Acta Veterinaria (Beograd), Vol. 53. No. 2-3, 67-76, 2003.

UDK 619:577.214

IDENTIFICATION AND CHARACTERIZATION OF RAT β–DRE BINDING FACTORS INVOLVED IN ERYTHROID-SPECIFIC INDUCTION OF TRANSCRIPTION

PAVLOVIĆ SONJA, KOVAČEVIĆ GRUJIČIĆ NATAŠA and MITROVIĆ TATJANA

Institute of Molecular Genetics and Genetic Engineering, 11001 Belgrade, Serbia

(Received 9. January, 2003)

We have identified and characterized a DNA-binding activity with specificity for the β-globin direct repeat elemnt (βDRE) from rat β_b^{miny}-globin promoter, an evolutionarily conserved transcriptional regulatory element in mammalian adult β-globin promoters. We have also confirmed that the βDRE contributed to the transcriptional inducibility of rat β_b^{miny}- globin gene in rat erythroleukemia (REL) cells. By using gel mobility shift and South-Western blot competition studies we have shown that 60 kD erythroid-specific transcription factor and 35 kD and 20 kD ubiquitous factors from rat erythroleukemia cells specifically bind to the β-DRE from the rat β_b^{miny}- globin promoter. Additionally, a significant increase in the quantity of 60 kD transcription factor was observed upon erythroid differentiation of REL cells.

Key words: rat, β-globin genes, transcription regulation, induction of erythropoiesis, REL cells

INTRODUCTION

The developmental-stage and tissue-specific regulation of the mammalian β-globin locus has provided a challenging system for understanding the molecular mechanisms that mediate gene activation during terminal differentiation in erythroid cells. An important step in understanding this regulation is identification of the *cis*-acting regulatory sequences that are required for expression of globin genes in erythroid cells, as well as *trans*-acting protein factors which bind at different regulatory regions located through the β-globin gene locus.

The murine erythroleukemia (MEL) (Friend *et al*., 1971) and rat erythroleukemia (REL) cell model systems for adult erythrocyte development (Kluge *et al*., 1976) are very useful for this characterization of the adult rat β-globin genes. Although MEL cells are a widely used tissue-culture model for the study of β-globin gene expression, REL cells represent a homologous system for studying rat globin gene expression. REL cells are a permanent cell line derived from transplantable tumors from 7,12 dimethylbenz (α) anthracene-induced erythroleukemia in the Long-Evans rat (Kluge *et al*., 1976). This cell line maintains its erythroid nature. Erythroid differentiation can be induced by dimethylsulfoxide (DMSO), as demon-

strated by a decrease in cell size, the appearance of red and benzidine-positive cells and increased synthesis of adult globin chains (Yamaguchi *et al.*, 1981).

The inducibility of β-globin expression during erythroid cell differentiation does not appear to be controlled by any single regulatory element. Different regions within the promoter, the gene, and the locus control region (LCR) (Townes and Behringer, 1990), contribute to a variable extent to β-globin transcriptional induction in a context- and assay-dependent manner (Antoniou *et al*., 1988; Antoniou and Grosveld, 1990; Cowie and Mayers, 1988; deBoer *et al*., 1988; Schreck *et al*., 1990; Wright *et al*., 1984). Much of the complex regulation of the gene, however, appears to be mediated by the LCR with the proximal (minimal) promoter region. The promoter region spanning 100 bp 5' from the transcription initiation site (*cap* site) of the β-globin promoter is named the minimal promoter (de Boer *et al*., 1988). It consists of three conserved regions responsible for efficient transcription of the β-globin gene: a TATA element at -30 bp, CAAT box at -70 bp and CACC element at -90 bp from the *cap* site (reviewed in reference Popovic *et al*., 1993). Another *cis*-acting element found exclusively in the promoters of adult β-globin genes is named the βDRE (β-globin direct repeat element) (Stuve and Mayers, 1990). It is a ten base pair (5'AGGGCAGAGC 3') duplicated sequence. The β-DRE motif is highly conserved in all mammalian β-globin promoter sequences known up to date. The position and number of repeats are variable, e.g., in the human minimal globin promoter this sequence is present three times and once in the distal promoter. On the other hand, rat minimal β_b^{miny} -globin promoter contains only two repeats (Pavlovic *et al*. 1999). Deletion and mutations in this region lead to a decrease in transcriptional rate in induced MEL cells (Antoniou *et al*., 1995).

