3 are accessible for binding to Hg^{II}) from the compactly beaded nucleosomes of transcriptionally inert DNA sequences (in which the H3 sulfhydryl groups are not accessible). The DNA sequence contents of the Hg^{II}-bound and unbound nucleosome fractions were compared by slot-blot hybridizations to ³²P-labeled cloned probes for *c-fos* and *c-myc*. The binding of the *c-fos* and *c-myc* nucleosomes to the Hg^{II} column accurately reflected both the timing and the degree of their expression, as determined by run-off transcription assays with the isolated nuclei. The superinduction of *c-fos* and *c-myc* expression by an inhibitor of protein synthesis (cycloheximide) was reflected in the persistence of the unfolded, transcriptionally active state of their component nucleosomes. These results provide direct evidence that rapid and reversible changes in nucleosome topography accompany the program of oncogene expression, and they suggest a way to monitor aberrant gene activity during malignant transformation.

The experiments to be described deal with the relationship between chromatin structure and transcription of the protooncogenes *c-fos* and *c-myc*. We used an affinity-chromatographic procedure for the purification of nucleosomes containing transcriptionally active DNA sequences (1) to monitor the changes in conformation of the nucleosomes along the protooncogenes as their transcription is activated or repressed. The technique is based on earlier observations that nucleosomes in transcriptionally active ribosomal genes unfold to uncover the sulfhydryl groups of histone H3, making them accessible to sulfhydryl reagents (2). This suggested that the unfolded nucleosomes of active genes could be retained selectively on organomercurial agarose columns. The feasibility of this approach has been confirmed by studies showing that liver nucleosomes retained by a Hg^{II} column are enriched in DNA sequences transcribed by hepatocytes (e.g., serum albumin and transferrin genes) but lacking in DNA sequences not transcribed in the liver, but in the brain (e.g., preproenkephalin DNA) (1).

What was not clear from those experiments was whether the SH-reactive state of the nucleosome is a dynamic configuration, unfolded only during transcription and refolding

when transcription is terminated, or whether the unfolded state persists after transcription has ceased. To answer this question requires the analysis of nucleosome conformation along the same gene in its active and inactive states. For this reason we selected a system in which the timing of expression of well-defined DNA sequences can be controlled experimentally.

The response of quiescent murine fibroblasts (3T3 cells) to growth factors involves a transient activation of the protooncogenes *c-fos* and *c-myc* as the cells are stimulated to reenter the growth cycle (3-8). Transcription of the *c-fos* gene in BALB/c 3T3 cells is activated within minutes after the addition of fresh serum, or platelet-derived growth factor (PDGF), to the culture medium. Transcription peaks within 10-20 min and then declines abruptly, returning to initial low levels by 30 min (4, 5). The transcription of *c-myc* begins at about 30 min, peaks at 1-2 hr, and declines to preactivation levels at about 4 hr (4). Because all of these events take place before DNA synthesis begins (4, 6), the analysis of changes in nucleosome structure due to transcription is not complicated by changes taking place during nucleosome assembly at the replication fork.

We have used ³²P-labeled cDNA probes for *c-fos* and *c-myc* to analyze the DNA sequence contents of nucleosome fractions separated by Hg^{II}-affinity chromatography at different times after serum or PDGF stimulation of quiescent BALB/c 3T3 cells. We show that the transient expression of both protooncogenes, as detected in run-off transcription experiments, is reflected accurately in the changing affinity of their constituent nucleosomes for the Hg^{II} column. The chromatographic separation of transcriptionally active nucleosomes has also been applied to studies of the mechanism of "superinduction" of oncogene expression when 3T3 cells are exposed to inhibitors of protein synthesis (3, 5, 6, 8). We show that the enhanced expression of *c-fos* and *c-myc* following exposure to cycloheximide involves transcriptional control, as evidenced by the unfolded, SH-reactive state of their nucleosomes and by the synthesis of the cognate RNAs in isolated nuclei.

