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In Embryonic Chicken Erythrocytes Actively Transcribed Alpha Globin Genes Are not Associated With the Nuclear Matrix

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ABSTRACT

The spatial organization of a 250 Kb region of chicken chromosome 14, which includes the alpha globin gene cluster, was studied using in situ hybridization of a corresponding BAC probe with nuclear halos. It was found that in non-erythroid cells (DT40) and cultured erythroid cells of definite lineage (HD3) the genomic region under study was partially (DT40 cells) or fully (HD3 cells) associated with the nuclear matrix. In contrast, in embryonic red blood cells (10-day RBC) the same area was located in the crown of DNA loops surrounding the nuclear matrix, although both globin genes and surrounding house-keeping genes were actively transcribed in these cells. This spatial organization was associated with the virtual absence of RNA polymerase II in nuclear matrices prepared from 10-day RBC. In contrast, in HD3 cells a significant portion of RNA polymerase II was present in nuclear matrices. Taken together, these observations suggest that in embryonic erythroid cells transcription does not occur in association with the nuclear matrix. J. Cell. Biochem. 106: 170–178, 2009. © 2008 Wiley-Liss, Inc.

KEY WORDS: NUCLEAR MATRIX; NUCLEAR HALO; GLOBIN GENES; EMBRYONIC ERYTHROCYTES

The eukaryotic cell nucleus is a highly organized system where most processes, such as transcription, repair, replication or splicing are localized to specific compartments [for review see Cremer and Cremer, 2001]. The nuclear matrix or scaffold is an operationally defined skeletal structure underlying the nucleus [Berezney et al., 1995; Berezney and Wei, 1998]. Although the nature of the nuclear matrix is still unclear and the existence of this structure has been questioned over the years [for review see Hancock, 2000; Misteli, 2007], it seems obvious that nuclear compartmentalization should have some structural support [Brown, 1999]. Transcription of the majority of genes in eukaryotic cell nuclei is mediated by RNA polymerase II organized in complexes known as transcription factories [Jackson, 1997; Carter et al., 2008]. It has been shown that transcription factories are associated with the nuclear matrix, that is, that they remain bound to nuclear remnants after chromatin removal by nuclease treatment and high salt extraction [Jackson, 1997]. This finding correlates well with previous observations demonstrating that active genes are associated with the nuclear matrix [Ciejek et al., 1983; Robinson et al., 1983]. In our recent study we have demonstrated that activation of transcription of several tissue-specific genes within a large DNA loop domain induces an association with the nuclear matrix of the whole looped DNA fragment [Iarovaia et al., 2005]. In the same study it was observed that genes transcribed at a very low level were not necessarily associated with the nuclear matrix [Iarovaia et al., 2005]. In connection with these results, it was interesting to study

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the pattern of association with the nuclear matrix of a large genomic area containing both tissue-specific and house-keeping genes. In the present study we have characterized the spatial organization in erythroid and non-erythroid cells of a large (250 Kb) region of chicken chromosome 14 which includes a cluster of erythroid cell-specific alpha globin genes and a number of other open reading frames that are transcribed and believed to represent essential house-keeping genes [Flint et al., 2001; Tufarelli et al., 2004]. Using in situ hybridization with nuclear halos [Iarovaia et al., 2004, 2005], we have demonstrated that in lymphoid cells the region under study was partially associated with the nuclear matrix. In AEV-transformed cultured erythroid cells derived from bone marrow of anemic chickens this region was fully associated with the nuclear matrix both before and after induction of alpha-globin gene transcription. In contrast, in 10-day chick embryo red blood cells (10-day RBC), which actively transcribe globin genes, [Bruns and Ingram, 1973; Weintraub et al., 1981; Singal et al., 2002] the whole 250 Kb genomic region under study was preferentially located outside the nuclear matrix. This related with the apparent absence of RNA polymerase II association with the nuclear matrix. A conclusion has been drawn that the spatial organization of transcription in embryonic cells drastically differs from that typical for cells of an adult organism.