Antoniou *et al*. (1995) found no evidence for βDRE binding activity in the promoter of the human b-globin gene. In contrast, Stuve and Mayers (1993) identified DNA binding activity with specificity for the βDRE from the promoter of the mouse β^{maj} -globin gene. We have detected several complexes binding at the βDRE element from the rat β_b^{miny} -globin promoter in REL cells (Pavlovic *et al*., 1999).

In this paper we show that the βDRE contributes to the transcriptional inducibility of rat β_b^{miny}-globin gene in REL cells. We have also identified and characterized the transcription factors that specifically bind to the β-DRE from rat β_b^{miny} -globin promoter and postulated their involvement in erythroid induction of transcription.

MATERIALS AND METHODS

Oligonucleotides

The following oligonucleotides were used in gel shift and South-Western analysis:

1. 5'-CTCACACAGG ACAGAGTGAT CAGGGGCCAG AATTT – rat βDRE. The oligonucleotide represents a part of the promoter of rat β_b^{miny}-globin gene located from –68 to –34 bp upstream of the *cap* site.

2. 5'-ACCCTCACAT TGCCCAATCT GCTCACACA – rat CCAAT. The oligonucleotide represents a part of the promoter of rat β_b^{miny}-globin gene located from –89 to –61 bp upstream of the *cap* site.

DNA constructs

The parent vector used in the constructions contained 881 bp of rat β_b^{miny}-globin promoter sequence cloned upstream of the *cat* reporter gene in pCAT-Basic vector (Promega) (Pavlovic *et al*., 1999). The first step was the insertion of a 1.9 kb *Hind* III fragment containing hypersensitive site 3 of the human β-globin LCR (Ellis *et al*., 1996) into the parent vector in order to achieve position-independent full expression of *cat* reporter gene in transfected REL cells. After the excision of distal parts of β_b^{miny}-globin gene promoter by exonuclease III digestion (Pharmacia), the construct used in this paper was generated. DNA sequencing analysis using universal reverse primer (ALFExpress DNA Sequencer, Pharmacia Biotech) revealed that the reporter construct contained a 96 bp-fragment of rat β_b^{miny}-globin promoter (from +27 to –69) and it was designated as pCATβ_b^{miny} (–69).

Cell culture and cell transfections

The REL and Hela cell lines were cultured as previously described (Pavlovic *et al*., 1996; Pavlovic *et al*., 1999). pCATβ_b^{miny}(-69) plasmid (100 μg) was linearized at *Aat*II site and cotransfected with GSE 1417 plasmid (Talbot *et al*., 1989) containing *tk-neo*^{R} (20 μg) into REL cells by electroporation, as follows: 3 x 10^7 cells in rapid, log phase of growth were washed in PBS and resuspended in 1 ml of ESB (140 mM NaCl, 25 mM HEPES pH 7.15, 0.75 mM Na_2HPO_4). The cells were electroporated in a 0.4 cm cuvette in a BioRad Gene Pulser apparatus set to deliver a 250 V, 960 μF. After electroporation cells were divided into three independent populations and selected in 800 μg/ml G418. Populations of transfected cells were ready for further use 3 weeks after the application of drug selection. At this stage, each population was further subdivided, with one half maintained in normal selection medium. The other half was induced to differentiate by culturing for 5 days in the presence of 1.5 % (v/v) DMSO.

CAT assays

Uninduced and induced REL cells transfected with pCATb $_b^{miny}$(-69) plasmid were pelleted and washed in PBS. Cells were resuspended in 100 μl 0.25 M Tris buffer (pH 7.8) per 10^7 cells and subjected to three cycles of freezing and thawing. Extracts were incubated at 65°C for 10 minutes to inactivate endogenous deacetylases. Cell extracts, containing equal amounts of proteins (400 μg) were incubated with [^{14}C] chloramphenicol (0.125 μCi) and unlabelled acetyl coenzyme A (4mM) for 3 hours at 37°C. [^{14}C] chloramphenicol and its acetylated forms were separated by thin layer chromatography (TLC), cut out from TLC plates and measured for CAT activity in a liquid scintillation counter. CAT activity was measured by thin layer chromatography (Gorman *et al*., 1982).

