Acta Veterinaria (Beograd), Vol. 46. No. 4, 173–184, 1996.

UDK 619:577.214

NUCLEAR EXTRACT FROM REL CELLS CONTAINS GATA-1 TRANSCRIPTION FACTOR

SONJA PAVLOVIĆ, TATJANA MITROVIĆ, M. JOKSIMOVIĆ, GORDANA NIČEVIĆ and ZVEZDANA POPOVIĆ

Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 283, P. O. Box 794, 11001 Beograd, Yugoslavia

(Received,

Preparation of nuclear extracts from different tissues may provide the proteins that regulate gene expression in those tissues. REL (rat erythroleukemia) cells represent a permanent erythroid cell line. They are the most convenient model system for studying rat globin gene expression. Nuclear extracts from REL cells were prepared for the first time in our laboratory. We also determined the most important steps in the procedure of nuclear protein isolation, as well as the way to control these steps. In this paper we show that nuclear extracts from REL cells contain the transcription factor GATA-1.

Key words: REL cells, nuclear proteins, nuclear extract preparation, GATA-1

INTRODUCTION

The processes of expression of globin genes from different species can be mimicked in transgenic mice or established erythroid cell lines (MEL, K562, HEL, HD3, REL cells). The established erythroid cell lines are especially convenient for studying globin gene expression. MEL (mouse erythroleukemia) cells (Friend, 1957) are commonly used in these experiments. Thus, this model system has been used in studies on the expression of globin genes from different species (deBoer et al., 1988.; Stuve and Myers, 1990.; Macleod and Plumb, 1991.; Galson and Housman, 1988.; Fong and Emerson, 1992.). However, the results obtained in MEL cells could be ambiguous even incorrect, for heterologous DNAs (e. g. human, chicken, goat, rat etc.).

We are investigating in the expression of rat β-globin genes. Fortunately, a cell line originating from rat erythroid tissue is already established. Thus, REL (rat erythroleukemia) cells should be very useful for us because they represent a homologous system for studying rat globin gene expression. REL (rat erythroleukemia) cells represent a permanent cell line from transplantable tumors from 7-12 dimethylbenz(α)anthracene-induced erythroleukemia of the Long-Evans rat (Kluge et al., 1976.). This cell line maintains its erythroid nature. Erythroid differentiation can be induced by dimethylsulfoxide (Me_2SO). This is shown by a decrease in cell size, the appearance of red and benzidine positive cells, and induced synthesis of adult globin chains.

The control of globin gene expression is a great challenge for scientists. The search for potential activators and repressors of these genes has led to the discovery of proteins called transcription factors which bind to DNA upstream and downstream of the coding sequences (genes). These proteins are nonhistone chromosomal proteins. Isolation of transcription factors is indispensable for studying gene expression. Thus preparation of nuclear extracts from the cells whose gene expression we are interested in, particularly, the isolation of the nonhistone protein fraction, may provide us with gene regulatory proteins.

The GATA-1 transcription factor is a DNA binding protein found in erythroid, mast and megakaryocytic cell lineages (deBoer et al., 1988; Martin et al., 1990; Romeo et al., 1990.). GATA-1 binds to a core DNA consensus, GATA, in the promoter and enhancer sequences of various erythroid genes such as globin, porphobilinogen deaminase and erythropoietin receptor genes (deBoer et al., 1988; Mignotte et al., 1989; Chiba et al., 1991). GATA-1 is required for the correct differentiation of the erythroid lineage (Pevny et al., 1991) and is a potent transcriptional regulator of its target genes. It is capable of transactivating promoters containing GATA-1 binding sites in transient assays (Evans and Felsenfeld, 1991; Chiba et al., 1991). GATA-1 also binds in the human β-globin locus control region (LCR) (Philipsen et al., 1990; Talbot et al., 1990; Pruzina et al., 1991).

The preparation of nuclear extract from REL cells was done for the first time in our laboratory. We modified the method used for the isolation of nuclear proteins from MEL cells, described by Gorski et al. (1986.). We also applied some modifications introduced in Gorski's method by deBoer et al. (1988.). Having had many problems during REL nuclear extract preparation, we determined the most important steps in this procedure, as well as the way to control these steps. Finally, we determined that nuclear extract from REL cells contained the GATA-1 transcription factor.

