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The 5'-flanking *cis-acting* elements of the human ε-globin gene associates with the nuclear matrix and binds to the nuclear matrix proteins

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ABSTRACT

The nuclear matrix attachment regions(MARs) and the binding nuclear matrix proteins in the 5'-flanking cis-acting elements of the human ε-globin gene have been examined. Using in vitro DNA-matrix binding assay, it has been shown that the positive stage-specific regulatory element (ε-PREII, -446bp ~ -419bp) upstream of this gene could specifically associate with the nuclear matrix from K562 cells, indicating that ε-PREII may be an erythroid-specific facultative MAR. In gel mobility shift assay and Southwestern blotting assay, an erythroid-specific nuclear matrix protein (ε-NMP_k) in K562 cells has been revealed to bind to this positive regulatory element (ε-PREII). Furthermore, we demonstrated that the silencer (-392bp -177bp) upstream of the human ε-globin gene could associate with the nuclear matrices from K562, HEL and Raji cells. In addition, the nuclear matrix proteins prepared from these three cell lines could also bind to this silencer, suggesting that this silencer element might be a constitutive nuclear matrix attachment region (constitutive MAR). Our results demonstrated that the nuclear matrix and nuclear matrix proteins might play an important role in the regulation of the human ε-globin gene expression.

Key words: *Human ε-globin gene, nuclear matrix attachment regions, nuclear matrix proteins, K562 cells.*

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INTRODUCTION

The nuclear matrix appears as a network of thick, polymorphic anastomosing filaments in the nuclei of eukaryotic cells and has been demonstrated to play a very important role in DNA three-dimensional organization, DNA replication, RNA synthesis, RNA processing and so on. Previous studies demonstrated that actively transcribed genes were preferentially associated with the nuclear matrix, indicating that the nuclear matrix might be involved in the regulation of gene expression. Further investigation into the association of active genes with the nuclear matrix has revealed a DNA loop anchorage site in the enhancer regions or intronic sequences of several genes. These sequences have been termed matrix attachment regions (MARs) or scaffold attached regions (SARs)[1, 2]. MAR_S often cohabit with other regulatory elements, eg, enhancers, silencers or promoters[3-5]. Interaction of these MARS with nuclear matrix may result in the formation of chromatin loops that juxtapose regulatory elements in proximity to one another[6]. The MARs have also been shown to functionally confer increased transcriptional activity in transfected genes. These data demonstrate the importance of MARs for appropriate gene expression[7, 8].

Nuclear matrix proteins which bind to MAR sequences have been identified by a number of laboratories. These nuclear matrix proteins may serve as the attachment points for these matrix-associated DNA sequences, and it is possible that some of these proteins may be responsible for forming the DNA loop domains[4]. In addition, many transcription factors have been found to be associated with the nuclear matrix[9]. Recent evidence has further demonstrated that several transcription factors are localized or sequestered on the nuclear matrix[10]. These results revealed that the nuclear matrix proteins might play an important role in regulation of gene expression.

Studies on transgenic mice or on differentiating embryoid bodies have shown that the erythroid- and developmental stage-specific expression of the human ε-globin gene is controlled, in part, by the 5'-flanking sequences of this gene. Both the positive stage-specific regulatory element (from -446bp to -419bp, termed ε-PREII) and the silencer (from -392bp to -177bp) upstream of the human ε-globin gene have been identified[11, 12]. However, little is known about the molecular regulatory mechanism of the human ε-globin gene expression. In this study, we have examined the nuclear matrix attachment regions (MARs) and the binding nuclear matrix proteins in the 5'-flanking cis-acting elements of the human ε-globin gene. Our results have shown that the positive stage-specific regulatory element (ε-PREII) of this gene might be an erythroid-specific MAR, which could specifically bind to an erythroid-specific nuclear matrix protein (ε-NMP_k) in K562 cells. Also, the silencer element of this gene might be a nuclear matrix attachment region, while the nuclear matrix proteins from K562, HEL and Raji cells could all bind to this silencer element.

