Tissue-Specific Binding of Total and β-Globin Genomic Deoxyribonucleic Acid to Non-Histone Chromosomal Proteins from Mouse Erythropoietic Cells†

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ABSTRACT: The synthesis and DNA binding activity of purified nuclear non-histone proteins from mouse erythroblasts and myoblasts have been compared by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, affinity chromatography, and protein blotting. The labeled non-histone proteins bound to mouse total DNA clearly differ between erythroid and muscle cell lines, but these differences mainly reflect the qualitative changes observed in their pattern of synthesis. By contrast, a cloned genomic mouse β-globin DNA fragment binds specifically several proteins (100K, 65K, 50K, 45K, and 34K) from erythropoietic Friend cells and does not bind any protein in the corresponding fraction from myoblasts. The specificity of these DNA protein interactions requires a NaCl concentration of 0.1 M and a low protein/DNA ratio. In these conditions λ DNA binds the above proteins to only a small extent. During the dimethyl sulfoxide induced terminal differentiation of Friend mouse erythroleukemia (MEL) cells, there is an apparent overall decrease of total as well as globin DNA binding to the nuclear non-histone proteins but not to the histones, whereas no significant qualitative changes are detected.

In bacteria, gene activity is regulated at the transcriptional level by proteins that bind to DNA at specific sites known as operator sites (Jacob & Monod, 1961) and thereby control the expression of adjacent genes (Gilbert & Mueller-Hill, 1967; Ptashnē, 1967). The complex mechanism of eukaryotic cell differentiation involves the regulation of gene expression at many levels by still largely unknown interactions. Proteins must therefore exist that recognize specific sites as signals, both on DNA, for its replication or transcription, and on its newly synthesized RNA transcripts for their splicing, maturation, and transport. Thus it seems likely that in higher organisms DNA and RNA binding nuclear proteins should exist that are capable of irreversibly selecting among the total genetic information a sequence of developmental programs.

Nuclear non-histone proteins (NHP) have been extensively studied as such possible control elements for genetic expression [for a review, see Elgin & Weintraub (1975)]. Among them, some proteins of the HMG group (Weisbrod & Weintraub, 1979) behave like DNA binding proteins, the apparent role of which is to maintain DNA in an "opened" configuration at the chromatin level (Weintraub & Groudine, 1976). Numerous attempts have also been made to demonstrate species (Teng et al., 1971; Wakabayashi et al., 1973; Chiu et al., 1974; Sevall et al., 1975; Dastugue & Crépin, 1979) or tissue specificity of NHP binding to DNA or a specific control exerted by this fraction on in vitro transcription by RNA polymerase (Teng et al., 1971; Shea & Kleinsmith, 1973; Barrett et al., 1974; Chiu et al., 1975; Crépin & Dastugue, 1979). However, data from these studies are of limited relevance, since they usually are dealing with a heterogeneous group of unpurified proteins and involve as experimental target the entire genome rather than specific coding regions.

Recent progress has been achieved in our knowledge of the eukaryotic gene organization [for a review, see Breathnach & Chambon (1981)]. More particularly, the availability of partially or completely sequenced, genomic fragments, including the noncoding adjacent regions, and the possibility of analyzing the DNA binding of individual proteins by the "blotting" technique (Bowen et al., 1980) offer the opportunity to identify one or several NHP bound to specific sequences in a well-characterized DNA genomic fragment. Moreover, the way this specific DNA binding pattern correlates with the process of cell differentiation can be readily explored.

In this paper we have compared the one-dimensional sodium dodecyl sulfate ($NaDodSO_4$) electrophoresis pattern and the DNA binding properties of the non-histone chromosomal proteins purified from mouse cells committed to express different genetic programs and able to undergo terminal differentiation in vitro: myoblasts and Friend erythroid cells (MEL).

Using a cloned mouse β-globin genomic DNA fragment or the λ vector fragments as radioactive probes, we have identified by protein blotting on nitrocellulose filters several non-histone tissue-specific DNA binding proteins in the chromosomal fraction of Friend erythropoietic cells.

Materials and Methods

Cell Culture. Mouse Friend erythroleukemia cells (MEL) (clone 707 1C) were cultivated in Ham's F.13 medium with 15% horse serum. Induction of globin synthesis was carried out by addition of dimethyl sulfoxide (Me_2SO) to a final concentration of 1.5% at a cell density of 5×10^4 cells/mL. After 4 days, induced or uninduced cells reached a saturation density of about 10^6 cells/mL and were harvested. Both types of cells were used as a source of erythroblasts (Ross et al., 1974). Mouse muscle cells from clone 10 T984 (Jakob et al., 1978) were cultivated in plastic dishes in a mixture of modified Eagle's medium and medium 199 plus 10% fetal calf serum.

Labeling Conditions. MEL cells were harvested from their growth medium by centrifugation for 10 min at 600*g* and washed in phosphate-buffered saline (PBS). They were resuspended in methionine-free medium plus [^{35}S]Met (10

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μCi/mL) at a density of 4×10^7 cells/mL in 75-mL tissue-culture flasks (Falcon N 3024). Incubations of varying lengths of time were performed at 37 °C in a humidified atmosphere containing 5% CO_2. Muscle cells were labeled for 5 h with [^{35}S]methionine [50 μCi dish^{-1} (10 mL)$^{-1}$] in medium containing 5×10^{-6} M methionine.