Preparation of nuclear extracts

HeLa cell nuclear extracts were prepared by the standard method of Dignam *et al.* (1983). The preparation of nuclear extracts from REL cells was done as previously described by Pavlovic *et al*. (1996).

Gel shift assays and competition study

Gel shift assays were performed as previously described (Pavlovic *et al*., 1999). Briefly, each 20 μl gel shift assay contained 0.1 ng of ^{32}P-labelled oligonucleotide (rat βDRE oligonucleotide) and 0.4 ng of the complementary unlabelled oligonucleotide, 2 μg of poly(dI):poly(dC), 10 – 14 μg of protein extract and 2 μl of the binding buffer (50 mM Tris (pH 8.0), 5 mM DTT, 5 mM EDTA, 250 mM NaCl and 10 % glycerol). 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 cyanole and 0.05% bromophenol blue, the samples were run on 4% acrylamide: 0.13% methylene bisacrylamide gel for 1 hr at 60 V/cm^2 in 1x TBE running buffer.

For competition experiments, the unlabelled oligonucleotide (rat CCAAT oligonucleotide) in 1 μl of annealing buffer was added to the assay mix before the addition of the extract. The competitor oligonucleotide was added in 100-fold molar excess.

Each assay was performed at least three times using nuclear protein extracts from different isolations.

South-Western blot analysis and competition study

South-Western blot analysis was done as previously described (Pavlovic *et al.*, 1999). Briefly, nuclear proteins extracted from uninduced and induced REL cells as well as from HeLa cells (15 μg each) were electrophoretically separated on 8% SDS PAGE and electrically transferred to a nitrocellulose membrane (Hybond C). Hybridization buffer contained 300 000 cpm/ml of ^{32}P-labelled probe (rat βDRE oligonucleotide), 20 μg/ml polydI:polydC and unlabelled competitor (rat CCAAT oligonucleotide) in 100-fold molar excess. The incubation was at room temperature overnight. Signals were visualized by autoradiography.

RESULTS

The βDRE of rat β_b^{miny}-globin promoter contributes to its transcriptional inducibility in REL cells

In order to determine the functional role of the βDRE of the rat β_b^{miny}-globin promoter, we tested whether the region from +27 to –69 bp relative to the transcriptional start site conferred transcriptional inducibility in REL cells (Figure 1).

This promoter fragment was cloned in the CAT reporter vector and introduced into REL cells by electroporation. For selection of transformants, the plasmid GSE 1417, containing a thymidine kinase promoter driven G418 resistance marker was cotransfected. Three independent stable populations of REL cells were established by selection in G418. Each population was split, and half was in-

duced to differentiate for 5 days by the addition of 1.5 % DMSO. The levels of CAT activity were measured before and after DMSO-induced differentiation by CAT assay.

-69 β **DRE** β **DRE** **TATA**

GCTCACAC**AGGACAGAGT**GATC**AGGGGCCAGA**ATTTGGCA**TATA**AAGCAG

+1 +27

AACAGAACCAGTTGCTTCTTATATTTGCTTCTGATACTGTTGTGTT

Figure 1. Sequence (+27 to -69) of the rat β_b^{miny}-globin promoter. Cap site is indicated as +1. The conserved consensus sequences are in blodscript

The results of CAT assay analysis demonstrated that the promoter fragment containing only the βDRE and TATA box had barely detectable activity in uninduced REL cells. Interestingly, we observed significant activation of the *cat* reporter gene upon erythroid differentiation of REL cells (Figure 2).