MATERIALS AND METHODS

Oligonucleotides. The following oligonucleotides were used:

1. TAGTTATGGC TATCATCTCT GAACCC - rat GATA. The probe represents a part of the promoter of rat β_b^{miny} -globin gene located from - 160 to - 185 bp upstream of the first ATG codon.

2. TCAGGGCTTT GATAGCACTA TCTGCAGAGC CAGGGCC - human GATA. The probe represents the GATA-1 binding site from DNase I hypersensitive site 4 of the human LCR. Gel mobility shift analysis showed that this fragment bound GATA-1 protein (Pruzina et al., 1991).

Cell lines and culture conditions. The MEL and REL cell lines were maintained in a modification of Eagle's minimal medium with 10% fetal

calf serum. The medium was enriched with nonessential amino acids (ala, asp, asn, glu, gly, pro, ser), all in final concentrations of 0.1 mM. Cells were grown under logarithmic phase growth conditions (at the density between 0.5×10^6 and 1.5×10^6 cells per ml) at 37^0 C. After the total amount of 10^9 cells (which represents the minimum for nuclear protein extraction) had been reached, the cells were harvested from cell culture media.

MEL and REL cells harvest. MEL and REL cells were harvested from cell culture media by centrifugation (at 4^0C), for 10 min at 2000 rpm in a Sorvall SS34 rotor. Pelleted cells were then suspended in five volumes of phosphate buffered saline (PBS) at 4^0C and collected by centrifugation as detailed above. Washing cells in PBS was done twice. The cells may be stored in liquid nitrogen if the preparation of nuclear extract is not continued immediately.

Nuclear extract preparation from MEL cells. Nuclear extract from MEL cells was prepared by a modification of the method described by Gorski et al. (1986). The first step of the procedure included the lysis of cells in an all-glass Dounce homogenizer (B type pestle) and the isolation of nuclei in the homogenization buffer-HB. The same buffer was used for preparation of the sucrose cushion through which the nuclei sediment. The pelleted nuclei were resuspended in lysis buffer (LB) and homogenized in an all-glass Dounce homogenizer by A type pestle. 1/10 volume of 4M $(NH_4)_2SO_4$ pH 8.0 was added to complete nuclear lysis and chromatin precipitation. The lysate was left in ice-water for 30 minutes with occasional mixing. It was spun in an ultracentrifuge. The supernatant contained nuclear proteins which were precipitated in the usual way, by the addition of solid $(NH_4)_2$ SO_4 (0.3 g of solid $(NH_4)_2SO_4$ per ml of supernatant) (Wildeman et al., 1984). The pellet of nuclear proteins was resuspended in dialysis buffer (DB), and dialysed for 2 hours, twice in the cold, each time against 100 volumes of the same dialysis buffer. Aliquots of supernatant were immediately frozen in liquid nitrogen. An aliquot was kept to determine the concentration of proteins in the nuclear extract by a colorimetric procedure (Bradford, 1976).

Buffers: HB - 2.2 M sucrose, 10 mM Hepes pH 7.6, 15 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 2 mM EDTA and 10% glycerol (Gorski et al., 1986.). The following endogenous proteases were added to homogenization buffer immediately before use: 0.5 mM DTT, 1% trasylol (aprotinin), 0,7 μg / ml pepstatin A, 0.7 μg/ ml leupeptin, 0.1 mM benzamidine and 0.5 mM PMSF. LB - 10 mM Hepes pH 7.6, 100 mM KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol (Gorski et al., 1986.). 1 mM DTT, O.1 mM PMSF and 0,1% trasylol were added just before use. DB - 8 mM Hepes pH 7.9, 8%glycerol, 40 mM KCl, 0.08 mM EDTA pH 8.0 and 0.2 mM DTT (de Boer et al., 1988.).

"UNNA" staining. The use of the UNNA stain (stain recipe from Dawn Kelly, 010-1-215-728-2471), made it possible to detect whether the preparation of nuclei was successful or not. The mixture of nuclear fraction and UNNA stain

was examined under high magnification under a microscope. Blue staining is characteristic for nuclei and red / pink staining for cytoplasm or nucleoli. Thus, cytoplasmic contamination showed up pink, as were whole cells.

