Vol. 130, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1985 Pages 364-371

PRESENCE OF NUCLEOSOMAL REPEAT IN THE TRANSCRIBED α GLOBIN GENE OF INDUCED MURINE ERYTHROLEUKEMIA CELLS

N.Kirov, I.Tsaneva and R.Tsanev

Institute of Molecular Biology, Bulgarian Academy of Sciences
1113 Sofia, Bulgaria

Received May 16, 1985

The sensitivity of the mouse α-globin gene to micrococcal nuclease and its nucleosomal repeat were studied in three different functional states of the gene: inactive in EAT cells, potentially active in uninduced MEL cells and active in induced MEL cells. The results show that: 1.The nuclease sensitivity of the gene differs in the three different functional states. 2.Both the coding and the 5′-flanking regions of the induced actively transcribed gene show a typical nucleosomal repeat pattern. 3.Hypersensitive sites for micrococcal nuclease and for an endogenous nuclease appear upstream of the gene after induction of differentiation.

The data concerning the nucleosomal structure of transcribing nonribosomal chromatin are controversial. Several studies support the idea that active genes are organized in nucleosomes (1-4), while other data show the absence of nucleosomal repeat in active heat-shock genes (5,6), in immunoglobulin genes (7), and a disturbed repeat in the yeast galaktokinase gene (8). Evidently, more studies on different models are needed to draw a clear picture of the nucleosomal organization of active genes in chromatin.

In the present report we have studied the sensitivity to micrococcal nuclease and the nucleosomal repeat of the mouse α-globin gene in three different functional states: inactive in Ehrlich ascites tumor cells, potentially active in murine erythroleukemia (MEL) cells, and actively transcribed in induced MEL cells. The results show that the conformation of the α-globin chromatin changes upon induction, as demonstrated by its increased nuclease sensitivity, but its nucleosomal repeat pattern is preserved. At the same time, in induced MEL cells several nuclease hypersensitive sites appear - one close to the 5′-flanking region of the α-globin gene, and others from 1 kb up to about 100 kb away from the gene.

0006-291X/85 $1.50

MATERIALS AND METHODS

Friend MEL cells, clone F4N, were grown in Eagle's minimum essential medium (Gibco) supplemented with 10% fetal calf serum. For induction of hemoglobin synthesis, exponentially growing cells were treated with 2% dimethylsulfoxide for 5-6 days. The percentage of induced cells, as determined by the benzidine reaction (9), was about 80% after 6 days of induction. The viability of the cells was determined by the trypan blue dye exclusion and was about 90%. Ehrlich-Lettré hyperdiploid ascites tumor (EAT) cells were propagated in albino mice and collected 8 days after inoculation. Nuclei of all cellular types were isolated as previously described (10).

For nuclease digestion one volume of the nuclear suspension (about 30 A_{260} units/ml) was mixed with two volumes of the digestion buffer (0.2M sucrose, 80 mM NaCl, 10 mM tris-HCl (pH8), 1 mM $CaCl_2$) and 5-7 enzyme units of micrococcal nuclease (Sigma, 90 U/mg) were added per 1 A_{260} unit of nuclei. The digestion was carried out at 25°C for different periods of time and the reaction was stopped by EDTA and cooling on ice. Acid-soluble products were determined in aliquots from the incubation mixture and DNA from the acid-insoluble pellet was extracted twice with 0.67 N $HClO_4$ for 10 min at 80°C. DNA was determined by the optical density, assuming 1 A_{260} unit equal to 50 µg of DNA. Isolation and purification of DNA was as previously described (10).

For quantitative determination of nuclease sensitivity of the α-globin gene, DNA from the nuclease digests was applied to filters, hybridized to the labelled DNA probe from the coding region as described below, and the radioactivity retained on the filters was measured. To this end, aliquots of 15 µg of DNA, heat-denatured at 100°C for 10 min, were applied either to nitrocellulose filters (11), or to Zetabind filters (kindly supplied by AMF, Inc., Cuno, Microfiltration Products Division, Meriden, Conn., USA; control experiments showed that Zetabind filters retained more DNA than nitrocellulose). Radioactivity was measured in a LKB Ultrobeta 1210 scintillation counter using a toluene/Triton X100 scintillation cocktail (12). The value obtained for the control filters, containing aliquots of denatured salmon sperm DNA, was substracted from all the other values. The percentage of hybridization was calculated from the proportion of filter-bound radioactivity of digested to undigested nuclear DNA. The nuclease sensitivity of the α-globin gene was finally expressed as a ratio of the percentage of remaining hybridizable sequences to the percentage of remaining total DNA.