Preparation of DNA and Non-Histone Proteins. Bacteriophages λgtWES C and the certified EK2 recombinant λgtWES G2 (Tilghman et al., 1977) containing the mouse *Eco*RI globin fragment were propagated in *Escherichia coli* C600 with a P2 facility (Wood, 1966). DNA was purified as described by Gross-Bellard et al. (1973) and labeled by nick-translation synthesis with [^{32}P]dCTP (Amersham) as precursor in the reaction (Rigby et al., 1977). The integrity of the DNA was checked by agarose gel electrophoresis of the *Eco*RI restriction fragments (Enquist et al., 1976). Nuclei and chromatin were prepared as already described (Dastugue & Crêpin, 1979). Non-histone chromosomal proteins were obtained according to MacGilliwray et al. (1972). Briefly, the final pellet of chromatin was homogenized in 2 M NaCl, 5 M urea, and 1 mM phosphate (pH 7.1). After dialysis against the same buffer the solution was sonicated and centrifuged at 15000*g* for 15 min. The supernatant was applied to a column of hydroxylapatite previously equilibrated with the same buffer. After an extensive washing for elimination of histones, the non-histone protein fractions were eluted stepwise with 50, 100, and finally 200 mM phosphate, pH 7.1, in a salt–urea buffer as above.

*DNA–Cellulose Chromatography. Eco*RI-restricted mouse DNA was covalently bound to [(aminobenzoxy)methyl]cellulose (Miles Yeda) by the diazotation procedure described by Noyes & Stark (1975). Usually, 18 μg of DNA was bound per mg of cellulose with a yield of 90%. Non-histone proteins and DNA–cellulose were dialyzed together against the binding buffer containing 0.1% glycine for 1 h at 20 °C; cellulose was pelleted, washed several times with binding buffer, and finally eluted with a minimum volume of electrophoresis sample buffer containing 2% $NaDodSO_4$. Bound proteins were analyzed on one-dimensional 0.1% $NaDodSO_4$–12.5% acrylamide–1% bis(acrylamide) slab gels (O'Farrell, 1975).

Detection of specific DNA binding proteins was carried out by the protein blotting technique of Bowen et al. (1980) in the conditions described in the legends of the figures.

Results

Electrophoretic Analysis of Purified Chromosomal Non-Histone Proteins Synthesized in Erythropoietic and Muscle Cells. ^{35}S-Labeled proteins were extracted with 5 M urea–2 M NaCl from the chromatin of two different committed mouse cell types, MEL cells, either uninduced or induced to synthesize globin, and dividing myoblasts or fusioned myotubes. Non-histone proteins (NHP) were purified by hydroxylapatite chromatography. Figure 1A shows the comparative pattern of synthesis of NHP fractions eluted at 50, 100, and 200 mM phosphate, after the bulk of the histones has been washed off with a 1 mM phosphate flow through (panel A, lane 1). MEL cells are found to differ according to whether they are induced or not by only one band, at 34K, which is nearly missing in uninduced Friend cells (Fc^-) in the 50 mM phosphate eluate (panel A, lane 2). However, clear tissue-specific differences between MEL cells and myoblasts are found only in the more acidic NHPs eluted later from hydroxylapatite, at 100 mM (panel B, compare lanes 2 and 3) and 200 mM (panel C, compare lanes 2 and 3). For instance, the 100K protein is eluted later in myoblasts (panel C, lane 3) than in MEL cells (panel B, lanes 1 and 2), and in addition, several proteins