Gel mobility shift assays and competition studies. Gel mobility shift assays were performed as previously described (deBoer et al., 1988). Briefly, each 10-μl gel mobility shift assay contained 0.1 ng of ^{32}P-labeled oligonucleotide and 0.4 ng of the complementary unlabeled oligonucleotide, 2 μg of poly (dI): poly (dC), 10 μg of protein extract, and 1 μl of 50 mM Tris (pH 8.0), 5 mM DTT, 5 mM EDTA, 250 mM NaCl, and 10% glycerol (Wall et al., 1988.). All other components were mixed on ice and then the extract was added and the assay mix was incubated at room temperature for 30 min. After the addition of 1 μl 10% glycerol containing 0.05% xylene cyanol and 0.05% bromophenol blue, the samples were run on 4% acrylamide: 0.13% methylene bis-acrylamide gel for 1 - 1.5 hr at 60 V/cm^2 in 1 x TBE running buffer.

For competition experiments, the unlabeled oligonucleotides were added to the assay mixes in a hundredfold molar excess.

RESULTS

Our attempts to isolate nuclear proteins from REL cells by the method that was used for nuclear extract preparation from MEL cells by Gorski et al., (1986) were unsuccessful. We did not obtain detectable amounts of nuclear proteins from REL cells using this method. Therefore, we decided to find out in which steps of the procedure we lost the proteins and to modify the procedure according to those findings.

For successful nuclear isolation a few points are of major importance:

1. The choice of homogenizer, speed and number of strokes for homogenizing
2. The composition of homogenization buffer
3. The composition and the amount of lysis buffer.

All the factors mentioned above can be determined and checked using the UNNA stain which differently stains cytoplasm and nuclei. Thus we can easily determine whether the cells are lysed, how clean and abundant the nuclear pellet is or whether the nuclei are lysed.

First, we determined the effects of sucrose concentration in homogenization buffer and the type of homogenizer, speed and number of strokes for homogenizing.

1. We used either a glass Dounce homogenizer with a B type pestle or a motor-driven glass teflon homogenizer and varied the speed and the number of strokes in order to lyse REL cells. The lysis of cells was checked under the microscope (Figure 1).

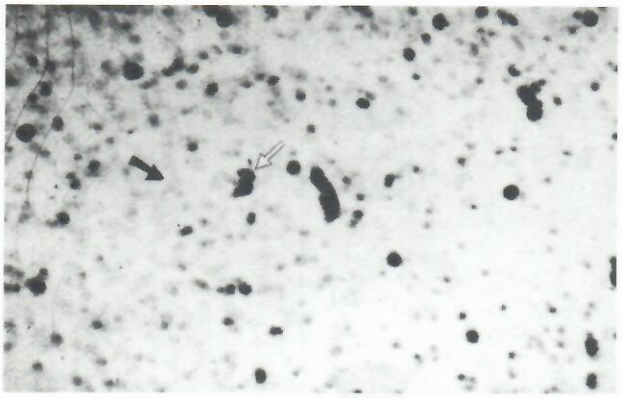

Figure 1. Photomicrograph of REL cell lysis: black arrow indicates scattered cytoplasm; white arrow indicates nuclei

The results are shown in Table 1.

Table 1. Influence of different homogenizing conditions on the lysis of REL cells.

Homogenizer	Speed (rpm)	Number of strokes	Ceel lysis
Glass Dounce, pestle B	/	/	—
Motor-driven glass teflon	60	6	—
Motor-driven glass teflon	60	12	—
Motor-driven glass teflon	90	6	—
Motor-driven glass teflon	90	12	+
Motor-driven glass teflon	120	6	+
Motor-driven glass teflon	120	12	+++

The symbols indicating cell lysis are: (—) - unsignificant; (+) - 20–30%; (++) - 50-60%; (+++) - 80-90%.

2. After the lysis of REL cells, the homogenate was laid on the top of cushions made of homogenization buffers with different concentrations of sucrose and spun in an ultracentrifuge. After centrifugation there were several layers in the tube (Figure 2). Our intention was to determine the composition of the homogenization buffer in which:

a) the solid disc of unbroken cells was insignificant

b) an interface existed but was not compact and broad

c) the sucrose cushion was free of cytoplasm and nuclei

d) the nuclei pellet was significant and free of cytoplasmic contamination.