METHODS

Cell Culture. BALB/c 3T3 mouse embryo fibroblasts were cultured as previously described (4). Confluent, quiescent cells were stimulated by replacing the old medium with fresh Dulbecco's modified Eagle's minimal essential medium containing 20% newborn calf serum or PDGF (Collaborative Research, Waltham, MA) at 3 units/ml. Cells were collected

Abbreviations: *c-fos*, the cellular homologue of FBJ and FBR mouse osteosarcoma virus *fos* genes; *c-myc*, the cellular homologue of the viral *myc* gene of avian myelocytomatosis virus; PDGF, platelet-derived growth factor.

*To whom reprint requests should be addressed.

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.

at 0, 15, 30, 60, 180, 240, and 360 min after addition of fresh medium. For the experiments using cycloheximide, the cells were initially stimulated with 20% newborn calf serum, incubated for 30 or 180 min prior to the addition of cycloheximide (10 μ g/ml), and then incubated for 30 min or 180 min.

Preparation of Nucleosomes. Nuclei were isolated from cultures containing 3×10^8 cells as described (4) except that the lysis buffer was supplemented with 5 mM sodium butyrate (to inhibit histone deacetylases) and with 0.1 mM 1,2-epoxy-3-(*p*-nitrophenoxy)propane and 0.1 mM phenylmethanesulfonyl fluoride. The nuclei were washed and suspended in buffer A [10 mM Tris-HCl, pH 7.4/25 mM KCl/25 mM NaCl/5 mM sodium butyrate/0.1 mM 1,2-epoxy-3-(*p*-nitrophenoxy)propane/0.1 mM phenylmethanesulfonyl fluoride] containing 0.5 mM CaCl_2 and 5 mM MgCl_2 , at a concentration of 1 mg of DNA per ml, as measured by absorbancy at 260 nm. Micrococcal nuclease (Cooper Bio-medical) was added at a concentration of 10 units/ml and, after 5 min at 37°C, the reaction was stopped with 2 mM EGTA (pH 7.5) and rapid cooling to 0°C (9). The nuclear suspension was centrifuged at $10,000 \times g$ for 20 min at 4°C (10), and the supernatant, which contained $\approx 10\%$ of the total DNA, was analyzed. DNA sizing was carried out as described by Maniatis *et al.* (11).

Hg^{II}-Affinity Chromatography of Nucleosomes. The supernatant fraction was adjusted to 5 mM with EDTA (pH 7.5) and applied to an organomercurial agarose column (Affi-Gel 501; Bio-Rad) preequilibrated with buffer A containing 5 mM EDTA. The column was washed with the EDTA-containing buffer at a flow rate of 30 ml/hr to remove the unbound nucleosome fraction, as described (1). The Hg^{II}-bound nucleosomes were then eluted with buffer A containing 5 mM EDTA and 10 mM dithiothreitol (IBI) (1).

DNA Purification. The bound and unbound nucleosome fractions from Hg^{II}-affinity chromatography were digested with RNase A (50 μ g/ml) for 1 hr at 37°C, followed by proteinase K (100 μ g/ml) for 2 hr at 37°C in the presence of 0.1% NaDodSO₄. DNA was then extracted as described by Maniatis *et al.* (12). After dialysis, concentration of the samples, and precipitation in ethanol (12), the DNA was collected by centrifugation at $25,000 \times g$ for 30 min at 4°C. The DNA content of each sample was determined by the diphenylamine reaction (13).