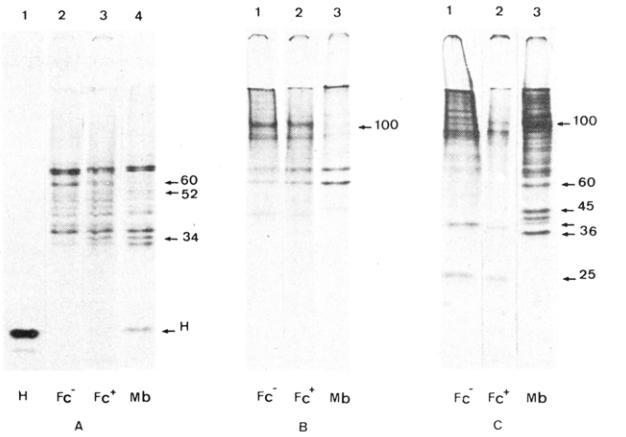

FIGURE 1: $NaDodSO_4$ gel electrophoresis of [^{35}S]methionine-labeled non-histone proteins derived from MEL cells and myoblasts. Proteins were prepared and partially purified as described under Materials and Methods, from cells labeled for 4–5 h with [^{35}S]methionine (sp act. 10 μCi/mL) in methionine-free medium supplemented with 15% serum. The samples were dialyzed against 2 M NaCl–0.5% $NaDodSO_4$, precipitated with 3 volumes of acetone at −20 °C, redissolved in electrophoresis sample buffer (O'Farrell, 1975) heated at 100 °C for 3 min in the presence of 5% β-mercaptoethanol, and loaded on a 0.1% $NaDodSO_4$–12.5% acrylamide–1% bis(acrylamide) gel. After electrophoresis, the gel was fixed in 40% methanol–10% acetic acid and then dried and autoradiographed. (A) NHP eluted at 50 mM phosphate, pH 7: (lane 1) (Mb) Histone fraction not retained on hydroxylapatite at 1 mM phosphate (15 000 cpm); (lane 2) NHP from uninduced MEL cells (FC^-) (33 000 cpm); (lane 3) NHP from Me_2SO-induced MEL cells (FC^+) (40 000 cpm); (lane 4) NHP from myoblasts (Mb) (34 000 cpm). (B) NHP eluted at 100 mM phosphate, pH 7.1: (lane 1) (FC^-) NHP (25 000 cpm); (lane 2) (FC^+) NHP (27 000 cpm); (lane 3) (Mb) NHP (24 000 cpm). (C) NHP eluted at 200 mM phosphate, pH 7.1: (lane 1) (FC^-) NHP (58 000 cpm); (lane 2) (FC^+) NHP (47 000 cpm); (lane 3) (Mb) NHP (70 000 cpm).

indicated by arrows in the range between 25K and 60K appear to characterize either MEL cells or myoblasts.

When these fractions are further purified by DNA–cellulose chromatography, the electrophoretic pattern obtained after eluting DNA-bound material derived from either erythroblasts or myoblasts confirms and extends their tissue-specific differences (Figure 2B). Interestingly, whereas no striking differences in the *synthesis* of 50 mM NHPs could be detected between MEL cells and myoblasts (Figure 1A), the elution of the corresponding *DNA-bound* material of the 50 mM fraction revealed relatively different affinities for some proteins: As protein input increases, at a fixed DNA concentration and providing saturation was not reached, the 60K and 38K proteins appear to be bound earlier in MEL cells, whereas the opposite is true for the 52K protein in myoblasts. Therefore, at a fixed protein/DNA ratio, the elution pattern of these proteins appears *quantitatively* but not necessarily *qualitatively* different between MEL cells and myoblasts (Figure 2A). Moreover, it is clear enough that the 45K DNA binding protein in MEL cells has no counterpart in myoblasts, where instead is found a protein migrating slightly faster at 44K (Figure 2A).

Although is must be pointed out that a number of these apparent tissue-specific differences could be related to the different activity of DNA replication within the two cell populations concerned (since, contrary to MEL cells, whether induced or not, cell division still occurred in myoblasts), these results could suggest a role of these chromosomal NHPs in the expression of at least a certain group of genes differentially expressed in the myogenic and erythrogenic programs. By contrast, at least in the 50 mM NHP fraction, we were not able to detect any modification of the elution pattern of

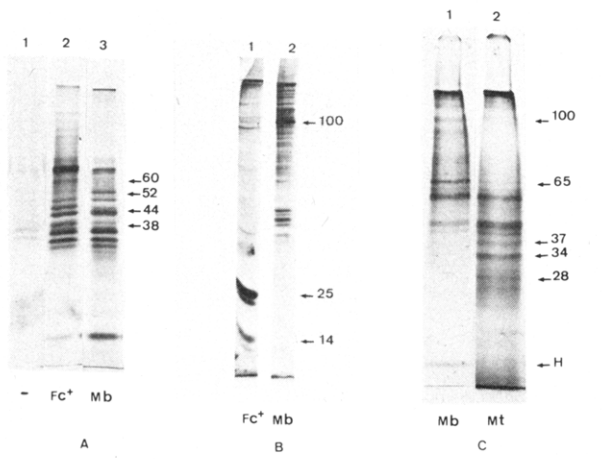

FIGURE 2: $NaDodSO_4$ gel electrophoresis of nuclear non-histone proteins bound to mouse total DNA. ^{35}S-Labeled non-histone proteins eluted from hydroxylapatite at 50 and 200 mM phosphate (pH 7.1) from induced MEL cells (FC^+), myoblasts (Mb), and myotubes (Mt) were dialyzed 1 h at room temperature against 10 mM Tris-HCl, pH 7.8, 100 mM NaCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM dithiothreitol, and 0.1% glycine in the presence of mouse *Eco*RI-restricted DNA–cellulose prepared as described under Materials and Methods. The cellulose was centrifuged, washed twice in the above binding buffer, and transferred to a second Eppendorf tube. The bound proteins were then eluted with electrophoresis sample buffer containing 2.1% $NaDodSO_4$ and separated by electrophoresis on a 12.5% acrylamide slab gel. Usually 10–25% of the input NHP was bound to DNA, depending on the NHP/DNA ratio and the source of NHP. (A) DNA-bound NHP from the 50 mM phosphate fractions (Figure 1A): (lane 1) 15 μg of FC^+ NHP eluted from cellulose without DNA (sp act. 18 000 cpm/μg); (lane 2) 15 μg of FC^+ NHP and (lane 3) 27 μg of Mb NHP (sp act. 10 000 cpm/μg). The amount of DNA was 3 μg in both cases. Apparent molecular weights are indicated at arrows; H, histones. (B) DNA bound NHP from the 200 mM phosphate fraction (Figure 1C): (lane 1) 10 μg of FC^+ NHP; (lane 2) 30 μg of Mb NHP. The amount of DNA was 3 μg in both cases. (C) DNA bound NHP from the 50 mM phosphate fraction: (lane 1) 10 μg of Mb NHP; (lane 2) 9 μg of Mt NHP. The amount of DNA was 20 μg in both cases.