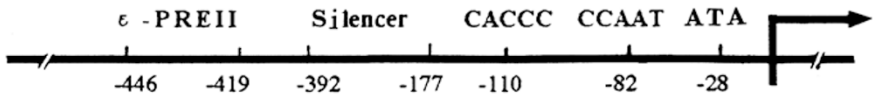
MATERIALS AND METHODS

Preparation of the nuclear matrices and nuclear matrix proteins

K562, HEL and Raji cells were grown in RPMI1640 medium supplemented with 10% fetal bovine serum. The nuclear matrices were prepared from these three cell lines according to the method described by Cockerill et al[13], and the nuclear matrix proteins were prepared according to the method described by Fey et al[14].

Preparation of DNA probes

The following diagram represents the structure of the upstream regulatory region of the human ε-globin gene. Sequences are numbered relative to the cap site at +1. The DNA fragment (from -446 to -419bp) is a positive stage-specific regulatory element ε-PREII, and the DNA fragment (from -392bp to -177bp) is a silencer element.



The ε-PREII DNA fragment was synthesized. The silencer DNA fragment and the promoter DNA fragment (-177bp ~ +1bp) of the human ε-globin gene were prepared from pε-GLCAT-SV plasmid by restriction enzymes (HincII/BamHI/HindIII). These three fragments were 5'-end labeled with γ-[^{32}P]-ATP respectively.

DNA-matrix binding assay

A standard in vitro MAR binding assay was performed as described by Cockerill et a1[13] and Rui-Ting ZONG et a1[15]. Briefly, matrices were washed 3 times in washing buffer (50 mM NaCl, 1 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, 0.5 mM PMSF, 0.25 mg/ml BSA). Binding was performed by incubation of the nuclear matrix with 90μl of assay solution (50 mM NaCl, 2 mM EDTA, 10 mM Tris-HCl, pH7.4, 0.5 mM PMSF, 0.25 mg/ml BSA, 5 ~ 10 ng of ^{32}P-end-labeled DNA fragment mixture, and 20μg of unlabeled, sonicated E. coli DNA) shaking for 1 h at 23 ℃. DNA fragments that interact with the matrix were separated from free DNA by 10min of centrifugation at 10,000 g at 4 ℃. After washing in 1 ml of final washing buffer (50 mM NaCl, 2 mM EDTA, 10 mM Tris-HCl, pH 7.4, 0.5 mM PMSF, 0.25 mg/ml BSA), the protein-DNA complex were solubilized in 15μl of solubilizing buffer (2 mM EDTA, 40 mM Tris-HCl, pH 7.4, 0.4 mg/ml proteinase K, 0.5% SDS, and 5 μg/ml sonicated salmon sperm DNA) and incubated overnight at 37 ℃. Resulting matrix-bound DNA fragments were resolved by polyacrylamide gel electrophoresis.

Gel mobility shift assay

Gel mobility shift assay was carried out as described by Strauss et a1[16]. The end-labeled DNA probe (~ 5 fmol) was incubated with 2 or 6 μg of nuclear matrix proteins in binding buffer (10 mM Tris-HCl, pH 7.6, 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 10 mM DTT, 4 % glycerol and 0.1% Triton X- 100) for 45 min on ice. Poly dI-dC (1μg) was added as a nonspecific competitor. DNA-protein complex was resolved by 5% non-denaturing polyacrylamide gel electrophoresis.

Southwestern blotting assay

Southwestern blotting assay was carried out according to the method described by Gao et al[17].

The nuclear matrix proteins (~ 100μg) from K562, HEL and Raji cells were separated by 12% SDS-polyacrylamide gel, transferred to a nitrocellulose filter (Schleicher and Schuell) in transfer buffer (50 mM Tris-HCl, 40 mM glycine, 0.04 % SDS, 20 % methanol) at 125 mA for 16 h at 4℃. After the transfer was completed, the filter was soaked in blocking buffer (50 mM Tris-HCl, 1 mM DTT, 5% Carnation non-fat milk powder, 5 μg/ml denatured and sonicated calf thymus DNA) at room temperature for 1 h, then the filter was incubated in binding buffer (50 mM Tris-HCl,pH 8.0, 50 mM NaCl, 1 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.25% Carnation non-fat milk powder) containing 5 x 10^5 cpm/ml DNA probe labeled with γ-[^{32}P]-ATP at 4 ℃ for 3 h. The filter was washed four times (8 min/each wash) at 4 ℃ with binding buffer and subjected to autoradiography.