MATERIALS AND METHODS

CELL CULTURE

The avian erythroblastosis virus-transformed chicken erythroblast cell line HD3 (clone A6 of line LSCC [Beug et al., 1979a,b]) and the chicken lymphoid cell line DT40 (CRL-2111, ATCC) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% chicken serum and 8% fetal bovine serum at 37°C in 5% CO_2 atmosphere. In the case of DT40 cells the medium additionally contained 50 μM β-mercaptoethanol. To induce erythroid differentiation, HD3 cells at a density of 8×10^5 cells/ml were incubated in the above medium additionally containing 10 mM HEPES (pH 8.0) and 20 μM iso-H-7 (1-(5-Isoquinolinylsulfonyl)-3-methylpiperazine dihydrochloride, Fluka) at 42°C in 100% air atmosphere [Nicolas et al., 1991]. Ten-day chicken embryonic red blood cells (RBC) were prepared as described previously [Mass et al., 2003].

PREPARATION OF NUCLEAR HALO FOR FISH

To isolate the nuclei, the cells were pelleted at 200 g for 5 min and permeabilizied in a buffer containing 10 mM PIPES pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 0.5% Triton X-100, 0.1 mM $CuSO_4$, 0.1 mM PMSF for 15 min on ice. Then the nuclei were centrifuged onto glass slides. The slides were sequentially incubated in a buffer containing 10 mM PIPES pH 6.8, 2 M NaCl, 10 mM EDTA, 0.1% digitonin, 0.05 mM spermine, 0.125 mM spermidine for 4 min and passed through 10×, 5×, 2×, 1× PBS and then through 10%, 30%, 50%, 70% and 95% ethanol solutions, air-dried, and finally fixed at 70°C for 2 h.

DNA FISH ANALYSIS

BAC DNA was biotinylated using Biotin-Nick Translation Mix (Roche, Penzberg, Germany) according to the manufacturer protocol.

Nuclear halos were treated sequentially with RNase A (100 μg/ml in 2× SSC) and pepsin (0.01% in 10 mM HCl), post-fixed with 1% paraformaldehyde, and rinsed in 70%, 80%, and 96% ethanol. To denature DNA, the slides were incubated in 70% formamide, 2× SSC for 5 min at 74°C, dehydrated in cold 70%, 80%, and 96% ethanol, and air-dried.

The hybridization mixture (in a final volume of 10 μl) contained 60% (v/v) formamide, 2× SSC, 10% dextran sulfate, 0.1% Tween-20, 10 μg sonicated salmon sperm DNA, 2 μg yeast tRNA and 25–50 ng of the labeled probe. In all experiments chicken total DNA (0.1–1 mg) was added. Before hybridization, the mixture was incubated for 10 min at 74°C to denature DNA. Hybridization was carried out overnight at 37–40°C. After hybridization the samples were washed twice in 50% formamide, 2× SSC at 40–42°C for 20 min.

For detection of a hybridization signal we used anti-biotin mouse antibodies conjugated with AlexaFluor 488 (Molecular Probes—Invitrogen, Eugene, OR), and a signal amplification kit for mouse antibodies (Molecular Probes—Invitrogen). The nuclear halos were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 0,5 μg/ml) in Vectashield antifade mounting medium (Vector Laboratories, USA). The slides were examined with a DMR/HC5 fluorescence microscope (Leica, Wetzlar, Germany) equipped with a HCX PZ Fluotar 100 ×/1.3 objective and recorded using a CCD DC 350 F camera (Leica, Wetzlar, Germany).

RNA FISH ANALYSIS

The cells were spread on silane-coated microscopic slides using a "Cytospin" centrifuge. All samples were fixed with paraformaldehyde as described [De Conto et al., 1999]. We used 27 bp long oligos (intron α^A: GGACGGGTAAGGCAGGGAGGGATAGGA-bio, ovalbumin exon: TTCTGTCTACACTCCAACATAAAAAGG-bio) with a single biotin label at the 3′ end. In situ hybridization with biotin-labeled oligonucleotides was carried out as described previously [Beatty et al., 2002] with modified concentration of formamide (40%) in the hybridization buffer and in the wash. After hybridization and removing the non-bound probe, the biotinylated probe was visualized using the NeutrAvidin-tetramethylrhodamin—biotinilated antiavidin amplification system. In all cases the DNA was counterstained with DAPI (40,6-diamidino-2-phenylindole) in Vectashield antifade mounting medium (Vector Laboratories). The results were analyzed as described above.