DNA-bound NHPs related to Me_2SO-induced maturation of MEL cells (data not shown).

Figure 2 (panel C) shows that, contrary to the situation observed during MEL cells induction, myoblast DNA-bound NHPs differ, at low protein/DNA ratio, from those of myotubes, since, several proteins being present in roughly identical amounts, by comparison, others are clearly preferentially bound according to the particular cell type.

Identification of Tissue-Specific Non-Histone Proteins That Bind Preferentially to the Purified Mouse β-Globin Gene. Both the complexity of the eukaryotic genome and the heterogeneity of non-histone proteins render it difficult to draw precise conclusions regarding the specificity of their interactions. However, we took advantage of the technique of protein transfer from polyacrylamide gels to nitrocellulose filters, recently developed by Bowen et al. (1980), in an attempt to detect a specific binding of the individual non-histone proteins analyzed above to a purified gene. Non-histone proteins were separated by $NaDodSO_4$ gel electrophoresis and then transferred to nitrocellulose filters after renaturation. The filters were incubated with ^{32}P-labeled DNA probes: for this purpose we used the purified DNA of the recombinant phage λgtWESM G2, containing a 7-kb genomic mouse β major region inserted between the two remaining *Eco*RI restriction sites in the middle of the 42-kb phage DNA. The globin DNA fragment contains the structural gene, the introns, and the 5′ and 3′ adjacent sequences (Tilghman et al., 1977; Edgell et al., 1981). As a control for globin sequence specificity we used also the DNA of the original phage λgtWES (λc), which does

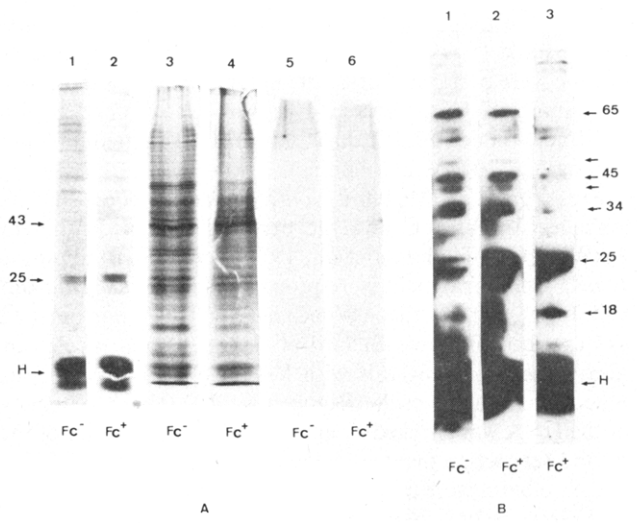

FIGURE 3: Protein transfer from polyacrylamide gel to nitrocellulose filters and binding of mouse β-globin DNA to the unfractionated chromosomal proteins from MEL cells. After gel electrophoresis as described in Figure 1, the gel was cut into identical strips. (A) (Lanes 1 and 2) Staining pattern and (lanes 3 and 4) 28-day autoradiograph of ^{35}S-labeled unfractionated chromosomal proteins from 16 μg of FC^- (lanes 1 and 3) and 15 μg of FC^+ (lanes 2 and 4). Lanes 5 and 6 show the simultaneous autoradiograph of the corresponding proteins remaining untransferred to nitrocellulose filters. (B) Autoradiograph of the nitrocellulose filters incubated with DNA for 60 min in 30 mL of binding buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, 0.02% Ficoll 400, 0.02% BSA fraction I, and 0.02% polyvinyl pyrolidone), containing 0.1 M NaCl, and then washed for 3 h with three changes of the same buffer: (lane 1) FC^- NHP and (lane 2) FC^+ NHP both incubated with ^{32}P-labeled globin DNA (1 ng, 25 000 cpm/mL); (lane 3) FC^+ NHP incubated with ^{32}P-labeled λWES DNA (1 ng, 28 000 cpm/mL).

not contain the globin insert. These DNA probes were labeled with ^{32}P by nick translation and incubated with the nitrocellulose filters containing the transferred non-histone proteins. Figure 3A shows the gel staining pattern of the unfractionated chromosomal proteins (lanes 1 and 2), which were transferred from an identical gel to nitrocellulose filters. The source of NHP was respectively from uninduced (lane 1) and Me_2SO-induced MEL cells (lane 2). The efficiency of protein elution from the gel to nitrocellulose was checked by comparing the ^{35}S-labeled protein patterns of two identical strips of the same gel, one having been transferred (lanes 5 and 6) and the other having been incubated in the same conditions without nitrocellulose transfer as a control (lanes 3 and 4). From their comparison, it is clear that, except for the intense 43K band, the nitrocellulose filters were not oversaturated and the proteins were faithfully and extensively transferred, albeit somewhat less completely in the high molecular weight region.