RESULTS

Previous studies have shown that the patterns of the human β-like globin gene expression in K562, HEL and Raji cells are different. The K562 cells, a human erythroleukemia cell line, express the human embryonic (ε) and fetal (γ) globin genes, but not adult (β) globin gene[18]. However, the HEL cells, also a human erythroleukemia cell line, express mainly the human fetal (γ) globin gene and trace amount of the embryonic (ε) globin gene, but not adult (β) globin gene[19]. The Raji cells are nonerythroid cells. Therefore, these three cell lines provide an ideal model for studying the regulatory mechanism involved in the erythroid-specific and developmental stage-specific expression of the human ε-globin gene. In order to clarify the molecular regulatory mechanism of the human ε-globin gene expression, we have examined the nuclear matrix attachment regions (MARs) and their binding nuclear matrix proteins in the 5'-flanking cis-acting elements of the human ε-globin gene.

The positive stage-specific regulatory element (-446bp ~ -419bp, ε-PREII) of the human ε-globin gene associates specifically with the nuclear matrix from K562 cells

It has been shown that the positive stage-specific regulatory element (from -446bp to -419bp, ε-PREII) upstream of the human ε-globin gene is not only an AT-rich sequence but also contains a palindromic structure. So we assumed that positive stage-specific regulatory element (ε-PREII) might be a nuclear matrix attachment region (MAR). In order to clarify this speculation, the in vitro DNA-matrix binding assay was carried out. The mixture of ^{32}P-end-labeled DNA fragments, including 28bp ε-PREII DNA fragment and 178bp promoter DNA fragment of the human ε-globin gene, was incubated with insoluble nuclear matrix preparations from K562, HEL and Raji cells in the presence of E.coli competitor DNA. The matrix-associated DNA was analyzed by 5% polyacrylamide gel electrophoresis. Our results repeatedly demonstrated that only nuclear matrix from K562 cells could specifically associate with this positive stage-specific regulatory element, ε-PREII (Fig 1, lane 2), however, the nuclear matrix prepared from HEL and Raji cells could not associate with this positive regulatory element (Fig 1, lanes 3 and 4), indicating that ε-PREII might be an erythroid-specific nuclear matrix attachment region (MAR) which might play

an important role in activating the human ε-globin gene expression. In addition, we have observed that the promoter DNA fragment of the human ε-globin gene can not associate with the nuclear matrices from K562, HEL and Raji cells (Fig 1, lanes 2-4), suggesting that this promoter region may not be a MAR.

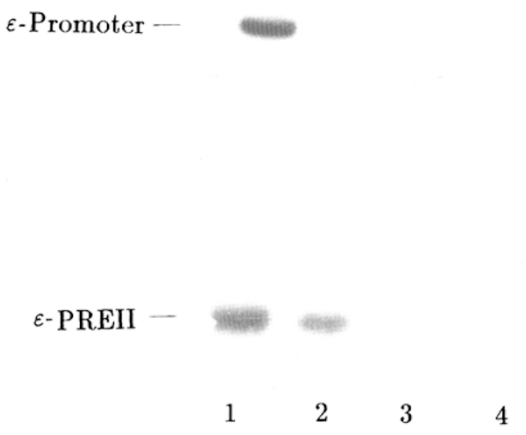


Fig 1. *In vitro* DNA-matrix binding assay: Specific binding of the positive regulatory element (ε-PREII) of human ε-globin gene to nuclear matrix from K562 cells. Lane 1: preincubation of DNA mixture(including a 28bp ε-PREII DNA fragment and a 178bp ε-promoter DNA fragment); Lane 2: DNA recovered in the pellet after incubation of DNA mixture with K562 cell nuclear matrix; Lane 3: DNA recovered in the pellet after incubation of DNA mixture with HEL cell nuclear matrix; Lane 4: DNA recovered in the pellet after incubation of DNA mixture with Raji cell nuclear matrix.