All the layers were examined under the microscope using UNNA stain. The results are shown in Table 2.

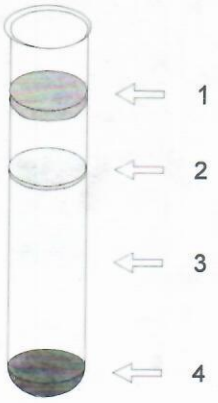

Figure 2. Appearance of the tube after centrifugation of the REL cell homogenate through a sucrose cushion: 1. solid disc of unbroken cells; 2. interface; 3. sucrose suchion; 4. pelleted nuclei

Table 2. Influence of different concentrations of sucrose in the homogenization buffer on the amount of REL nuclei and the presence of cytoplasmic contamination in the pellet after centrifuging the homogenate of lysed REL cells through a sucrose cushion.

Sucrose concentrationin HB	Disc of unbroken cells	Interface	Sucrose cushion		Pellet	
			cytoplasm	nuclei	cytoplasm	nuclei
1.80 M	+	—	+	—	+	+++
1.90 M	+	—	+	—	+	+++
2.00 M	+	—	+	—	+	+++
2.05 M	+	+	—	—	—	+++
2.10 M	+	+	—	+	—	+
2.20 M	+	+++	—	+	—	+

The symbols are as follows: (—) - absence of cellular material in the layer; (+) - presence of cellular material in the layer, (++) and (+++) - increasing amounts of cellular material in the layer.

Finally, we determined the optimal conditions for the first step of nuclear extract preparation from REL cells:

10^9 REL cells were thawed in five packed cell pellet volumes (circa 5 ml) of 2.05 M sucrose homogenization buffer. After homogenization by 12 strokes of the motor-driven homogenizer at 120 rpm, the homogenate was checked under the microscope for cell lysis. 80-90% of the cells had been lysed. The volume of homogenate was increased with the same buffer to 10 ml per starting 10^9 REL cells. Then 4 ml cushions of 2.05M sucrose - 10% glycerol homogenization buffer were made in the tubes and 1.5-2 ml of homogenate was laid on the top of each cushion. After centrifugation we noticed that the interface was not too large and was free of nuclei. The clean nuclei were pelleted.

3. In the next step of the procedure we varied the amount of lysis buffer usually used for MEL cell nuclear extract preparation (Gorski et al., 1986.)

examining 3, 5 and 10 ml per 10^9 REL cells. The lysis of nuclei was the most efficient and the final amount and concentration of nuclear proteins most satisfactory when 10 ml of lysis buffer per 10^9 REL cells was used. We also noticed that the homogenization had to last long enough if complete nuclear lysis was expected. Nuclear lysis was always checked under the microscope (Figure 3).

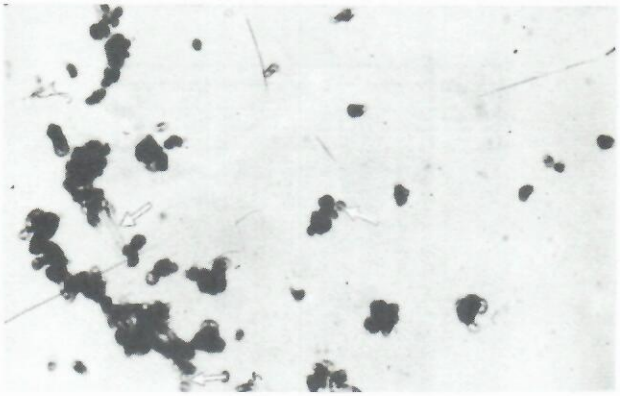

Figure 3. Photomicrograph of REL cell nuclear lysis: arrows indicate nuclear contents leaking through nuclear membranes

Removing the chromatin, precipitation of nuclear proteins and dialysis were done in a way similar to the one used for MEL cells.

Finally, in Table 3, we present the improvement that is achieved in the preparation of nuclear extract from REL cells by our modifications of the method of Gorski et al. (1986).

Table 3. The amounts of nuclear proteins obtained from 10^9 REL cells by different modifications of the procedure used for MEL cells by Gorski et al. (1986).