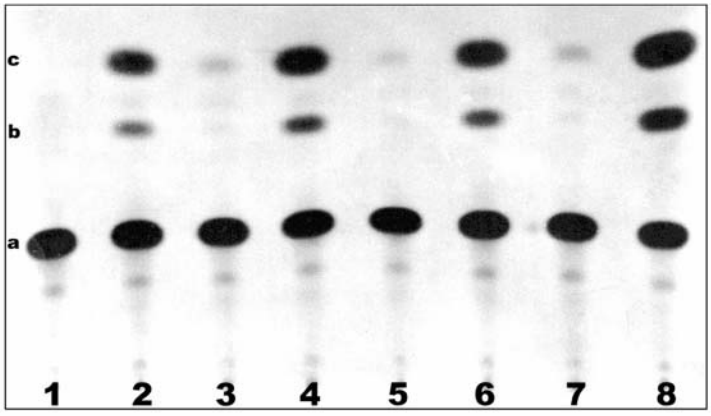

Figure 2. CAT enzymatic activities of recombinant CAT plasmid containing 95 bp (from +27 to -69) of rat β_b^{miny}-promoter in REL cells. The levels of CAT activity in mock transfected REL cells (lanel), REL cells transformed with pCATβ_b^{miny}-globin plasmid before (lanes 3, 5, 7) and after the induction to differentiation with dimethyl sulfoxide (lane 4, 6, 8) were determined by TLC assay. Lane 2 represents the level of activity of CA enzyme (IU). Unacetylated chloramphenicol spots in the chromatograph are mrked by a, and acetylated chloramphenicol sport are marked by b and c.

Gel shift and competition analysis of bDRE reveals regulatory element specific transcription factors

First gel shift studies of the interactions between transcription factors isolated from REL cells and rat βDRE showed four distinct complexes on the gel (Pavlovic *et al*., 1999).

We wished to know if some of the observed complexes could be the result of protein binding to adjacent promoter sequences. We performed a gel shift assay with nuclear extract from DMSO-induced REL cells using the rat βDRE oligonucleotide as the probe and the partially overlapping CCAAT box oligonucleotide from the rat β_b^{miny}-globin promoter as the competitor (Figure 3). We observed that only the fastest, erythroid-specific β4 complex did not compete with the CCAAT box oligonucleotide, indicating that transcription factors which take part in forming the β4 complex specifically bind to the βDRE from the rat β_b^{miny}-globin promoter. This competition study enabled us to identify transcription factors which form a β4 complex.

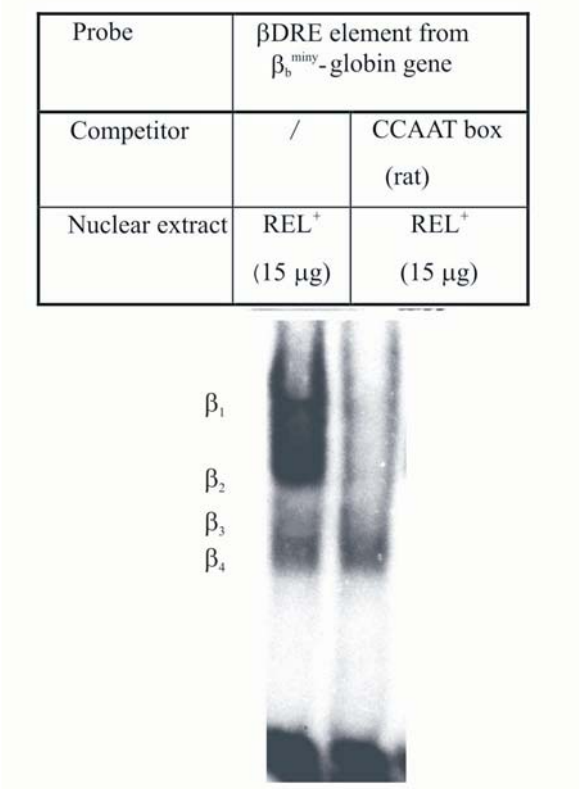

Figure 3. Gel shift assay of βDRE element with nuclear extract from induced REL cells and competition by adjacent CCAAT boy oligonuleotide from rat β_b^{miny}-globin promoter. The competitior oligonucleotide was used in 100-fold greater molar concentraton than the probe. The protein DNA complexes were separated on 4% polyacrilamide gel. Formed complexes are designated as β_1–β_4.

Characterization of sequence-specific βDRE binding proteins in REL cells

We performed South-Western blot analysis using the βDRE as a probe and the partially overlapping CCAAT box oligonucleotide as competitor (Figure 4). Nuclear extract from HeLa cells was used as the nonerythroid control.