An erythroid-specific nuclear matrix protein binds specifically to the positive stage-specific regulatory element ε-PREII of the human ε-globin gene

In order to further examine the interaction between the nuclear matrix proteins and cis-acting elements in the 5'-flanking sequences of the human ε-globin gene, the binding of nuclear matrix proteins prepared from K562, HEL and Raji cells to the positive stage-specific regulatory element ε-PREII was analyzed by gel mobility shift assays. Our data repeatedly showed that only one shift band A could be detected in the nuclear matrix proteins prepared from K562 cells (Fig 2, lanes 2 and 3). However, nothing happened with the nuclear matrix proteins from HEL and Raji cells (Fig 2, lanes 4-7), suggesting that this nuclear matrix protein ε-NMP_k in K562 cells may be an erythroid-specific nuclear matrix protein and the interaction between the ε-NMP_k and ε-PREII DNA may play a critical role in activating the human ε-globin gene expression.

To further define the molecular weight of the ε-NMP_k identified above, Southwestern blotting assay was performed. As indicated in Fig 3, we observed that there were two polypeptide bands which could specifically bind to the ε-PREII DNA probe, and their molecular weights were near 40 kD (Fig 3, lane 1). Similarly, nothing could be found with the nuclear matrix proteins from HEL and Raji cells (Fig 3, lanes 2 and 3). Therefore, we tentatively assume that this erythroid-specific nuclear matrix protein (ε-NMP_k) in K562 cells may be composed of two polypeptides of approximate 40 kD in size.

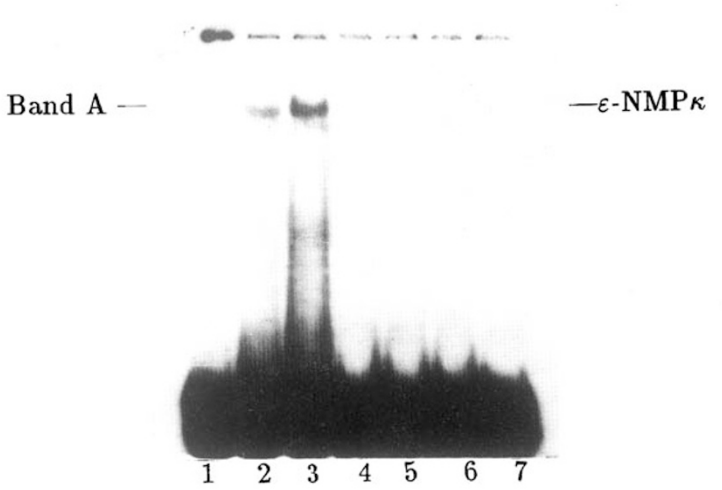


Fig 2. Gel mobility shift assay of the labeled DNA probe (ε-PREII, from -446bp to -419bp) with the nuclear matrix proteins from K562, HELand Raji cells. Lane 1, labeled fragment without nuclear matrix proteins; Lanes 2 and 3, labeled fragment with 4 and 6μg of K562 cell nuclear matrix proteins; Lanes 4 and 5, labeled fragment with 4 and 6μg of HEL cell nuclear matrix proteins; Lane 6 and 7, labeled fragment with 4 and 6 μg of Raji cell nuclear matrix proteins.