DNA-DNA Hybridization Procedures. Nucleosomal DNA from each sample was blotted onto a nylon membrane (Zeta-Probe; Bio-Rad) in a slot-blot apparatus (Schleicher & Schuell), in quantities of 1 μ g and 5 μ g, following the alkaline procedure recommended by Bio-Rad (14). Membrane-bound DNA was hybridized following the Southern procedure, as described (12). The cDNA probes employed were a 1-kilobase (kb) *Pst* I-*Pvu* II restriction fragment of FBJ osteosarcoma proviral DNA (Oncor, Gaithersburg, MD) and a 1.5-kb *Pst* I-*Aha* III restriction fragment of avian myelocytomatosis virus MC29 (Oncor). The probes, nick-translated with [α -³²P]dCTP to a specific activity of 10^8 dpm/ μ g of DNA (15) and purified on an Elutip affinity column (Schleicher & Schuell), were heat-denatured and added to the hybridization fluid. Hybridization was carried out for 16 hr at 68°C. The filters were washed (12), dried at 25°C on Whatman 3MM paper, covered with Saran wrap, and applied to Kodak X-Omat AR5 film. The film was exposed for various times with a Dupont Cronex I-G Plus intensifying screen at -80°C and developed in Kodak X-Omat M4 developer. The autoradiograms were scanned with a laser densitometer (LKB Ultrascan 2202), and peak areas corresponding to each slot blot were integrated.

Nuclear Run-Off Transcription Assays. Nuclei were isolated as described (4) from fibroblasts at 0, 15, 60, and 210 min after serum stimulation. Cells treated with cycloheximide at

30 min or 180 min after serum stimulation were incubated for an additional 30 min before isolation of the nuclei. Each nuclear preparation was incubated with 100 μ Ci of [α -³²P]UTP (800 Ci/mmol; New England Nuclear; 1 Ci = 37 GBq) and unlabeled nucleoside triphosphates for 30 min at 30°C, as described (4, 16), and treated with DNase I and proteinase K prior to extraction of the RNA and further purification steps (4). RNA samples were hybridized to a 4.8-kb *Xba* I-*Bam*HI restriction fragment from plasmid pSVc-myc-1 (ATCC 41029) or to a mixture of the 1.5-kb *Sst* I-*Eco*RI and 5.3-kb *Eco*RI fragments from plasmid pc-fos(mouse)-3 (ATCC 41041).

RESULTS

Separation of Transcriptionally Active and Inactive Nucleosomes. Unfolding of the nucleosomes in transcriptionally active genes affects the central domain of the nucleosome "cores," where the SH groups of the two histone H3 molecules lie in close proximity (17-23). These groups are not accessible to SH reagents in the compactly beaded nucleosomes of inactive chromatin (2, 18, 21, 24-26), but they become accessible as the nucleosomes unfold during transcription (1, 2). This change in nucleosome topography and SH reactivity along transcriptionally active genes makes it possible to separate the unfolded nucleosomes by Hg^{II}-affinity chromatography (1). Fig. 1 shows a typical separation of the mixture of "active" and "inactive" nucleosomes generated by a brief micrococcal nuclease digestion of isolated 3T3 cell nuclei. After the flow-through fraction of unbound nucleosomes was collected, the Hg^{II}-bound nucleosomes were displaced from the column with 10 mM dithiothreitol. The procedure is rapid and reproducible, and it allows the recovery of >96% of the nucleosomes containing accessible H3 sulphhydryl groups (1).

FIG. 1. Fractionation of nucleosomes by Hg^{II}-affinity chromatography. Nucleosomes released from 3T3 cell nuclei by micrococcal nuclease digestion were applied to an organomercurial agarose column. After elution of the unbound nucleosome fraction (first peak), the Hg^{II}-bound nucleosomes were displaced from the column with 10 mM dithiothreitol (DTT) (second peak). The DNA and histone complements of both fractions were compared in DNA-sizing experiments (Inset A) and in electrophoretic separations of the histones in NaDodSO₄/15% polyacrylamide gels (Inset B). (A) Lane 1: *Hae* III restriction fragments of ϕ X174 DNA used as size markers. Lane 2: DNA of unbound nucleosome fraction. Lane 3: DNA of Hg^{II}-bound nucleosome fraction. DNA was visualized with ethidium bromide. (B) Lane 1: protein standards used as molecular mass markers. Lane 2: histones extracted from the unbound nucleosome fraction. Lane 3: histones extracted from the Hg^{II}-bound nucleosome fraction. All proteins were stained with Coomassie brilliant blue R.