RT PCR ANALYSIS

RNA was extracted from cells with the Trizol kit (Invitrogen, Life Technologies, Eugene, OR). All RNA samples were further treated with DNase I to remove residual DNA. The efficiency of cDNA synthesis was equal in all preparations, as verified by RT-PCR with 18S RNA-specific primers.

RNA (1 μg) was then reverse transcribed in a total volume of 20 μl for 60 min at 42°C, using 0.4 μg random hexamer primers, 200 U reverse transcriptase (Fermentas, Vilnius, Lithuania) in the presence of 20 U ribonuclease inhibitor (Fermentas, Vilnius, Lithuania). The

TABLE I. Primers Used for the RT-PCR Analysis

Gene	PCR-primer
Primers to exons	
TFIIIC	CTACCACGATGAAGCTGACC
	GGATTCCTGACAATGTAACG
Loc425933	GAGGCCGAGGAGGAGCTGCT
	CGCCTTCTTCAGGTCCAGCAC
RHBDF1	CGTCACTCTGCTCCTTCA
	TCCACTGTATCCTCCTCCA
MPG	AACTATGCCAAGAAAAAGAA
	TGTCCCAGGAAGGATTTG
α^D	TGCCGAGGACAAGAAGC
	AGGTTGTAGGCATGCAGGTT
α^A	AACCAAGACCTACTTCCCC
	GTACTTGGCGGTCAGCAC
TMEM8	AGCTGCACAGACGATACCA
	TTTCAGAATCTTCTTCACCC
P15	GGATGGACTTGAAGCGAAC
	AAGCCTCTGTTTTTCTATTGC
Axin1	ATCAAACCAGCCACAAAAAG
	ATTCAGAGTAGGCAGATAACC
Primers to introns	
TFIIIC	AACCACAGGTAGGAGGGA
	GATTTAAGCCATTAGAAGC
Loc425933	TGGCGGACGACAGAAAGAAG
	TCCTACGGCTGCAACAACG
RHBDF1	GCCAAAATGAGCTTCCG
	AGCAGTCACAGAGCAAAA
MPG	GCTGCCTTGTATGCTCTA
	TGTTGGGATAAGGGTGAA
ggPRX	TGAAGAATTCAGAACATCAC
	CTAGTTTCCAGAATGTTCTG
α^D	TGGAGGTCTTGGGGTCAG
	AGGCTCAGTACCTTTGCTC
α^A	TCCCCTGATCCTCTTCCC
	GCACAGTGAGTCAGGGCTA
TMEM8	TCTGTCTACACCTACACCAT
	ATGTAAAGAAGCAGAAGAAT
P15	TGCCTGGATGCCATTGTT
	CCCATTCCGCTTCCTGTG
Axin1	GTGTTTCTTGCAGTGGGTT
	CAGCAGGTCAATGGAGTG
Primers for controls	
Ovalbumin	AATGGACCAGTTCTAATGT
	GTCAGCCCTAAATTCTTC
18S RNA	GGACATCTAAGGGCATCACAGACC
	CGTAGAGGTGAAATTCTTGGACC

synthesized cDNAs were amplified with Taq DNA polymerase (Fermentas, Vilnius, Lithuania), using a combination of "rev" and "dir" PCR primers (Table I). After 14 cycles of RT-PCR (when using primers to exons) or after 23 cycles (when using primers to introns), 5 μl of the reaction mixture was taken every three cycles, and the amplification was continued up to the 35th cycle. The products of PCR reactions were analyzed by agarose gel electrophoresis.