We detected three nuclear factors which were specifically bound to the labelled rat βDRE probe and did not compete with the unlabelled CCAAT oligonucleotide. They were 60 kD erythroid-specific transcription factor and 35 kD and 20 kD ubiquitous factors (present both in REL and HeLa nuclear extracts). We conclude that these three proteins form the β4 complex observed earlier in gel mobility shift assays. Again, we observed a significant increase in the quantity of 60 kD erythroid-specific transcription factor upon erythroid differentiation of REL cells.

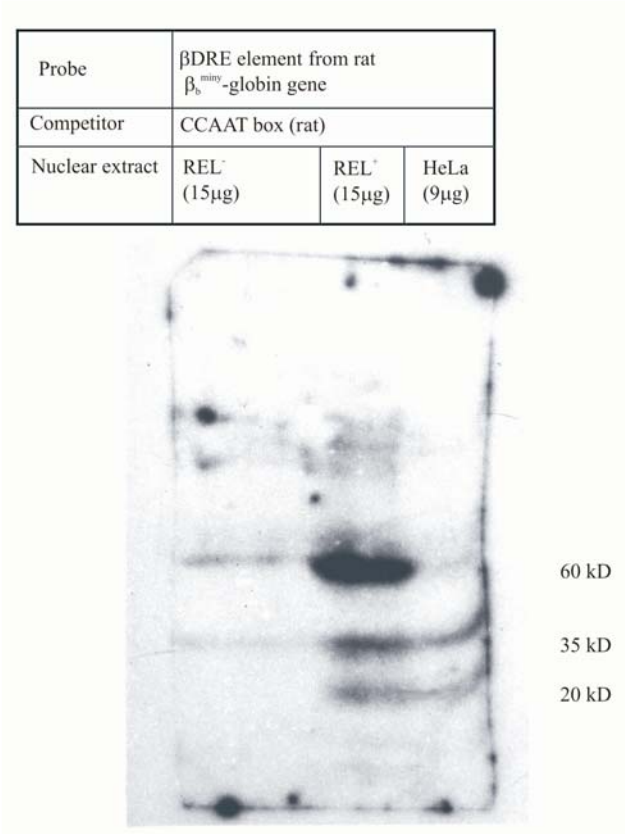

Figure 4. Analysis of the interactions between the βDRE promoter from rat β_b^{miny}-globin gene and transcriptional factors from uninduced and DMSO-induced REL and Hela cells by the South-Western method and the competition by adjacent CAAT box oligonucleotide. Molecular weights of proteins are indicated. Nuclear proteins were separated on 8% SDS-PAGE. Unlabelled oligonucleotide competitor was added in 100 molar excess.

DISCUSSION

In this paper we have demonstrated that a -69 bp fragment of the β_b^{miny}-globin promoter containing only the TATA box and βDRE is transcriptionally induced during REL cell differentiation. In contrast with our results, deletion experiments on the human β-globin promoter demonstrated that the minimal promoter of this gene is not inducible during MEL cell differentiation (Antoniou *et al*., 1988). However, if combined with the β-LCR, the human minimal β-globin promoter does provide erythroid-specific induction of transcription (Antoniou and Grosveld, 1990), as does the -69 bp rat β_b^{miny}-globin promoter in the absence of the LCR. 5' deletion mutants of the murine β^{maj} -globin promoter indicate that the murine minimal promoter is involved in transcriptional induction upon MEL cell differentiation (Mayers *et al*., 1986; Cowie and Mayers, 1988; Stuve and Mayers, 1990). More interestingly, a drop in inducibility occurs when the βDRE is removed, indicating that this element contributes significantly to induction (Stuve and Mayers, 1990). Therefore, these results strongly support the presumption that the rat β_b^{miny}-globin minimal promoter resembles the murine β^{maj} -globin promoter. The human β-globin minimal promoter appears to be differentially regulated during DMSO-induced erythroid differentiation.

No evidence of β-DRE binding activity in the promoter of human β-globin gene has been obtained (Antoniou *et al*., 1995). In contrast, DNA-binding activity with specificity for the β-DRE from the promoter of the mouse β^{maj} -globin gene has been identified (Stuve and Mayers., 1993). This binding activity, termed βDRf, for β-globin direct repeat factor, was detected in fractionated nuclear extracts from MEL cells, and has been partially purified from undifferentiated cells. We have found several complexes binding at the β-DRE from the rat β_b^{miny}-globin promoter in MEL and REL cell nuclear extracts (Pavlovic *et al*., 1999).