The DNA autoradiographs shown in Figure 3B identify those proteins of the chromatin that bind λ-globin DNA (lanes 1 and 2) and λWES DNA (lane 3) at a salt concentration of 0.1 M NaCl. It is clear from a comparison of the stained or labeled gel patterns shown in Figure 3A with these autoradiographs that some proteins bind while many do not, thus indicating that DNA does not bind indiscriminately. An intense and indiscriminating DNA binding to the 25K region, where H1 and protein IP 25 (Keppel et al., 1977) migrate, as well as to the large excess of histones, easily visible on the stained pattern (Figure 3A, lanes 1 and 2) but poorly labeled by [^{35}S]methionine (lanes 3 and 4), obscured the autoradiographs in Figure 3B. In spite of this, it remains obvious that some proteins, indicated by arrows at 65K, 45K, and 34K, preferentially bind λ-globin DNA in uninduced (lane 1) as well as in Me_2SO-induced MEL cells (lane 2) (compare to

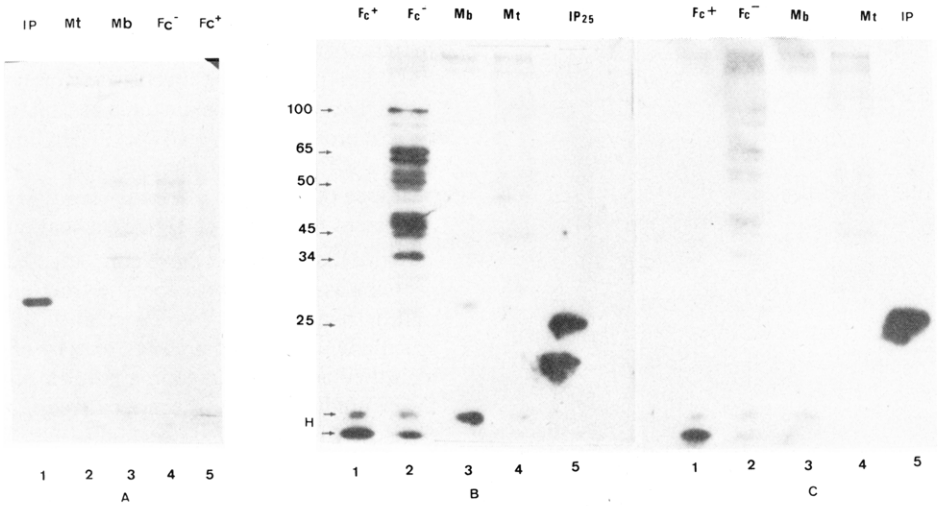

FIGURE 4: Binding of mouse β-globin DNA to the purified 50 mM NHP fraction from MEL cells and myoblasts. Purified NHPs eluted at 50 mM phosphate from hydroxylapatite, derived from either uninduced (FC^-) or induced Friend cells (FC^+), myoblasts (Mb), or fusioned myotubes (Mt), were separated by electrophoresis on a 12.5% acrylamide–0.1% $NaDodSO_4$ gel. Two identical gels were run in parallel; one gel was stained with Coomassie blue. (A) (Lane 1) FC^+ NHP (8 μg); (lane 2) FC^- NHP (14 μg); (lane 3) Mb NHP (7 μg); (lane 4) Mt NHP (6 μg); (lane 5) IP_{25} DNA binding protein (Keppel et al., 1977) (10 μg). Proteins in the other gel were transferred to nitrocellulose filters by the sandwich method (Bowen et al., 1980). Thus, each filter was the symmetric replicate of the same gel and was incubated as described in Figure 3 with the same amount of either ^{32}P-labeled β-globin DNA or ^{32}P-labeled λWES DNA (5 ng, 0.6×10^5 cpm/mL). The filters were then washed in binding buffer containing 0.1 M NaCl as described in Figure 3 and dried. (B) Autoradiograph of the filter containing transferred protein samples described in (A) incubated with ^{32}P-labeled globin DNA in 20 mL of binding buffer (5 ng, 0.6×10^5 cpm/mL). (C) Autoradiograph of the symmetric replicate filter incubated with ^{32}P-labeled λWES DNA in 20 mL of binding buffer (5 ng, 0.62×10^5 cpm/mL).

the λWES DNA binding to induced MEL cell NHPs shown in lane 3).

Since the large amount of histones, as well as contaminating RNA and DNA, present in the unfractionated chromosomal proteins might interfere with the detection of the specific DNA binding to non-histone proteins, we used in subsequent experiments, the purified non-histone fraction eluted at 50 mM phosphate from hydroxylapatite, which is free of nucleic acids and only slightly contaminated by histones. The non-histone proteins were analyzed by electrophoresis on two identical $NaDodSO_4$ gels. Figure 4A shows the stained pattern of purified chromosomal protein IP 25 (lane 1), NHP from myotubes (lane 2), myoblasts (lane 3), and uninduced (lane 4) and Me_2SO-induced MEL cells (lane 5). Since only a few micrograms of NHP have been loaded on the gels, in order to favor specific protein–DNA interactions occurring at a low protein/DNA ratio, one can visualize only a few accumulated major nuclear proteins that might be structural proteins. The autoradiographs shown in panels B and C identify those transferred proteins that bind DNA at a salt concentration of 0.1 M NaCl. Since the two nitrocellulose filters were symmetrical replicates of the same gel and were incubated with the same amount of radioactive DNA probe, they are strictly comparable. At this relatively high-salt concentration, DNA remains bound only to high-affinity proteins. Marker proteins, including lysozyme, were completely transferred and did not pick up any DNA (data not shown) although some DNA binding to lysozyme was reported by others (Bowen et al., 1980) at a lower salt concentration. The comparison of the two autoradiographs of Figure 4 clearly shows that at least ten proteins, distributed in five groups, namely, at 100K, 65K, 50K, 45K, and 34K, in uninduced MEL cells (lane 2) bind intensively the globin-containing DNA fragment in panel B, for a relatively much lower level of binding to the λWES DNA control is observed in panel C. As expected, the histones and chromosomal protein IP 25 (lane 5) bind tightly but indiscriminately both types of DNA. Surprisingly, although we have used half as much Fc^+ NHP as Fc^- NHP, no DNA binding to the Me_2SO-induced MEL cell NHP fraction was observed (lane 1). However, this apparent overall nonspecific decrease of the DNA binding activity does not seem to involve the histones, whose binding to DNA is still detected. When histone binding was used as a control, the signal ratio of the histones to Fc^- NHP shows that these latter proteins are indiscriminately tightly bound to the DNA. The absence of a similar signal from Fc^+ cells strongly suggests that the DNA binding capacity of the proteins is lost in the Me_2SO-treated cells.