PREPARATION OF NUCLEAR MATRICES AND WHOLE CELL EXTRACTS

For DNAse I/NaCl extraction 7×10^7 cells (cycling HD3 cells, differentiated HD3 cells and 9-day chicken RBC) were collected by centrifugation and washed twice with PBS. Then the cells were incubated for 30 min on ice in permeabilization buffer (10 mM PIPES, (pH 7.8), 0.5% Triton X-100, 100 mM NaCl, 0.3 M sucrose, 0.2 mM PMSF and 3 mM $MgCl_2$). After incubation, the cells were washed three times with TM buffer (50 mM Tris–HCl pH 7.5, 3 mM $MgCl_2$) and then treated with RNase-free DNase I (100 μg/ml) (Sigma, St. Louis, MO) for 30 min at 37°C in 200 μl of TM buffer. An equal volume of TM buffer supplemented with 4 M NaCl was added (final concentration of NaCl was 2 M). After 20-min incubation on ice, the pellets were washed twice with the above buffer and resuspended in TM buffer. For preparing a whole cell extract (input fraction) the cells were washed twice with PBS, lysed in an IP buffer (50 mM Tris–HCl pH 8.0, 5 mM EDTA, 1% SDS, protease inhibitors) and sonicated for 30 s (VirSonic 100).

IMMUNOBLOTTING

Equal aliquots of each fraction (corresponding to $\sim 12 \times 10^6$ cells for the nuclear matrix fraction and $\sim 4 \times 10^6$ cells for the input fraction) were separated by 10% SDS–PAGE and blotted onto polyvinylidene difluoride membranes (Hybond-P, Amersham Biosciences, USA). The membranes were blocked overnight in 5% dry milk in PBS containing 0.1% Tween-20 (PBS-T) and incubated for 1 h with a primary antibody (mouse monoclonal antibodies to RNA polymerase II CTD repeat, Abcam ab5408) diluted in PBS containing 0.02% Tween-20 and 1% bovine serum albumin. After three washes with PBS-T, the membranes were incubated for 1 h with secondary antibodies (horseradish peroxidase-conjugated anti-mouse IgG) in PBS containing 0.02% Tween-20 and 5% dry milk. The immunoblots were visualized using an Amersham ECL kit. For data presentation, the films were scanned and processed with Adobe Photoshop CS software.

RESULTS

ESTIMATION OF THE TRANSCRIPTION LEVELS OF GENES PRESENT IN THE AREA UNDER STUDY

The map of the 250 Kb region of chicken chromosome 14 studied in the present work is shown in Figure 1A. The erythroid-specific domain of alpha-globin genes is located approximately in the middle of this genomic region. Just upstream to this domain a ubiquitously expressed gene ggPRX (CGTHBA) is located [Vyas et al., 1995; Sjakste et al., 2000]. This apparently housekeeping gene is highly conserved in evolution. In humans it is known as gene "-14" [Vyas et al., 1995]. Further upstream four other ORFs (MPG1, RHBDF1, snRNP 25K protein, and TFIIIC) are present. Downstream to the alpha-globin gene domain there are also several ORFs of which two encode putative transmembrane proteins (TMEM6, and TMEM 8), one encodes Melanoma antigen P15 and the last one encodes axin 1. We first checked if transcripts of all the above-mentioned reading frames were present in total mRNA isolated from DT40 cells, cycling HD3 cells, differentiated HD3 cells and 10-day RBC. The test fragments within the exon sequences were PCR-amplified after reverse transcription reaction and a minimum of PCR cycles necessary to observe a band was determined. The results shown in Figure 1B demonstrate that in non-erythroid cells all ORFs located upstream and downstream to the alpha-globin gene domain were transcribed, although the quantities of RNA transcribed from different ORFs varied significantly. No traces of globin RNAs were detected in RNA samples from DT40 cells even after 35 cycles of PCR. In RNA samples from all erythroid cells tested no traces of ovalbumin mRNA were detected even after 35 cycles of PCR. As expected, the levels of globin RNAs were quite prominent already in both cycling and differentiated HD3 cells, and they were even more