It is our opinion that the β-DRE does not take part in the transcriptional regulation of the human β-globin gene, while it has an active role in the inducibility of the rat β_b^{miny}- globin-as well as the murine β^{maj} -globin minimal promoter.

We have shown that among the interactions between transcription factors isolated from REL cells and rat βDRE only the fastest, erythroid-specific β4 complex did not compete with adjacent promoter elements, indicating that transcription factors which participate in forming the β4 complex specifically bind to βDRE element from rat β_b^{miny}-globin promoter. Moreover, we have characterized those transcription factors as: the 60 kD erythroid-specific transcription factor and 35 kD and 20 kD ubiquitous factors. However, it seems that there is only a significant increase in the quantity of the 60 kD erythroid-specific transcription factor upon erythroid differentiation of REL cells.

The observed quantitative difference is in agreement with what deBoer *et al*. (1988) described for the transcription factors involved in the induction of transcription of the human β-globin gene during erythroid differentiation.

We presume that the 60 kD transcription factor which binds to the βDRE from the rat β_b^{miny}-globin gene is GATA-1. It is well known that GATA 1 is an erythroid-specific transcription factor. It is the universal activator of β-globin genes

(reviewed in Pavlovic and Popovic, 1996). Additionally, the experiments of Matsuda *et al*. (1994) suggest that the rat GATA-1 transcription factor is approximately 60 kD in size. Anyway, confirmation may be achieved by using antibodies and supershift analysis.

Taking all these data into consideration, we postulate that the 60 kD erythroid-specific transcription factor (probably GATA-1) is involved in tissue-specific transcriptional activation of the rat β_b^{miny}-globin promoter. Furthermore, we conclude that interaction between βDRE and the 60-kD erythroid-specific transcription factor is of utmost importance for erythroid-specific induction of transcription by the rat β_b^{miny}-globin promoter in differentiating REL cells.

Address for correspondence:
Dr Sonja Pavlović
Institute of Molecular Genetics and Genetic Engineering
Vojvode Stepe 444a, P.O. Box 446
11001 Belgrade, Serbia & Montenegro
E-mail: sonya@sezampro.yu

REFERENCES

1. *Antoniou M, Grosveld F,* 1990, β-globin dominant control region interacts differently with distal and proximal promoter elements, *Gen Dev*, 4:1007-13.
2. *Antoniou M, deBoer E, Habets G, Grosveld F, 1988, The human* β-globin gene contains multiple regulatory regions: identification of one promoter and two downstream enhancers. *EMBO J,* 7:377-84.
3. *Antoniou M, deBoer E, Spanopoulou E, Imam A, Grosveld F, 1995, TBP binding and the rate of transcription initiation from the human* β-globin gene, *Nucl Acids Res*, 23:3473-80.
4. *Cowie A, Myers R M,* 1988, DNA sequences involved in transcriptional regulation of the mouse β-globin promoter in murine erythroleukemia cells, *Mol Cell Biol*, 8:3122-8.
5. *deBoer E, Antoniou M, Mignotte V, Wall L, Grosveld F*, 1988,The human β-globin promoter; nuclear protein factors and erythroid specific induction of transcription, *EMBO J,* 7:4203-12.
6. *Dignam JD, Lebovitz RM, Roeder RG*, 1983, Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucl Ac Res*, 11:1475-89.
7. *Ellis J, Tan-Un KC, Harper A, Michalovich D, Yannoutsos N, Philipsen S and Grosveld F,* 1996, A dominant chromatin-opening activity in 5'hypersensitive site 3 of the human bin locus control region. *EMBO J*, 15:562-8.
8. *Friend C, Scher W, Holland JG, Sato T*, 1971, Hemoglobin synthesis in murine virus induced leukemic cells *in vitro*: stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc Natl Acad Sci, USA* 68:378-82.
9. *Gorman CM, Moffat LF, Howard BH*, 1982, Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol, 2:1044-51.*
10. *Kluge N, Ostertag W, Sugiyama T, Arndt-Jovin D, Steinheider G, Furusawa M,* Dube *SK,* 1976, Dimethylsulfoxide-induced differentiation and hemoglobin synthesis in tissue cultures of rat erythroleukemia cells transformed by 7,2-dimethylbenz(α)anthracene. *Proc Natl Acad USA,* 73:1237-40.
11. *Matsuda K, Kobune Y, Noda C. Ichihara A*, 1994, Expression of GATA-binding transcription factors in rat hepatocytes. *FEBS Lett*, 353 (3): 262-72.
12. *Myers RM, Tilly K, Maniatis T*, 1986, Fine structure genetic analysis of a β-globin promoter. *Science*, 232:613-18.
13. *Pavlovic S, Popovic Z,. Transcription factor GATA-1, 1996. Bulletin of Hematology*, 24 (3):63-8.