Number of experiments	procedure used for MEL cells by Gorski et al.	modification of the HB composition 2.05 M sucrose) (see this paper)	modification of the homogenizing conditions (motor-driven glass teflon homogenizer, 12 strokes, 120 rpm) (see this paper)	modification of the volume of LB (10 ml) (see this paper)	Amount of nuclear proteins (μg) Average values and standard deviations are shown
3	+	—	—	—	0.0
3	+	+	—	+	0.0
6	+	—	+	+	291 ± 217
12	+	+	+	+	1573 ± 396

The nuclear extract from REL cells was tested in gel mobility shift assays (Figure 4). We found that the probe from rat β-globin gene promoter, containing

the GATA motif (see Materials and methods), showed DNA-binding activity both in MEL and REL cell nuclear extracts. We demonstrated by competion assays that the band detected in these experiments was a result of the interaction between GATA-1 transcription factor and the probe. We used a GATA-1-specific competitor, derived from a known GATA-1 binding site in the human b-globin LCR (see Materials and methods). Thus, we successfully detected the GATA-1 transcription factor in our nuclear extract from REL cells.

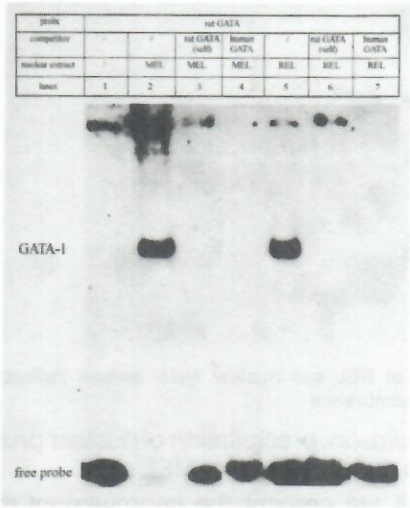

Figure 4. Gel mobility shift assays (see Materials and methods), using nuclear extracts from MEL and REL cells. The free probe (rat GATA, see Materials and methods) and the mobility of the bound GATA-1 complex are indicated. A 100 times molar excess of competitor was included in the binding reactions in lanes 3 and 6 (self competition) and lanes 4 and 7 (human GATA, see Materials and methods).

DISCUSSION

A nuclear extract from REL cells was prepared for the first time in our laboratory by the method of Gorski et al. (1986.) which was slightly modified in our procedure. The most important steps in the procedure as well as the way to control these steps are described. Our findings could be helpful in the isolation of nuclear proteins from different sources, either from cultured cells or cells obtained from tissues.

We consider that there are three important steps in the procedure of nuclear extract preparation:

1. The choice of homogenizer, speed and number of strokes for homogenizing intact cells

2. The composition of homogenization buffer

3. The composition and the amount of buffer for nuclear lysis.

1. The choice of homogenizer depends on the nature of the tissue. It is very important to choose the proper type of homogenizer and to determine the speed and number of strokes required for lysing 90% of the cells. For harder tissues, such as REL cells, a motor driven homogenizer is indispensable. In this case, the homogenization should be done by 12 strokes at the speed of 120 rpm.

2. The density of the nuclei is characteristic of the cell-type from which they are isolated. The correct concentration of sucrose in the homogenization buffer should be determined by preliminary experimentation because it is HB which forms the sucrose cushion through which the nuclei sediment. Determination of the required sucrose concentration in the homogenization buffer is the most important step in the procedure of isolation of nuclear extract. The correct sucrose concentration permits only nuclei, but not cytoplasm, intracellular membranes or organelles to pellet. The density of the sucrose cushion also influences the yield of pelleted nuclei.

The concentration of sucrose in the homogenate which is layered over the sucrose cushion is also important. It must be high enough to prevent the accumulation of intracellular membranes and organelles at the interface; otherwise the yield of nuclei is substantially reduced. The final sucrose concentration of the homogenate is governed by the volume of homogenization buffer added to it (Marzluff and Huang, 1984.).

We determined that the correct concentration of sucrose in the homogenization buffer for lysing REL cells and cleaning the nuclei by sedimentation through a sucrose cushion is 2.05 M. We also diluted the homogenate of REL cells to a final volume of 10 ml HB per 10^9 cells.