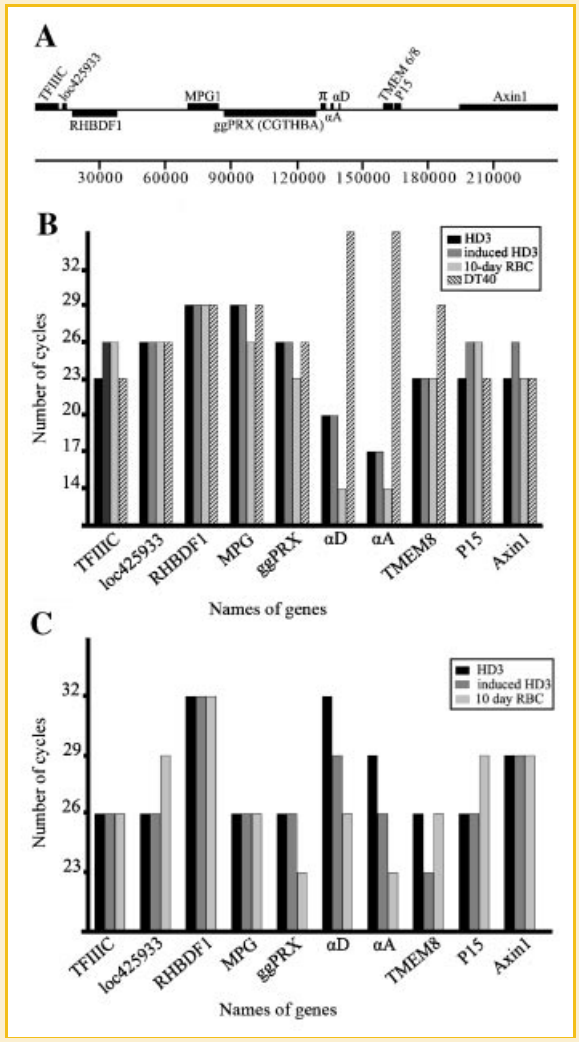

Fig. 1. RT-PCR analysis of the transcription level of alpha-globin genes and genes in surrounding areas. A: Relative positions of the genes studied. Designations on the scale of distances (bp) are given in accordance with the DNA sequence deposited in GenBank (accession number AN172304). The positions of genes are indicated by black rectangles. B: Histograms demonstrating the results of RT-PCR with exon-specific primers. The height of the columns corresponds to the PCR cycle when the product detectable by agarose gel electrophoresis appeared. C: Histograms demonstrating the results of RT-PCR with intron-specific primers. The designations are the same as in section "B".

prominent in 10-day RBC expressing globins. The transcripts of two overlapping genes (*TMEM6* and *TMEM 8*) located upstream to the alpha-globin gene domain were also overrepresented (about 50-fold) in all types of erythroid cells as compared to DT40 cells. It is clear that the above-described analysis makes it possible only to estimate the relative amounts of different transcripts in total RNA. This RNA may be present in cytoplasm even when transcription of the gene has long been terminated. This scenario looks quite probable for erythroid cells that undergo terminal differentiation. That is why it was important to analyze the transcription status in erythroid cells of the genes present within the area under study. With this aim in the next set of experiments the RT-PCR procedure was carried out with PCR primers designed to amplify the intronic sequences present in primary transcripts only. The same genes as in the above-described experiment were analyzed with the exception of the embryonic alpha-type globin gene π. Again we were interested in a minimal number of PCR cycles necessary to produce a visible band. The results shown in Figure 1C demonstrate that all of the above mentioned genes were expressed both in cultured HD3 cells and in 10-day RBC. The α^A gene was expressed much more intensively in 10-day RBC and in differentiated HD3 cells than in cycling HD3 cells. The estimated levels of transcription of other tested genes, with the exception of TMEM8, were quite different. Interestingly, in induced HD3 cells the *TMEM8* gene was expressed even at a higher level than the α^A gene.