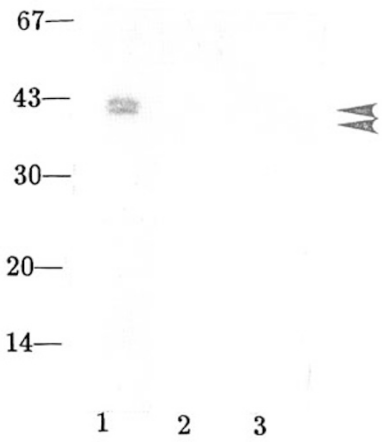


Fig 3. Southwestern blotting assay. The nuclear matrix proteins from K562, HEL and Raji cells were separated by 12% SDS-PAGE, transferred to a nitrocellulose filter and incubated with the labeled DNA fragment(C-PREII, from -446bp to -419bp). Lane 1, the nuclear matrix proteins from K562 cells; Lane 2, the nuclear matrix proteins from HEL cells; Lane 3, the nuclear matrix proteins from Raji cells.

The silencer (-392bp ~ -177bp) of the human ε-globin gene associates with the nuclear matrices and binds to the nuclear matrix proteins from K562, HEL and Raji cells

Previous studies have shown that the silencer between -392bp and -177bp upstream of the human ε-globin gene is indeed necessary for the silencing of the hu-

man ε-globin gene during later developmental stages. This silencer fragment is an AT-rich DNA sequence. In order to examine its interaction with the nuclear matrix, the *in vitro* DNA-matrix binding assay was also carried out. The mixture of ^{32}P-end-labeled DNA fragments, including 215bp silencer DNA fragment and 178bp

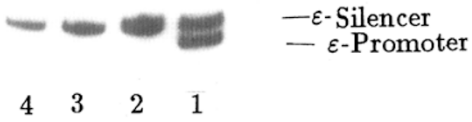


Fig 4. *In vitro* DNA-Matrix Binding Assay: Specific binding of the human ε-globin gene silencer to the nuclear matrices from K562, HEL and Raji cells. Lane 1: preincubation of DNA mixture(including a 215bp ε-silencer DNA fragment and a 178bp ε-promoter DNA fragment); Lane 2: DNA recovered in the pellet after incubation of DNA mixture with the nuclear matrix from K562 cells: Lane 3:DNA recovered in the pellet after incubation of DNA mixture with the nuclear matrix from HEL cells;Lane 4:DNA recovered in the pellet after incubation of DNA mixture with the nuclear matrix from Raji cells.

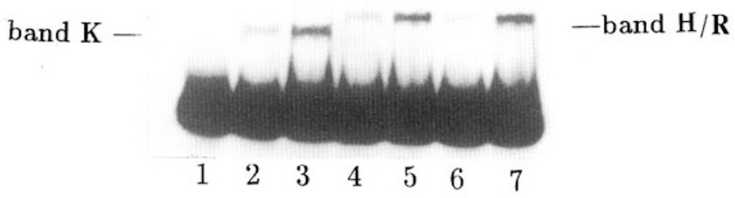


Fig 5. Gel mobility shift assay of the labeled silencer(-392bp to -177bp) of the human ε-globin gene with nuclear matrix proteins from K562, HEL and Raji cells. Lane 1: labeled DNA fragment without protein; Lanes 2 and 3: labeled DNA fragment with 2 μg and 6μg of nuclear matrix proteins from K562 cells; Lanes 4 and 5: labeled DNA fragment with 2 μg and 6μg of nuclear matrix proteins from HEL cells; Lanes 6 and 7: labeled DNA fragment with 2 μg and 6μg of nuclear matrix proteins from Raji cells.

promoter DNA fragment of human ε-globin gene, was incubated with insoluble nuclear matrix preparations from K562, HEL and Raji cells in the presence of E.coli competitor DNA as indicated. The matrix-associated DNA was analyzed by 10% polyacrylamide gel electrophoresis. Our results repeatedly demonstrated that all the nuclear matrices from K562, HEL and Raji cells could bind to this silencer DNA fragment (-392bp ~-177bp) (Fig 4, lanes 2-4), indicating that this silencer may also be a nuclear matrix attachment region (MAR), and the interaction between the silencer and nuclear matrix may play a critical role in silencing human ε-globin gene. On the other hand, we found in the gel mobility shift assay that nuclear matrix proteins from K562, HEL and Raji cells could also specifically bind to this silencer DNA respectively (Fig 5, lanes 2-7). However, the shift band K for K562 cells, indicated by the arrow in Fig 5, was different from the shift band H/R, which was common to HEL and Raji cells. Our data suggested that this common nuclear matrix protein might be partly responsible for the silencing of human ε-globin gene expression in HEL and Raji cells.