14. *Pavlović S, Mitrović T, Joksimović M, Nikčević G, Popović Z,* 1996, Nuclear extract from REL cells contains GATA -1 transcription factor. *Acta Veterinaria*, 46:173-84.
15. *Pavlovic S, Mutrovic T, Nikcevic G, Grujicic N, Lazic D, Glisin V, Popovic Z*, 1999, Tha rat β_b^{miny}-globin promoter: nuclear protein factors and erythroid-specific induction of transcription. *CMLS*, 56:871-81.
16. *Popovic Z, Nikcevic G, Savkovic S, Marjanovic J, Pavlovic S*, 1993, Transcriptional regulation of the β-globin gene. *Genetika*, 25:233-45.
17. *Schreck R, Zorbas H, Winnacker E, Baeuerle PA*, 1990, The NF-kappaB transcription factor induces DNA bending which is modulated by its 65 kD subunit. *Nucleic Acids Res*,18:6497-502.
18. *Stuve LL and Myers RM*, 1990, A directly repeated sequence in the β-globin promoter regulates transcription in murine erythroleukemia cells. *Mol Cell Biol*, 10:972-81.
19. *Stuve LL and Myers RM*, 1993, Identification and characterization of a β-globin promoter-binding factor from murine erythroleukemia cells. *Mol Cell Biol*, 13:4311-22.
20. *Talbot D, Collis P, Antoniou M, Vidal M, Grosveld F, Greaves DR*, 1989, A dominant control region from human β-globin locus conferring integration site-independent gene expression. *Nature*, 338:352-5.
21. *Townes T and Behringer RR*, 1990, Human globin locus activation region (LAR): role in temporal control. *Trends Genet*, 6:219-23.
22. *Wright S, Rosenthal A, Flavell R, Grosveld F*, 1984, DNA sequences required for regulated expression of b-globin genes in murine erythroleucemia cells. *Cell*, 38:265-73.
23. *Yamaguchi Y, Kluge N, Ostertag W, Furusawa M*, 1981, Erythroid differentiation in rat erythroleukemia cells with hypertonic culture conditions. *Proc Natl Acad. Sci USA,* 78:2325-9.

IDENTIFIKACIJA I KARAKTERIZACIJA β-DRE VEZUJUĆIH FAKTORA UKLJUČENIH U ERITROIDNO-SPECIFIČNU INDUKCIJU TRANSKRIPCIJE

PAVLOVIĆ SONJA, KOVAČEVIĆ GRUJIČIĆ NATAŠA i MITROVIĆ TATJANA

SADRŽAJ

Identifikovali smo i okarakterisali DNK vezujuće proteine koji specifično prepoznaju transkripcioni regulatorni element βDRE iz β_b^{miny}-globinskog promotora pacova. βDRE je evolutivno konzervisan u adultnim β-globinskim promotorima sisara. Potvrdili smo da βDRE regulatorni element doprinosi transkripcionoj inducibilnosti β_b^{miny}-globinskog gena pacova u pacovskim eritroleukemičnim ćelijama. Korišćenjem "gel mobility shift" i South-Western metoda i kompeticionih eseja, pokazali smo da se za βDRE pacovskog β_b^{miny}-globinskog promotora specifično vezuju eritroidno-specifični transkripcioni faktor od 60 kD i dva opšta transkripciona faktora od 35 i 20 kD poreklom iz pacovskih eritroleukemičnih ćelija. Takođe, eritroidna diferencijacija REL ćelija je praćena značajnim povećanjem količine transkripcionog faktora od 60 kD.