ANALYSIS OF THE SPATIAL ORGANIZATION OF THE GENOMIC REGION UNDER STUDY IN ERYTHROID AND NON-ERYTHROID CELLS

To analyze the spatial organization of the genomic area under study (in terms of association with the nuclear matrix), we carried out in situ hybridization with so-called nuclear halos of biotinylated DNA of the BAC clone CH261-75C12. The 250 Kb insertion of this clone includes the whole DNA region presented in Figure 1A. Nuclear halos representing the crown of extended DNA loops fixed at the nuclear matrix were prepared by 2 M NaCl extraction of cells cytospun on microscopic slides [Iarovaia et al., 2004, 2005]. In the first set of experiments nuclear halos were prepared from DT40 cells. The results of hybridization are shown in Figure 2A,B and Figure S1. One can see that the greater part of the probe is associated with the nuclear matrix while a short fragment is looped out. It was reasonable to assume that this looped fragment represents a part of the construct containing the repressed domain of α-globin genes and two closely located genes (*TMEM6* and *TMEM8*) that seem to be poorly expressed in DT40 cells (see the previous section, Fig. 1B). In the next set of experiments nuclear halos were prepared from HD3 cells. Here the whole area under study was closely associated with the nuclear matrix in both chromosomes (Fig. 3A,B and Fig. S2). This result could be expected taking into consideration the fact that in non-induced HD3 cells the levels of expression of alpha-globin genes are comparable with those of the surrounding genes present in the area under study. Besides, it is known that in these cells the so-called Full Domain Transcript covering all three alpha-globin genes and the upstream area is produced. It probably serves to potentiate the domain for active transcription of individual globin genes [Razin et al., 2004]. The results of hybridization of the CH261-75C12 probe with the nuclear halos prepared from induced HD3 cells are shown in Figure 4 and Figure S3. One can see that the association of the whole area under study with the nuclear matrix is not disturbed in these cells expressing globins at the protein level. A completely different hybridization pattern was observed when the CH261-75C12 probe was hybridized to the nuclear halos prepared from 10-day RBC (Fig. 5A,B and Fig. S4). Only in a small portion of the nuclear halos (~10%) the whole signal was concentrated on the nuclear matrix as in HD3 cells. In other cases the hybridization signal was present predominantly in the crown of DNA loops. In some cases the whole stretch of hybridizing dots was located outside the nuclear matrix.

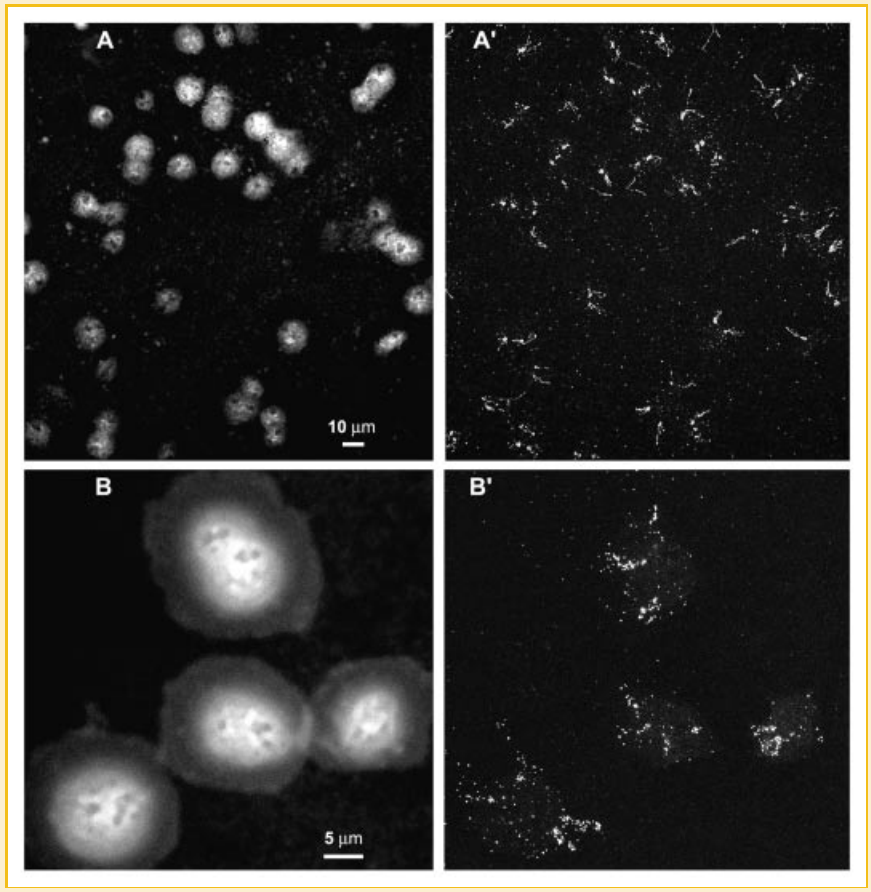

Fig. 2. Hybridization of the CH261-75C12 BAC DNA probe with nuclear halos from DT40 cells. A,B: DAPI staining of nuclear halos. A′,B′: Results of hybridization.