DISCUSSION

Privious studies have shown that the erythroid and developmental stage-specific expression of human E-globin genes is controlled, in part, by both the positive stage-specific regulatory element (from -446bp to -419bp, ε-PREII) and the silencer (from -392bp to -177bp) upstream of the human ε-globin gene[20-22]. However, the molecular regulatory mechanism of the human ε-globin gene expression is still unknow. Although several MARs have been detected in human β-globin locus[3, 23], the site of MARs within both ε-PREII and silencer upstream of the human ε-globin gene has not yet been identified. In order to further clarify the molecular mechanism involved in the regulation of human ε-globin gene expression, we first selected K562 cells and HEL cells with different human β-like globin gene expression pattern, together with nonerythroid Raji cells as an experimental model system. Then the nuclear matrix attachment regions (MARs) in the 5'-flanking cis-acting elements of human ε-globin gene and the related nuclear matrix proteins of these cells were compared by both in vitro DNA-matrix binding assay and gel mobility shift assay.

By using the in vitro DNA matrix binding assay, it was repeatedly demonstrated that the ε-PREII upstream of the human ε-globin gene could specifically associate with the nuclear matrix prepared from K562 cells, but not with the nuclear matrices prepared from both HEL and Raji cells, indicating that ε-PREII may be an erythroid specific-facultative MAR. To our knowledge, this is the first demonstration that the positive stage-specific regulatory element ε-PREII of human ε-globin gene can associate with the nuclear matrix from K562 erythroleukemia cells. By using gel mobility shift assay, an erythroid-specific nuclear matrix protein (designated as ε-NMP_k) was first detected in K562 cells, which could specifically bind to the positive stage-specific regulatory element (ε-PREII). Southwestern blotting assay indicated

that the ε-NMP_k in K562 cells was composed of two polypeptides of about 40 kD in size. We tentatively suggested that ε-NMP_k of K562 cells, via its interaction with ε-PREII, may play a pivotal role in activating the specific expression of human ε-globin gene in K562 cells.

In addition, the present data also showed that the nuclear matrices prepared from K562, HEL and Raji cells could also associate with the silencer DNA, as evidenced from the in vitro DNA-matrix binding assay. Meanwhile, ε-promoter DNA fragment did not show any association with these nuclear matrices. Therefore, it seems that silencer DNA can slso act as a nuclear matrix attachment region and we considered this regulatory element as a constitutive MAR, which may play a critical, but yet undefined role in silencing the human ε-globin gene expression. Now, when the nuclear matrix proteins isolated from K562, HEL and Raji cells were analysed by using a gel mobility shift assay with labeled silencer DNA fragment (-392bp to -177bp), it was found that the nuclear matrix proteins of these cells could all bind to the silencer DNA. However, the shift band K from K562 cells differed slightly in mobility as compared with that of shift bands H/R from HEL and Raji cells. The reason for such difference is not clear, but it may be possible that nuclear matrix protein in shift band K is different from those in shift bands H/R. Further studies are needed.

On the whole, the present work has demonstrated the importance of MARs and their binding nuclear matrix proteins in the regulation of human ε-globin gene expression. Further studies of the interaction between, nuclear matrix attachment regions (MARs) and nuclear matrix proteins will surely throw much light to our understanding of the molecular mechanism of human ε-globin gene expression.