3. The composition and amount of lysis buffer, the choice of pestle type and the time of homogenization have to be determined individually for nuclei originating from different kinds of tissus or cultured cells. Usually, the nuclei solution is diluted with lysis buffer to reach a definite DNA concentration depending on the kind of cells from which the nuclei are because different tisues have nuclei with distinct DNA: nuclear protein ratios. If the nuclei contain about the same amount of extractable proteins as DNA, the solutution should be diluted with lysis buffer to reach a DNA concentration of 1 mg / ml (liver, for example). After lysis of nuclei in a small amount of lysis buffer, the concentration of DNA should be determined, and then, according to the DNA: nuclear protein ratio characteristic for the tissue, lysis buffer is added to dilute the nuclei solution. For example, for HeLa cells 3 ml of lysis buffer per 10^9 cells should be used (Dignam et al., 1983.).

We determined that 10 ml of lysis buffer per 10^9 REL cells allowed the most successful lysis of nuclei.

The GATA-1 transcription factor was identified in chicken erythroid cells (Evans et al., 1988), mouse erythrorid (MEL) cells (deBoer et al., 1988; Wall et al., 1988) and human erythroid cells K562 (Tsai et al., 1989). A homologous GATA-1 protein exists also in the frog (Zog et al., 1991). In this paper it is shown, for the first time, that the GATA-1 transcription factor is present in rat erythroid cells (REL cells). The structure and function of rat GATA-1 protein remains to be determined in future experiments.

Acknowledgments. We are grateful to F. Grosveld and S. Philipsen for their generous gift of an oligonucleotide probe from human β-globin LCR. We also wish to thank our colleagues from the Institute of Physiology for lending the motor-driven glass teflon homogenizer to us.

REFERENCES

1. *Bradford, M.* 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem 72,* 248.
2. *Chiba.T., Ikawa, J. and Todokoro, K.* 1991. GATA-1 transactivates erythropoietin receptor gene, and erythropoietin receptor-mediated signals enhance GATA-1 gene expression. *Nucleic Acids Res. 19,* 3843 - 3848.
3. *deBoer, E., Antoniou, M., Mignotte, V., Wall, L. and Grosveld, F.* 1988. The human β-globin promotor; nuclear protein factors and erythroid specific induction of transcription. *EMBO J. 7,* 4203-4212.
4. *Dignam, J. D., Lebovitz, R. M. and Roeder, R. G.* 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.11,* 1475 - 1489.
5. *Evans, T., Reitman, M. and Felsenfeld, G.* 1988. An erythrocyte - specific DNA binding factor recognizes a regulatory sequence common to all chicken globin genes. *Proc. Natl. Acad. Sci. USA 85,* 5976-5980.
6. *Evans, T. and Felsenfeld, G.* 1991. Trans-activation of a globin promoter in nonerythroid cells. *Mol. Cell. Biol. 11,* 843-853.
7. *Fong, T. C. and Emerson, B. M.* 1992. The erythroid specific protein cGATA-1 mediates distal enhancer activity through a specialized β-globin TATA box. *Genes and Dev. 6,* 521-532.
8. *Friend, C.* 1957. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exp. Med. 105,* 307-318.
9. *Galson, D. L. and Housman, D. E.* 1988. Detection of two tissue-specific DNA-binding proteins with affinity for sites in the mouse β-globin intervening sequence 2. *Mol. Cell. Biol. 8,* 381-392.
10. *Gorski, K., Carniero, M. and Schibler, U.* 1986. Tissue-specific in vitro transcription from the mouse albumin promoter. *Cell 47,* 767-776.
11. *Kluge, N., Ostertag, W., Sugiyama, T., Arndt-Jovin, D., Steinheider, G., Furusawa, M. and Dube, S. K.* 1976. Dimethylsulfoxide-induced differentiation and hemoglobin synthesis in tissue cultures of rat erythroleukemia cells transformed by 7,12-dimethylbenz(α anthracene. *Proc. Nat. Acad. Sci. USA 73,* 1237-1240.
12. *Macleod, K. and Plumb, M.* 1991. Derepression of mouse β-major-globin gene transcription during erythroid differentiation. *Mol. Cell. Biol. 11,* 4324-4332.
13. *Martin, D. I. K., Zon, L. I., Mutter, G. and Orkin, S. H.* 1990. Expression of an erythroid transcription factor in megakaryocytic and mast cell lineages. *Nature 344,* 444-447.
14. *Marzluff, W. F. and Huang, R. C. C.* 1984. Transcription of RNA in isolated nuclei in Transcription and translation, *Ed. B. D. Hames & S. J. Higgins. IRL Press, Oxford 'Washington, 90-95.*
15. *Mignotte, V., Wall, L., deBoer, E., Grosveld, F. and Romeo, P. H.* 1989. Two tissue-specific factors bind the erythroid promoter of the human porphobilinogen deaminase gen. *Nucleic Acids Res. 17,* 37-54.
16. *Revny, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S. F., D'Agati, V., Orkin, S. H. and Constantini, F.* 1991. Erythroid differentiation in chimeric mice blocked by a targeted mutation in gene for transcription factor GATA-1. *Nature 349,* 257-290.
17. *Philipsen, S., Talbot, D., Fraser, P. and Grosveld, F.* 1990. The β-globin dominant control region:hypersensitive site 2. *EMBO Journal 9,* 2159-2167.
18. *Pruzina, S., Hanscombe, O., Whyatt, D., Grosveld, F. and Philipsen, S.* 1991. Hypersensitive site 4 of the human β globin locus control region. *Nucleic Acids Res. 19,* 1413-1418.
19. *Romeo, P. H., Prandini, M. H., Joulin, V., Mignotte, V., Prenant, M., Vainchenker, W., Margueri, G. and Uzan, G.* 1990. Megakaryocytic and erythrocytic lineages share specific transcription factors. *Nature 344,* 447-450.
20. *Stuve, L. L. and Myers, R. M.* 1990. A directly repeated sequence in the β-globin promoter regulates transcription in murine erythroleukemia cells. *Mol. Cell. Biol. 10,* 972-981.