More typical was the observation of the V-shaped distribution of signals and a single stretch starting from the nuclear matrix. Keeping in mind the fact that the domain of α-globin genes is located approximately in the middle of the CH261-75C12 insertion, one can conclude that in most cases this domain is located outside the nuclear matrix. If the gobin genes were transcribed in all cells, this would indicate that in embryonic erythrocytes transcription occurs on looped DNA (i.e., that elongating RNA polymerase II complexes are not associated with the nuclear matrix). However, the population of 10-day RBC may be quite heterogenous. One may suggest that intensive transcription occurs only in a small portion of cells characterized by an association of the DNA region under study with the nuclear matrix. Other cells might represent different intermediate stages of terminal erythroid differentiation resulting in full inactivation of erythrocyte nuclei. In order to find out what portion of 10-day RBC studied in our experiments actually transcribes alpha globin genes, FISH with an intronic probe of the α^A gene was carried out. The results of this experiment are shown in Figure 6A,B. After examination of 200 cells we concluded that positive hybridization signals were present in ~80% of the cells. This means that the portion of cells that do not express globin genes can not exceed 20% and most probably is much lower as the efficiency of in situ hybridization can hardly amount to 100%. In some cells only one hybridizing spot was seen. This most likely reflects the fact that two signals are not always present in the same focal plane. In control experiment with the probe recognizing an intron of the ovalbumin gene, which is not active in erythroid cells, no hybridization signals were observed (not shown).

ANALYSIS OF RNA POLYMERASE II ASSOCIATION WITH THE NUCLEAR MATRIX IN HD3 CELLS AND 10-DAY RBC

It was shown previously that in different cells the transcription factories as well as elongating RNA polymerase II are associated with the nuclear matrix [Jackson and Cook, 1985; Jackson, 1997]. Taking into consideration the results described in the previous section it was interesting to check whether this is the case also in 10-day chicken RBC and HD3 cells. To this end we prepared nuclear matrices from cycling and differentiated HD3 cells and also from 10-day RBC and subjected them to Western analysis with antibodies recognizing RNA polymerase II. The conventional high-salt extraction procedure was used to prepare nuclear matrices [Rzeszowska-Wolny et al., 1988]. The results of experiments carried out with cycling HD3 cells, differentiated HD3 cells and 10-day RBC are presented in Figure 7. It is evident that in HD3 cells (either cycling or differentiated) RNA polymerase II is retained in the nuclear matrix while in 10-day RBC most, if not all, of it is solubilized during chromatin extraction.