DNA-sizing experiments showed that both fractions contained predominantly monomer-length DNA (170–180 base pairs) (Fig. 1 *Inset A*). Electrophoretic analyses of the nucleosomal histones showed that the proportions of the major core histones (H2A, H2B, H3, and H4) were the same in the Hg^{II}-bound and unbound fractions (Fig. 1 *Inset B*).

Nucleosome Conformational Changes Reflect Protooncogene Activity. Hg^{II}-affinity chromatography was used to fractionate the nucleosomes of murine fibroblasts at successive times following stimulation of quiescent cell cultures with fresh serum or PDGF. The times selected for the analyses of nucleosome structure were based on the elegant studies of Greenberg and Ziff (4), who employed run-off transcription assays in isolated 3T3 cell nuclei to monitor *c-fos* and *c-myc* RNA synthesis after serum stimulation. Their work clearly established that *c-fos* transcription is a very early but transient phenomenon that is detectable within 5 min, peaks in about 15 min, and returns to initial low levels by 30 min. The transcription of *c-myc* follows a different time course; little or no *c-myc* transcription is observed for the first 30 min, then a surge of activity takes place, which peaks in 1–2 hr and regresses to prestimulation levels in about 4 hr. The differences in programming of these two protooncogenes provide an excellent experimental system to test whether the retention of the *c-fos* and *c-myc* nucleosomes on Hg^{II} columns reflects accurately the timing of their expression.

Nuclei were isolated from serum-stimulated 3T3 cells at 0, 15, 30, 60, 180, 240, and 360 min, and each nuclear preparation was treated with micrococcal nuclease to release about 10% of the total DNA. The released nucleosomes were fractionated on Hg^{II} columns to separate the compact nucleosomes of the flow-through fraction from the unfolded nucleosomes retained by the column and subsequently displaced with 10 mM dithiothreitol. The DNA in each of the nucleosome fractions was analyzed by slot-blot hybridizations to ³²P-labeled cloned cDNA probes for *c-fos* and *c-myc*. Comparisons of the slot-blot autoradiograms for the unbound and Hg^{II}-bound nucleosomes at each of the successive times clearly shows that the retention on the Hg^{II} column of the protooncogene nucleosomes depends on the timing of their expression (Fig. 2). For example, *c-myc* DNA sequences are not recovered in the Hg^{II}-bound nucleosomes at 0 or 15 min but are localized exclusively in the flow-through fraction. At 30 min, *c-myc* DNA begins to appear in the Hg^{II}-bound fraction, and by 60 min it is all recovered in the unfolded nucleosomes. Nuclear transcription assays (see Fig. 4B) confirm that the nucleosome conformational changes mirror the activity of the *c-myc* genes at 15 and 60 min. Later, at 4 or 6 hr, the situation is reversed and little or no *c-myc* DNA is associated with the Hg^{II}-bound nucleosome fraction. Quantitative densitometry of less-exposed autoradiograms allows an estimate of the proportions of the total *c-myc* DNA present in the Hg^{II}-bound and unbound nucleosomes at successive times following serum stimulation. A plot of these data (Fig. 3A) shows the reversible nature of the conformational changes taking place in the nucleosomes of the *c-myc* gene during its activation and subsequent repression.