DNA (20 µg per lane) was fractionated by electrophoresis in 1.2% agarose slab gels in 20 mM Na-phosphate buffer, pH 7.5, 1 mM EDTA. In each lane 0.1 µg of Hind III-digested λ-phage DNA were included as internal standards. After electrophoresis DNA was denatured and transferred to nitrocellulose filters by Southern blotting (13).

As hybridization probes Hinf1 restriction fragments of the α-globin gene (A and B in Fig.1) were isolated as described (14) from the plasmid pML2 containing a 2.1 kb genomic fragment of the adult mouse α_1-globin gene (kindly provided by Dr.Rougeon, Inst.Pasteur, Paris). The probes were labelled by nick-translation with [α^{32}P]-dCTP (Amersham kit PB 5100) to a specific radioactivity of 1-2.10^8 dpm/µg.

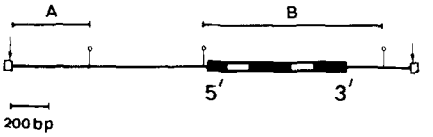


Fig. 1 Scheme of the 2.1 kb genomic fragment containing the mouse α-globin gene. $EcoR_1$ (↓) and Hinf1 (↑) restriction sites; $EcoR_1$ linkers (□ □).

Prehybridization, hybridization and washing of the nitrocellulose filters were carried out as described (10) and of the Zetabind filters as prescribed by the manufacturer.

To visualize the molecular-weight standards, the filters were rehybridized with radioactive λ-phage DNA after washing three times with boiling 0.1 xSSC, 0.1% SDS and cooling down to room temperature.

The dry filters were autoradiographed for 4 to 10 days at -70°C on HSII X-ray films (ORWO) with an itensifying screen EU-B3-U4.2 (USSR).

RESULTS

The sensitivity to micrococcal nuclease digestion was defined as degradation of the sequences to fragments which cannot hybridize to the labelled DNA probe. As shown in Fig.2, the sensitivity of the α-globin gene depended on its functional state. The inactive gene in EAT cells was digested at the same rate as total DNA while the rate of digestion of the gene in MEL cells was much greater than that of bulk chromatin. Moreover, the active gene in induced MEL cells was digested more rapidly than the potentially active gene in uninduced cells.

The repeat lengths of total chromatin from EAT, uninduced and induced MEL cells were evaluated from the plots of nucleosome number vs DNA length, measured on the electrophoregrams (not shown). The values obtained were 168±2 bp for MEL cells (both induced and uninduced) and 181 ± 2 bp for EAT cells.

To study the periodic structure of the α-globin gene, DNA from the nuclear digests was fractionated on agarose gels and subjected to blot-hybridization

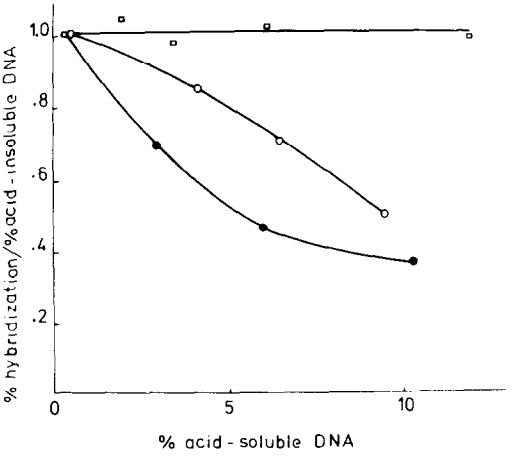


Fig. 2 Sensitivity of the α-globin chromatin to digestion with micrococcal nuclease in nuclei from EAT cells (-□-□-), uninduced (-o-o-) and induced (-●-●-) MEL cells. Hybridization with the DNA probe from the coding region on Zetabind filters.

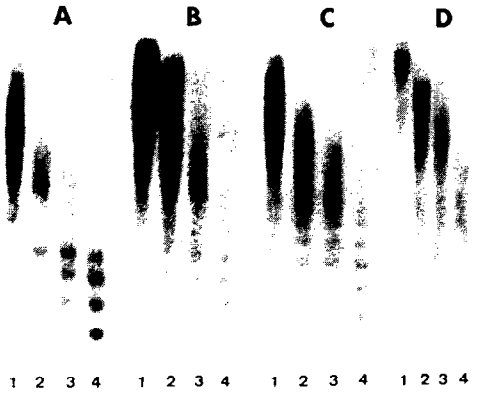
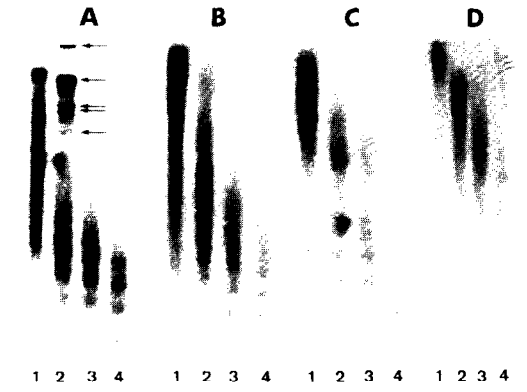