21. *Talbot, D., Collis, P., Antoniou, M., Vidal, M., Grosveld, F. and Greaves, D. R.* 1989. A dominant control region from the human β-globin locus conferring integration site-independent gene expression. *Nature 338,* 352-355.
22. *Tsai,S. F., Martin, D. I. K., Zon, L. I., D'Andrea, A. D.,Wong, GG and Orkin, S. H. 1989. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. Nature 339,* 446-451.
23. *Wall, L., de Boer, E. and Grosveld, F.* 1988. The human β-globin gene 3'enhancer contains multiple binding sites for an erythroid-specific protein. *Genes & Dev. 2,* 1089-1100.
24. *Wildeman, A. G., Sassone - Corsi, P., Grundstrom, T., Zenke, M. and Chambon, P.* 1984. Stimulation of in vitro transcription from the SV40 early promoter by the enhancer involves a specific trans - acting factor. *EMBO J., 3,* 3129-3133.
25. *Zon, L. I., Mather, C., Burgess, S., Bolce, M. E., Harland, R. M. and Orkin, S. H.* 1991. Expression of GATA-binding proteins during embryonic development in Xenopus laevis. *Proc. Natl. Acad. Sci. USA 88,* 10642-10646.

JEDARNI EKSTRAKT IZ REL ĆELIJA SADRŽI TRANSKRIPCIONI FAKTOR GATA-1

SONJA PAVLOVIĆ, TATJANA MITROVIĆ, M. JOKSIMOVIĆ, GORDANA NIKČEVIĆ i ZVEZDANA POPOVIĆ

SADRŽAJ

Proteini koji regulišu ekspresiju gena u različitim tkivima izoluje se iz jedara ćelija tih tkiva. REL (pacovske eritroleukemične) ćelije su permanentna eritroidna ćelijska linija. One predstavljaju najpogodniji model-sistem za izučavanje ekspresije globinskih gena pacova. U našoj laboratoriji su, po prvi put, izolovani jedarni proteini iz REL ćelija. Takođe su utvrđeni i najvažniji koraci u postupku ekstrakcije jedarnih proteina kao i način da se taj postupak kontroliše. Pored toga, u ovom radu je pokazano da jedarni ekstrakt iz REL ćelija sadrži transkripcioni faktor GATA-1.