A similar analysis of the distribution of *c-fos* DNA sequences in the Hg^{II}-bound and unbound nucleosomes also confirms that an unfolding of the nucleosome cores takes place when transcription begins. Hybridizations to ³²P-labeled *c-fos* DNA show a more rapid kinetics of nucleosome unfolding than observed for the *c-myc* nucleosomes (Fig. 2). All of the *c-fos* hybridization signal is recovered in the Hg^{II}-bound fraction at 15 min when transcription is at its maximum (Fig. 4B) but at 30 or 60 min, when *c-fos* transcription is barely detectable (ref. 4 and Fig. 4B), its nucleosomes have reverted to the compact configuration and they pass through the Hg^{II} column. At later times, all of the *c-fos* DNA remains associated with the compact nucleosomes of the

FIG. 2. Comparisons of the protooncogene DNA sequence contents of the unbound and Hg^{II}-bound nucleosome fractions. DNA was extracted from each nucleosome fraction prepared at the indicated times after serum stimulation of quiescent BALB/c 3T3 cells. The DNA was hybridized to ³²P-labeled cDNA probes for *c-myc* (Upper) and *c-fos* (Lower). The resulting slot-blot autoradiograms are compared for the unbound and Hg^{II}-bound nucleosomes at each time point. Note that the retention by the column of nucleosomes containing the protooncogene DNA sequences corresponds with the timing of their expression (see text) and that *c-myc*-containing nucleosomes remain in the unfolded configuration after prior cycloheximide treatment of the cells.

flow-through peak. Quantitative densitometry of the slot blots (Fig. 3B) confirms the close correspondence between the timing of *c-fos* transcription [as determined by run-off transcription assays (ref. 4 and Fig. 4B)] and the unfolded state of the nucleosomes.

When quiescent 3T3 cells are stimulated with PDGF, the program of *c-fos* and *c-myc* expression follows that observed after serum stimulation. We compared the DNA sequence contents of the Hg^{II}-bound and unbound nucleosomes prepared 15, 60, and 180 min after PDGF addition and observed the same close correspondence between nucleosome unfolding and transcription of the protooncogenes (data not shown).

Nucleosome Unfolding During Superinduction of Protooncogene Expression. The expression of *c-fos* and *c-myc* in PDGF-stimulated 3T3 cells is superinduced when inhibitors of protein synthesis such as cycloheximide (3, 5, 6, 8, 27, 28), puromycin (5), or anisomycin (27) are added to the culture medium. There is good evidence that this effect is due to a stimulation of transcription of the responding protooncogenes, since superinduction is blocked by actinomycin D (3) and can be monitored by the enhancement of activity of cycloheximide-treated 3T3 cell nuclei in run-off transcription assays (27).

We investigated the relationship between nucleosome structure and the superinduced state of *c-fos* and *c-myc* by Hg^{II}-affinity chromatography of the nucleosomes of serum-stimulated 3T3 cells at different times after exposure to cycloheximide. The times selected were chosen to introduce the protein synthesis inhibitor when virtually all of the *c-fos* and *c-myc* nucleosomes had reverted to the compact, inactive form; i.e., at 30 min for *c-fos* and at 3 hr for *c-myc* (Fig. 3).

FIG. 3. Reversible changes in protooncogene nucleosome structure following serum stimulation of quiescent 3T3 cells. Nucleosomes fractionated by Hg^{II} -affinity chromatography were analyzed for their contents of *c-myc* and *c-fos* DNA by quantitative slot-blot hybridizations to the ^{32}P -labeled cDNA probes. (A) The distribution of *c-myc* DNA in the bound (●) and unbound (○) nucleosome fractions is shown as a function of time. (B) The corresponding distributions of *c-fos* DNA are plotted for the bound (●) and unbound (○) nucleosomes.

Exposure to cycloheximide between 30 and 60 min resulted in reactivation of the *c-fos* gene, as evidenced by the binding of 99% of its constituent nucleosomes to the Hg^{II} column at the 60-min time point (Fig. 4A) and by the activity of the isolated nuclei in run-off transcription assays (Fig. 4B). A similar experiment, in which cycloheximide was added at 3 hr and the nucleosomes were fractionated 30 min later, also showed reactivation of *c-fos* transcription (Fig. 4B), but the effect was transient; 3 hr after cycloheximide addition the *c-fos* nucleosomes had reverted to the compact form and appeared in the flow-through fraction (Fig. 2).