Fig. 3 Hybridization of fractionated DNA from nuclear digests to the DNA probe from the coding region. Nuclei from induced (A), uninduced (B) MEL cells and EAT cells (C) digested with micrococcal nuclease to 1, 3, 6 and 9 % acid-soluble products (1 to 4). Total DNA from MEL cells (D) digested with 0.7, 1.3, 2.7 and 5.3 U of micrococcal nuclease per mg DNA (1 to 4)for 10 min at 25°C.

with two labelled probes - from the 5′-flanking region and from the coding region of the gene (A and B in Fig.1). As seen in Fig.3, hybridization with the coding region produced a typical nucleosomal repeat pattern in all three types of cells. DNA fragments up to pentamers only could be distinguished in MEL cells, while higher oligomers were present in the digests of EAT cells. No periodicity of the nucleosomal type could be observed in the autoradiographs of naked DNA from MEL cells digested with micrococcal nuclease.

To avoid incorrect evaluations of DNA mobilities due to overloading of the gels used for blot-hybridization, the size of a DNA band was calculated from autoradiographs containing internal standards of Hind III-digested λ-phage DNA. This permitted to establish that the repeat length of the coding region of the α-globin gene was 167 ± 5 bp for both induced and uninduced cells, i.e. identical to that of the total chromatin. Similarly, in EAT cells the gene showed a longer repeat - 176 ± 5 bp - practically the same as in total chromatin

Hybridization with the 5′-flanking region of the gene gave the same results (Fig.4): a clear nucleosomal repeat in all three types of cells and repeat lengths the same as those of the corresponding total chromatin. Specific of this probe was the detection of several sharp bands of high-molecular weights, which could be observed only in the nuclear digests of induced MEL cells



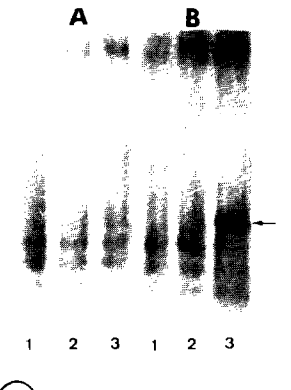


Fig. 4 Hybridization of fractionated DNA from nuclear digests to the DNA probe from the 5′-flanking region. Nuclei from induced (A), uninduced (B) MEL cells, EAT cells (C) and total DNA from MEL cells (D),digested as in Fig. 3.

Fig. 5 Hybridization of autodigested nuclear DNA to the DNA probe from the 5-flanking region. Nuclei from unindiced (A) and induced (B) MEL cells incubated at 25°C in the digestion buffer for 0, 10 and 40 min (1 to 3).

(Fig.4A). They appeared early in the course of digestion, then their intensities gradually changed and finally they disappeared. Thus, an optimal extent of digestion was needed for their detection. No such bands could be found in EAT cells, in uninduced MEL cells or in naked DNA.

A DNA fragment of about 1 kb, hybridizing to the 5′-flanking probe only, was produced by endogenous nucleolysis of induced MEL cells (Fig.5).

DISCUSSION

Our results show that micrococcal nuclease can distinguish between three different functional states of the α-globin gene, in agreement with the data for the β-globin gene of MEL cells (15,16). This demonstrates that some structural changes take place in the α-globin chromatin to make it potentially active and then active after induction. The increased nuclease sensitivity of the gene in induced differentiated cells as compared to uninduced MEL cells is a good indication that the bulk of the cells have their α-globin gene in an actual state of transcription.

The observation of a repeat hybridization pattern,on the other hand,shows that although the active α-globin gene is digested at a higher rate than the potentially active or inactive genes, still the basic difference in nuclease

protection between core and linker DNA is preserved. This result cannot be due to hybridization to the inactive α_3 and α_4 pseudogenes. $EcoR_1$ restriction fragments of these genes gave under our experimental conditions a cross-hybridization with the labelled probe 10 times less intensive as autoradiographic signal than the α-globin gene (not shown).