One of the most interesting features is the lack of strong DNA binding activity in the myoblast (lane 3) as well as in the myotube NHP (lane 4) for either β-globin DNA (panel B) or λWES DNA (panel C). Since the muscle cell NHPs were purified exactly by the same procedure as MEL cell NHPs and since they bind total mouse DNA as well (Figure 2), these results clearly demonstrate the specific ability of a class of non-histone proteins present in erythroid Friend cells for binding the β-globin gene preferentially.

Since Bowen et al. (1980) reported the importance of thoroughly renaturing the proteins in order to preserve their DNA binding specificity, we have analyzed native non-histone proteins in 6 M urea, pH 8.5, gels containing no $NaDodSO_4$. In these conditions we observed a preferential tight binding of globin DNA to high molecular weight components that were not separated on this type of gel (data not shown). Therefore, we analyzed the same preparation of native proteins on a 0.1% $NaDodSO_4$ gel as above. In addition, the protein/DNA ratio was considerably reduced by increasing the DNA concentration in order to favor specific interactions. Figure 5 shows the autoradiographs identifying DNA binding NHPs when the nitrocellulose filters are incubated with either λ-globin DNA or λWES DNA at a salt concentration of 0.1 M NaCl. Interestingly, the nonspecific binding to λ DNA appears almost completely abolished in these conditions (lanes 3 and 4) whereas the globin DNA binds almost exclusively to a protein from uninduced MEL cells with an apparent molecular weight of 45K (lane 2).

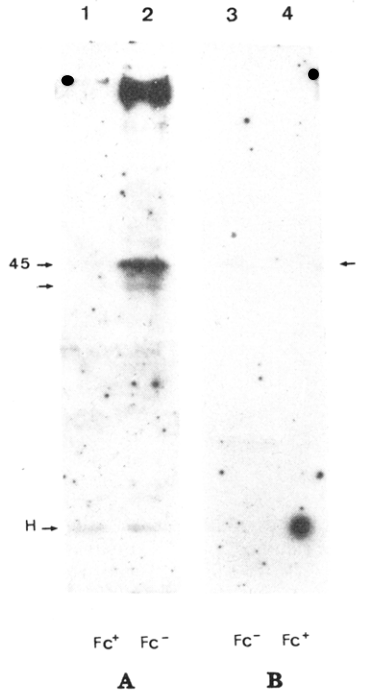

FIGURE 5: Binding of mouse β-globin DNA to NHP from MEL cells at a low protein/DNA ratio. 7.5 μg of NHP (50 mM fraction) from either uninduced or Me_2SO-induced MEL cells was loaded on a 12.5% acrylamide (0.1% $NaDodSO_4$) gel, but without any previous denaturation of the samples by $NaDodSO_4$ or heating. After electrophoresis the proteins were transferred to nitrocellulose filters by the sandwich method. The filters were then incubated with either the purified ^{32}P-labeled β-globin DNA fragment (200 ng, 0.1×10^5 cpm/mL) or an equivalent amount of the ^{32}P-labeled λ *Eco*RI DNA fragment containing the early promotors. The filters were washed in binding buffer containing 0.1 M NaCl, dried, and autoradiographed: (A) Autoradiograph of the filter, incubated with globin DNA; (B) autoradiograph of the filter, incubated with λ DNA; (lanes 1 and 4) FC^+ NHP (7.5 μg); (lanes 2 and 3) FC^- NHP (7.5 μg).

However, the highest specific globin DNA binding is observed with a high molecular weight, presumably multimeric component, which is present in both uninduced and Me_2SO-induced MEL cell NHPs, although it binds much more tightly to the uninduced MEL cell type (compare lanes 1 and 2).

Discussion

The comparative electrophoretic gel analysis of the nonhistone chromosomal proteins presented in this paper confirms an earlier report [for a review, see Elgin & Weintraub (1975)] showing a limited tissue specificity of this nuclear protein fraction. More precisely, we observed a few tissue-specific proteins when mouse erythroblasts and myoblasts are compared, the greatest specific differences being found among acidic chromosomal proteins or the NHPs that bind to mouse total DNA. These findings might suggest that the structural and/or enzymatic functions played by the bulk of NHP common to all tissues might be modulated in order to acquire a limited tissue and/or sequence specificity as expected for possible gene regulatory molecules.