For *c-myc*, the addition of cycloheximide at the 3-hr time point reactivated the gene and all of the *c-myc* hybridization signal was recovered in the Hg^{II} -bound nucleosomes prepared 3 hr later (Fig. 4A). In this case, superinduction leads to a relatively stable unfolded configuration of the *c-myc* nucleosomes, which remain active, as judged by run-off transcription assays (data not shown). Thus, for both *c-fos* and *c-myc*, the inhibition of protein synthesis following transient expression and regression to the inactive state results in reactivation of the protooncogenic chromatin and unfolding of the nucleosomes.

DISCUSSION

The present experiments were designed to determine whether the kinetics of nucleosome unfolding and refolding were compatible with the timing of changes in the transcription of well-defined DNA sequences having different programs of expression. The results are clear-cut. They show that unfolding of the nucleosomes is a rapid process that occurs within minutes when *c-fos* transcription is activated. They show that the effect is gene-specific. No unfolding of the

FIG. 4. (A) Effects of prior exposure to cycloheximide (CHX) on the distribution of protooncogene DNA sequences in the unbound and Hg^{II} -bound nucleosomes of serum-stimulated 3T3 cells. The DNA sequence contents of the nucleosome fractions were compared by slot-blot hybridizations to ^{32}P -labeled probes for *c-myc* (Upper) and *c-fos* (Lower). As judged by the binding of its component nucleosomes to the Hg^{II} column, *c-myc* is fully expressed at 60 min (after addition of cycloheximide at 30 min) and at 360 min (after addition of cycloheximide at 180 min); i.e., all of the *c-myc* hybridization signal is recovered in the Hg^{II} -bound nucleosomes. For *c-fos*, the addition of cycloheximide at 30 min leads to recovery of 99% of the *c-fos* DNA in the Hg^{II} -bound nucleosomes prepared at 60 min. A similar reactivation of *c-fos* is observed 30 min after addition of cycloheximide at 180 min. (B) Synthesis of protooncogene RNAs in isolated fibroblast nuclei. Nuclei were isolated at the indicated times after stimulation and incubated with [α - ^{32}P]UTP in run-off transcription assays (4). The nuclear RNA was extracted and hybridized to a 4.8-kb probe for *c-myc* DNA (Left) or to probes containing 6.8 kb of *c-fos* DNA (Right). Note that transcription of each protooncogene at 15 min and at 60 min parallels the binding of its component nucleosomes to the Hg^{II} column (Fig. 2). Addition of cycloheximide at 30 min leads to reactivation of *c-fos* transcription as measured at 60 min (*60). Cycloheximide added at 180 min also reactivates *c-fos* transcription as assayed 30 min later (**210). Note that *c-myc* has not yet been reactivated under these conditions, a result in accord with its original slow response to serum stimulation.

c-myc nucleosomes is observed during the activation of the *c-fos* gene, but later, when *c-fos* transcription is suppressed and *c-myc* reaches its peak of activity, all of the *c-myc* nucleosomes bind to the Hg^{II} column. The results also show that the nucleosome "cores" along each protooncogene revert to the compact, beaded state as its transcriptional activity ceases. Therefore, unfolding of the nucleosomes is a dynamic and reversible process that accurately reflects the timing and extent (Figs. 3 and 4) of transcription of the associated DNA sequences. Evidence that this change in topography of the nucleosome core depends on the hyperacetylation of histones H3 and H4 is presented elsewhere (1).