The finding of a nucleosomal repeat in the coding region of the active α-globin gene is in disagreement with data concerning some other types of active nonribosomal genes - heat-shock (5,6) and immunoglobulin (7) genes,but is in accordance with the observed repeat in the active β-globin gene of MEL cells (4), and in the active ovalbumin gene (2). A reasonable explanation of this controversy would be that the preservation of the nucleosomal repeat should depend on the rate of transcription. Some reversible conformational changes in the nucleosome may occur only when it interacts with the polymerase. These changes could be very transient and will remain undetected by the enzyme when the rate of initiation is low enough, or may become a permanent state of the transcribed chromatin when this rate is very high (6,21).

It is also worth noting that the length of the repeat of the α-globin gene coincided with the value for the bulk chromatin in both EAT and MEL cells, and was not affected by the induction process. The total chromatin repeat length of our cells is shortened, compared to the repeat lengths reported for some mouse tissues: 190 bp for spleen (4) and 195 for liver (17). This is especially true for our MEL cell line (168 bp) but a similarly shortened repeat length (174 bp) was found for another MEL cell Line (4).

It has been reported that the repeat length of the β-globin gene in MEL cells is longer than that of bulk chromatin and some other inactive genes and it was argued that an increased repeat length may be an intrinsic property of active or potentially active genes (4).Since the developmental control of the events leading to the expression of the α-globin gene seems to be different from that of the β-globin gene and the two genes reside in different chromatin domains (18) it should not be surprising that their repeat lengths are dif-

ferent. However, in the light of our data the validity of the idea that activation of a gene is related to a longer repeat is strongly questioned.

The presence of several sharp nonnucleosomal DNA bands arising from the α-globin gene in induced MEL cells only,(Fig.4) demonstrates the appearance of nuclease hypersensitive sites in the differentiated cells. The existence of DNAse I-hypersensitive sites was found to be a universal feature of genes which are active or have a history of transcription (19). Our data show that there is one hypersensitive site close to the 5′-end of the α-globin gene (between our probes A and B in Fig.1) which is recognized by an endogenous nuclease and by the micrococcal nuclease. In this region a DNAse I-hypersensitive site has been reported for the same gene (20). The endogenous nuclease recognizes also a second site, upstream of the globin gene, which is about 1 kb away from the first. The other nuclease hypersensitive sites recognized by the micrococcal nuclease are located far away - up to 100 kb - from the gene, and might be related to the activation in the differentiated cells of other genes clustered upstream of the α-globin gene.

ACKNOWLEDGMENT

The authors are grateful to Dr.F.Rougeon for his kind gift of the plasmid.

REFERENCES

1. Lacy, E. and Axel, R. (1975) Proc. Natl. Acad. Sci. USA 72, 3978-3982.
2. Bellard, M., Gannon, F. and Chambon, P. (1978) Cold Spring Harbor Symp. Q.Biol. 42, 779-791.
3. Barsoum, J., Levinger, L. and Varshavsky, A. (1982) J. Biol. Chem. 257, 5274-5282.
4. Smith, R.D., Seale, R.L. and Yu, J. (1983) Proc. Natl. Acad. Sci. USA 80, 5505-5509.
5. Wu, C., Wong, Y.-G. and Elgin, S.C.R. (1979) Cell 16, 807-814.
6. Karpov, V.L., Preobrazhenskaya, O.V. and Mirzabekov, A.D. (1984) Cell 36, 423-431.
7. Weischet, W.O., Glotov, B.O. and Zachau, H.G. (1983) Nucleic Acids Res. 11, 3593-3612.
8. Lohr, D. (1983) Nucleic Acids Res. 11, 6755-6773.
9. Orkin,S.H.,Haras,P.I. and Leder,P.(1975) Proc.Natl.Acad.Sci.USA 72,98-102.
10. Kirov,N., Djondjurov,L. and Tsanev,R. (1984) J.Mol.Biol. 180, 601-614.
11. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205.
12. Anderson, L.A. and McClure, W.O. (1973) Anal. Biochem. 53, 173-179.
13. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
14. Dretzen, G., Bellard, M., Sassone-Lorsi, P. and Chambon, P. (1981) Anal.Biochem. 112, 295-298.

15. Smith, R.D. and Yu, J. (1984) J.Biol. Chem. 259, 4609-4615.
16. Smith, R.D., Yu, J. and Seale, R.L. (1984) Biochemistry 23, 785-790.
17. Gottesfeld, J.M. and Melton, D.A. (1978) Nature 273, 317-319.
18. Rifkind, R.A., Sheffery, M. and Marks, P.A. (1984) in: Advances in Cancer Research 42, pp. 149-166, Academic Press.
19. Elgin, S.C.R. (1981) Cell 27, 413-415.
20. Sheffery,M. Marks,P.A. and Rifkind,R.A. (1984) J.Mol.Biol. 172, 417-436.
21. Levy, A. and Noll, M. (1981) Nature 289, 198-203.