Contrary to other reports (Peterson & McConkey, 1976; Lunadei et al., 1978; Neumann et al., 1978a), we have not observed reproducible differences in the pattern of synthesis in the total DNA binding activity of NHP derived from MEL cells before and after their Me_2SO-induced terminal differentiation. The reason why we have not observed these differences might be due to the fact that these authors have analyzed the NHP not only from different clones but also much earlier after induction, since the various changes observed might be transient as described for the protein 25K (Keppel et al., 1977). By contrast, we found a clearly different pattern of the bulk of DNA binding NHP following the transition from dividing myoblasts to fusioned myotubes. Since it is unlikely that trace amounts of contaminating sarcomeric muscle proteins could bind specifically to the DNA with such a high affinity, these changes might be related to the appearance of a new transcriptional activity in fused myotubes and/or to the arrest of DNA replication.

More recent studies have concentrated on the isolation of proteins associated with specific fractions of DNA or chromatin [reviewed by Weideli et al. (1980)]. Thus, several specific DNA binding proteins, or class of proteins, have been identified or purified, and in a number of cases, their specific DNA binding site has been localized (Engelke et al., 1980; Hsieh & Brutlag, 1979; Lee et al., 1979; Weideli et al., 1978, 1980; Pederson & Bhorjee, 1975; Jack et al., 1981). There are now more evidences for the existence, more or less far from the presumptive transcription initiation site at the 5′ gene extremity, of exposed DNA regions (Wu & Gilbert, 1981) or additional, sequence-specific, protein binding sites (Jack et al., 1981). However, a regulatory role of these sequences is still to be demonstrated.

In the present study, we have attempted to characterize among chromosomal NHP derived from either erythroblasts or myoblasts specific DNA binding proteins for a particular cloned genomic DNA fragment: the β globin that is in vivo transcriptionally active or inactive depending on the above type of cells examined (Tilgham et al., 1977). Our results show that among total NHP from MEL cells a limited number is able to bind preferentially the eucaryotic DNA. Moreover, the strength or the stability of these protein–DNA interactions differs between the type of cells studied: in protein blotting experiments using the *purified* 50 mM phosphate fractions of the NHP, we failed to detect any DNA binding to NHP from Me_2SO-induced MEL cells and muscle cells, whereas a strong specific eucaryotic DNA binding was observed to a limited number of polypeptides derived from uninduced MEL cells. The lack of β-globin DNA binding to the NHP derived from myoblasts or myotubes demonstrates the tissue specificity of the protein–DNA interactions that we have identified in erythropoietic cells.

Our results also show the critical importance of the protein/DNA ratio for the detection of specific DNA binding proteins. We found that lowering this ratio allows a selection of the most specific DNA binding proteins (compare experiments of Figures 4 and 5). This is in accordance with the results of Jack et al. (1981), which demonstrate sequence-specific binding of a protein component for a short region near a major *Drosophila* heat-shock protein gene.

Earlier reports showed that the induction of MEL cells to express globin genes after Me_2SO treatment is accompanied by a modification of the chromatin and a decreased content of NHP in the mononucleosomes obtained after micrococcal nuclease treatment (Neumann et al., 1978b). Nevertheless, we believe that the unexpected lack of DNA binding to the purified NHP fraction from mature MEL cells is not related to the smaller amount of Fc^+ NHP but might be due to our stringent experimental conditions selecting the most stable interactions. Thus, it might reflect a decreased DNA binding affinity of this particular NHP fraction after Me_2SO induction rather than a decreased NHP content. This assumption is based upon the following observations: (1) The patterns of NHP synthesis and the amount of NHP used in blotting experiments are roughly similar for mature and immature MEL cells. (2) Using less stringent binding conditions, and par-

ticularly a higher protein/DNA ratio, we have observed no significant differences in the pattern of globin DNA binding to unfractionated chromosomal proteins derived from MEL cells in both states of differentiation. This decreased DNA binding activity could be explained by some modification of this NHP fraction following Me_2SO induction such as phosphorylation. We are presently testing this hypothesis.

Finally, it must be clearly emphasized that the above-described DNA specificity of NHP binding must not be overinterpreted. We have used a cloned β-globin gene as a useful tool in order to further characterize the tissue and gene specificity of the NHP. As a first step toward this direction, the present results demonstrate the existence of a class of NHPs that binds globin DNA but not λ DNA. However, these proteins could well be mouse DNA binding proteins that have no specificity for a particular gene. However, their tissue specificity would suggest that they could be related to the differential expression of at least certain groups of genes. There is presently no doubt that isolation of tissue-specific DNA binding polypeptides such as those we have described for a cloned eucaryotic gene should be a new general approach to study regulatory proteins involved in the control of gene expression at the transcriptional level. We are presently investigating the existence of DNA binding proteins with both specific binding sites and in vitro transcription modulating activity on the β-globin gene (Triadou et al., 1981).

References

Barrett, T., Maryanka, D., Hamlyn, P. H., & Gould, H. J. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 5057–5061.

Bowen, B., Steinberg, J., Laemmli, U. K., & Weintraub, H. (1980) *Nucleic Acids Res. 8*, 1–19.