Of particular interest is the correspondence between nucleosome conformation, as determined by Hg^{II} -affinity chromatography, and the superinducing effects of a protein synthesis inhibitor on *c-fos* and *c-myc* expression. We find that cycloheximide treatment results in a transient reactivation of *c-fos* that is reflected in the unfolding of its nucleosomes and in the enhanced synthesis of *c-fos* RNA in isolated nuclei. Similar conformational changes take place during the superinduction of *c-myc*. Our results not only confirm that a block in protein synthesis leads to an augmen-

tation of *c-fos* and *c-myc* expression (3, 5, 6, 8, 27, 28), they show that this effect must involve some aspect of chromatin reorganization to facilitate transcription. While there is compelling evidence that the stability of the *c-fos* and *c-myc* mRNAs are also key factors in the program of oncogene expression (29–35) and that the protooncogene mRNA lifetimes are extended in cycloheximide-treated cells (29, 31), we conclude that such posttranscriptional controls are not the only or primary basis for the superinducing effects of cycloheximide in serum-stimulated 3T3 cells. Instead, the changes in nucleosome structure and the enhancement of protooncogene transcription are more supportive of the hypothesis that cycloheximide prevents the synthesis of labile, transacting repressor proteins and thus maintains the genes in their active states (3, 36). The latter view is also supported by the identification of DNA sequences near the *c-myc* promoters that suppress its expression (36) and by the observation that factors in human cells in which *c-myc* is inactive can suppress an active mouse *c-myc* gene in mouse-human hybrid cells (37).

The changes in nucleosome conformation that accompany the activation of *c-fos* and *c-myc* are part of a larger program of chromatin structural changes, some of which affect regulatory DNA sequences (38–46). How the conformational changes within the nucleosomes of the *c-fos* and *c-myc* transcription units are coupled to alterations in DNA superhelicity and DNase I-hypersensitivity at specific sites in their flanking nontranscribed regions remains an open and challenging problem.

Unfolding of the nucleosome core particles has now been observed to correlate with transcription in species as diverse as myxomycetes (2) and mammals (1). The change in nucleosome topography can be monitored by Hg^{II}-affinity chromatography, a relatively simple and reproducible procedure that allows the selective recovery of the nucleosomes being transcribed at the time. In concert with DNA-DNA or DNA-RNA hybridization techniques, Hg^{II}-affinity chromatography should find many applications in studies of gene expression and its control. It should also prove to be a powerful approach to the detection of aberrant gene expression during malignant transformation.

We are greatly indebted to Anthony Cozzolino for expert and dedicated technical assistance. This research was supported by National Institutes of Health Grant CA14908-14 and by Grant NP-228 from the American Cancer Society.