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REFERENCES

[1] Getzenberg RH. Nuclear matrix and the regulation of gene expression: Tissue specificity. J Cell Biochem 1994; 55:22-31.
[2] Laemmli UK, Kas E, Polja KL et al. Scaffold-associated regione:cia-acting determinants of chromatin structural loops and functional domains. Curr Opin Genet Dev 1992; 2:275-85.
[3] Jarman AP, Higgs DR. Nuclear scaffold attachment sites in the human globin gene complex. EMBO J 1988; 7:3337-45.
[4] Dickinson L A, Joh T, Kohwi Y et al. A tissue-specific MAR/SAR DNA-binding protein with unusal binding site recognition. Cell 1992; 70:631-45.
[5] Kim MK, Lesoon-Wood LA, Weintraub BD et al. A soluble transcription factor, Oct-1 is also found in the insoluble nuclear matrix and possesses silencing activity in its Alanine-rich domain. Mol Cell Biol 1996; 16:4366-77.
[6] Hofmann JFX, Laroche t, Brand AH et al. RAP-1 factor is necessary for DNA loop formation in vitro at the silent mating type locus HML. Cell 1989; 57:725-37.

[7] Stief A, Winter DM, Straling WH et al. A nuclear DNA attachment elemant mediates elevated and position-independent gene activity. Nature 1989; 341:343-5.
[8] Blasquez VC, Xu M, Moses SC et al. Immunoglobulin kappa gene exppression after stable integration. I. Role of the intronic MAR and enhancer in plasmacytoma cells. J Biol Chem 1989; 264:21183-9.
[9] Nardozza TA, Quigley MM, Getzenberg RH. Association of transcription factors with the nuclear matrix. J Cell Biochem 1996; 61:467-77.
[10] van Wijnen AJ, Bidwell JPP, Fey EG et al. Nuclear matrix association of multiple sequence-specific DNA binding activities related to SP-1, ATF, CCAAT, E/EBP, OCT-1 and AP-1. Biochemistry 1993; 32:8397-402.
[11] Trepicchio WL, Dyer MA, Baron MH. Developmental region of the human embryonic β-like globin gene is mediated by synergistic interactions among multiple tissue and stage-specific elements, Mol Cell Biol 1993; 13:7457-68.
[12] Cao SH, Gutuman PD, Dave HPG et al. Identification of a transcriptional silencer in the 5'-flanking region of the human ε-globin gene, Proc Natl Acad Sci USA 1989; 86:5306-9.
[13] Cockerill PN, Garrard WT. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomermase II sites. Cell 1986; 44:273-82.
[14] Fey EG, Penman PS. Nuclear matrix proteins reflect cell type of origin in cultured human cells, Proc Natl Acad Sci USA 1988; 85:121-5.
[15] Rui-Ting Zong, Scheuermann RH. Mutually exclusive interaction of a novel matrix attachment region binding protein and the NF-uNR enhancer repressor. J Biol Chem 1995; 41:24010-8.
[16] Strauss F, Varshavsky A. A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutural proximity by DNA folding in the nucleosome. Cell 1984; 37:889-901.
[17] Erwei Gao, Joseph L, alcorn et al. Identification of enhancers in the 5'-flanking region of the rabbit surfactant protein A (SP-A) gene and characterization of their binding proteins, J Biol Chem 1993; 268:19697-709.
[18] Wada Y, Noguchi CT. In vitro differential expression of human globin gene. J Biol Chem 1988; 263:12142-6.
[19] Martin P, Papyannopoulou T. HEL cells: a new human erythroleukemia cell line with spontaneous and induced globin expression, Science, 1982; 216:12233-5.
[20] Yan ZJ, YD Chen, RL Qian. Developmental stage-specific trans-acting factors from the mouse haematopoises tissues binding to the 5' flanking cis-acting elements of the human ε-globin gene. Chinese Science Bulletin 1995; 40:778-83.
[21] Yan ZJ, YD Chen, RL Qian. Studies on DNA-protein interactions in the upstream regulatory region of the human ε-globin gene promoter. Cell research 1996; 6:101-14.
[22] Yan ZJ, C Jiang, RL Qian. Trans-acting factors from the human fetal liver binding to the human ε-glogin gene silencer. Cell Research 1997; 7:151-9.
[23] Cunningham JM, Purucker SM, Jane SM et al. The regulatory element 3' to the $^{A}\gamma$-globin gene binds to the nuclear matrix and interacts with special A-T-rich binding protein 1 (ASTB1), an SAR/MAR -associating region DNA binding protein. Blood 1994; 84:1298-308.

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