Breathnach, R., & Chambon, P. (1981) *Annu. Rev. Biochem. 50*, 349–383.

Chiu, J. F., Craddock, C., Morris, H. P., & Hnicila, L. S. (1974) *FEBS Lett. 42*, 94–97.

Chiu, J. F., Tsai, Y. H., Sakuma, K., & Hnilica, L. (1975) *J. Biol. Chem. 250*, 9431–9433.

Crêpin, M., & Dastugue, B. (1979) *Eur. J. Biochem. 99*, 499–505.

Dastugue, B., & Crêpin, M. (1979) *Eur. J. Biochem. 99*, 491–498.

Edgell, M. H., Weaver, S., Jahn, C. L., Padgett, R. W., Phillips, S. J., Voliva, C. F., Comer, M. B., Hardies, S. C., Haigwood, N. L., Langley, C. M., Racine, R. R., & Hutchison III, C. A. (1981) in *Organization and Expression of Globin Genes* (Stamatoyannopoulos, G., & Nienhuis, A. W., Eds.) pp 69–88, Liss, New York.

Elgin, S. R. C., & Weintraub, H. (1975) *Annu. Rev. Biochem. 44*, 725–774.

Engelke, D. R., Ng, S. Y., Shastry, B. S., & Roeder, R. G. (1980) *Cell (Cambridge, Mass.) 19*, 717–728.

Enquist, L., Teimeier, D., Leder, D., Weisberg, R., & Sernberg, N. (1976) *Nature (London) 259*, 596–598.

Gilbert, W., & Mueller-Hill, B. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 2415–2421.

Gross-Bellard, M., Oudet, P., & Chambon, P. (1973) *Eur. J. Biochem. 36*, 33–38.

Hsieh, T. S., & Brutlag, D. C. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 726–730.

Jack, R. S., Gehring, W. J., & Brach, C. (1981) *Cell (Cambridge, Mass.) 24*, 321–331.

Jacob, F., & Monod, J. (1961) *J. Mol. Biol. 3*, 318–329.

Jakob, H., Buckingham, M. E., Cohen, A., Dupont, L., Fiszman, M., & Jacob, F. (1978) *Exp. Cell Res. 114*, 403–408.

Keppel, F., Allet, B., & Eisen, H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 653–656.

Lee, D., Castro, C. E., Jagodzinsky, L. L., & Sevall, J. S. (1979) *Biochemistry 18*, 3160–3165.

Lunadei, M., Matteucci, R., Ullu, E., Gambari, R., Rossi, G. B., & Fantoni, A. (1978) *Exp. Cell Res. 114*, 468–471.

MacGillivray, A. J., Cameron, A., Krauze, R. J., Rickwood, D., & Paul, J. (1972) *Biochim. Biophys. Acta 277*, 384–402.

Neumann, J. R., Whittaker, R., Blanchard, B., & Ingram, V. (1978a) *Nucleic Acids Res. 5*, 1675–1687.

Neumann, J. R., Housmann, C. D., & Ingram, V. (1978b) *Exp. Cell Res. 111*, 277–284.

Noyes, B. E., & Stark, G. R. (1975) *Cell (Cambridge, Mass.) 5*, 301–310.

O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007.

Pederson, T., & Bhorjee, J. S. (1975) *Biochemistry 14*, 3238–3242.

Peterson, J. L., & McConkey, E. H. (1976) *J. Biol. Chem. 251*, 555–558.

Ptashnê, M. (1967) *Nature (London) 214*, 232–234.

Rigby, P. W. J., Dieckmann, M., Rhodes, C., & Berg, P. (1977) *J. Mol. Biol. 113*, 237–251.

Ross, J., Gielen, J., Packman, S., Ikawa, Y., & Leder, P. (1974) *J. Mol. Biol. 87*, 697–714.

Sevall, J. S., Cockburn, A., Savage, M., & Bonner, J. (1975) *Biochemistry 14*, 782–789.

Shea, M., & Kleinsmith, L. J. (1973) *Biochem. Biophys. Res. Commun. 50*, 473–477.

Teng, C. S., Teng, C. T., & Allfrey, V. G. (1971) *J. Biol. Chem. 266*, 3597–3609.

Tilghman, S. M., Tiemeier, D. C., Polsky, F., Edgell, M. H., Seidman, J. G., Leder, A., Enquist, C. W., Norman, B., & Leder, P. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4406–4410.

Triadou, P., Lelong, J.-C., Gros, F., & Crêpin, M. (1981) *Biochem. Biophys. Res. Commun. 101*, 45–54.

Wakabayashi, K., Wang, S., Hord, G., & Hnilica, L. S. (1973) *FEBS Lett. 32*, 46–48.

Weideli, H., Scheal, P., Artavanis-Tsakonas, S., Stewart, R., Yuan, R., & Gehring, W. J. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 693–700.

Weideli, H., Brack, C., & Gehring, W. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3773–3777.

Weintraub, H., & Groudine, M. (1976) *Science (Washington, D.C.) 93*, 848–853.

Weisbrod, S., & Weintraub, H. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 630–634.

Wood, W. B. (1966) *J. Mol. Biol. 16*, 118–133.

Wu, C., & Gilbert, W. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1577–1580.