- Allegra, P., Sterner, R., Clayton, D. F. & Allfrey, V. G. (1987) *J. Mol. Biol.* **196**, in press.
- Prior, C. P., Cantor, C. R., Johnson, E. M., Littau, V. C. & Allfrey, V. G. (1983) *Cell* **34**, 1033–1042.
- Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. (1983) *Cell* **35**, 603–610.
- Greenberg, M. & Ziff, E. B. (1984) *Nature (London)* **311**, 433–437.
- Kruijer, W., Cooper, J. A., Hunter, T. & Verma, I. M. (1984) *Nature (London)* **312**, 711–716.
- Muller, R., Bravo, R., Burckhardt, J. & Curran, T. (1984) *Nature (London)* **312**, 716–720.
- Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenschein, G. E. (1984) *Cell* **36**, 241–247.
- Cochran, B. H., Zullo, J., Verma, I. M. & Stiles, C. D. (1984) *Science* **226**, 1080–1082.
- Nelson, D., Covault, J. & Chalkley, R. (1980) *Nucleic Acids Res.* **8**, 1745–1763.
- Bloom, K. S. & Anderson, J. N. (1978) *Cell* **15**, 141–150.
- Maniatis, T., Jeffrey, A. & van deSande, H. (1978) *Biochemistry* **14**, 3787–3794.
- Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
- Burton, K. (1968) *Methods Enzymol.* **12**, 163–169.
- Bio-Rad (1986) *Technical Bulletin 1110* (Bio-Rad, Richmond, CA).
- Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
- Groudine, M., Peretz, M. & Weintraub, H. (1981) *Mol. Cell. Biol.* **1**, 281–288.
- Camerini-Otero, R. D. & Felsenfeld, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5519–5523.
- Zama, M., Olins, D. E., Wilkinson-Singley, E. & Olins, A. (1978) *Biochem. Biophys. Res. Commun.* **85**, 1446–1452.
- Mirzabekov, A. D., Shick, V. V., Belyavsky, A. V. & Bavykin, S. G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4184–4188.
- Eshagpour, H., Dieterich, A. E., Cantor, C. R. & Crothers, D. M. (1980) *Biochemistry* **19**, 1797–1805.
- Prior, C. P., Cantor, C. R., Johnson, E. M. & Allfrey, V. G. (1980) *Cell* **20**, 597–608.
- Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D. & Klug, A. (1984) *Nature (London)* **311**, 532–537.
- Burlingame, R. W., Lowe, W. E., Wang, B. C., Hamlin, R., Xuong, N. H. & Moundrianakis, E. N. (1985) *Science* **228**, 546–553.
- Wong, N. T. N. & Candido, E. P. M. (1978) *J. Biol. Chem.* **253**, 8263–8268.
- Dieterich, A. E., Axel, R. & Cantor, C. R. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 199–206.
- Bode, J., Henco, K. & Wingender, E. (1980) *Eur. J. Biochem.* **110**, 143–152.
- Greenberg, M. E., Hermanowski, A. L. & Ziff, E. B. (1986) *Mol. Cell. Biol.* **6**, 1050–1057.
- Bravo, R., Burckhardt, J., Curran, T. & Muller, R. (1980) *EMBO J.* **5**, 695–700.
- Dani, C., Blanchard, J. M., Piechaczyk, M., El Saboudy, S., Marty, L. & Jeanteur, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7046–7050.
- McCormack, J. E., Pepe, V. H., Kent, R. B., Dean, W., Marshak-Rothstein, A. & Sonenschein, G. E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5546–5550.
- Mitchell, R. L., Zokas, L., Schreiber, R. D. & Verma, I. M. (1985) *Cell* **40**, 209–217.
- Eick, D., Piechaczyk, M., Henglein, B., Blanchard, J. M., Traub, B., Kofler, E., Wiest, S., Lenoir, G. M. & Bornkamm, G. W. (1985) *EMBO J.* **4**, 3713–3725.
- Rabbitts, P. H., Forster, A., Stinson, M. A. & Rabbitts, T. H. (1985) *EMBO J.* **4**, 3727–3733.
- Piechaczyk, M., Yang, J.-Q., Blanchard, J. M., Jeanteur, P. & Marcu, K. B. (1985) *Cell* **42**, 589–597.
- Treisman, R. (1985) *Cell* **42**, 889–902.
- Chung, J., Randall, R. R. & Leder, P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7918–7922.
- Feo, S., ar-Rushdi, A., Heubner, K., Finan, J., Nowell, P. C., Clarkson, B. & Croce, C. (1985) *Nature (London)* **313**, 493–495.
- Saito, H., Hayday, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7476–7480.
- Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. (1984) *Cell* **37**, 381–391.
- Wiman, K. G., Clarkson, B., Hayday, A. C., Saito, H., Tonegawa, S. & Hayward, W. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6798–6802.
- Schubach, W. & Groudine, M. (1984) *Nature (London)* **307**, 702–708.
- Tuan, D. & London, I. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2718–2722.
- Fahrlander, P. D., Piechaczyk, M. & Marcu, K. B. (1985) *EMBO J.* **4**, 3195–3202.
- Dyson, P. J., Littlewood, T. D., Forster, A. & Rabbitts, T. H. (1985) *EMBO J.* **4**, 2885–2891.
- Renz, M., Neuberger, M., Kurz, C., Bravo, R. & Muller, R. (1985) *EMBO J.* **4**, 3711–3716.
- Treisman, R. (1986) *Cell* **46**, 567–574.