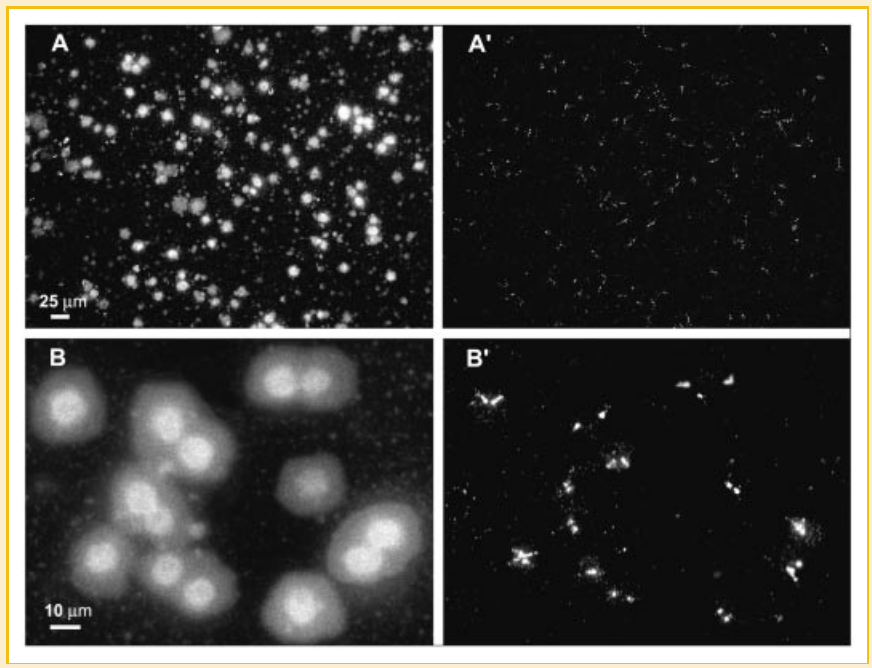

Fig. 3. Hybridization of the CH261-75C12 BAC DNA probe with nuclear halos from proliferating HD3 cells. A,B: DAPI staining of nuclear halos. A′,B′: Results of hybridization.

DISCUSSION

The most important result of this study is the demonstration of striking differences in the spatial organization of transcription in embryonic erythroblasts and erythroblasts of an adult organism.

Chickens have two erythroid cell types: primitive lineage cells, which predominate in the early stages of development (3–6 days), and definite lineage cells, which become the predominating cell type at later stages [Chapman and Tobin, 1979]. Primitive haematopoiesis occurs exclusively in the extra-embryonic blood islands of the yolk sac [Moore and Owen, 1965]. Definite haematopoiesis starts in the yolk sac [Moore and Owen, 1965] or within mesodermal cell clusters that line the mesentery and major blood vessels [Baumann and Dragon, 2005]. Later, the definite haematopoiesis is localized to bone marrow. For our discussion it is important to underline that in 10-day chick embryos immature definite red blood cells are released into the blood circulation system where they undergo several divisions and complete their terminal differentiation. These cells express adult alpha-globin genes and both fetal and adult beta-globin genes [Weintraub et al., 1981; Landes et al., 1982]. In adult birds the mature erythroblasts are concentrated in bone marrow and are not released into blood.

HD3 cells are a continuous cell line derived from mature erythroblasts of adult chickens by transformation with the temperature-sensitive mutant *ts*34 of the avian erythroblastosis virus [Beug et al., 1979a]. Cycling HD3 cells do not produce globins although the domain of alpha-globin genes is potentiated for transcription [Razin et al., 2004]. After induction of erythroid differentiation these cells stop proliferation and start transcription of globin genes and expression of hemoglobins which then gradually decreases, and the cells become similar to mature erythrocytes of adult birds which have completely inactive nuclei [Beug et al., 1979a]. We have demonstrated that in these cells transcription of globin genes, as well as transcription of surrounding house-keeping genes, occurs in association with the nuclear matrix. The whole 250 Kb area under study including the domain of alpha-globin genes flanked from both sides by several ubiquitously

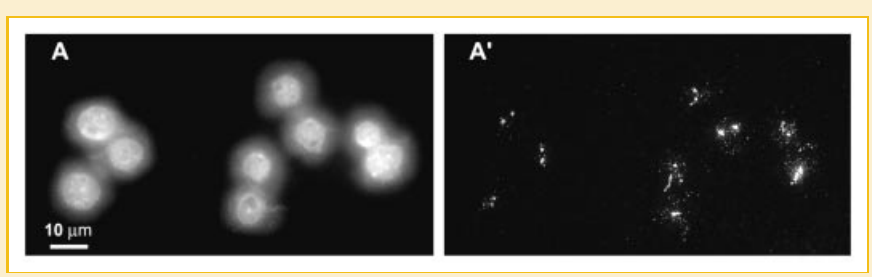

Fig. 4. Hybridization of the CH261-75C12 BAC DNA probe with nuclear halos from differentiated HD3 cells. A: DAPI staining of nuclear halos. A′: Results of hybridization.

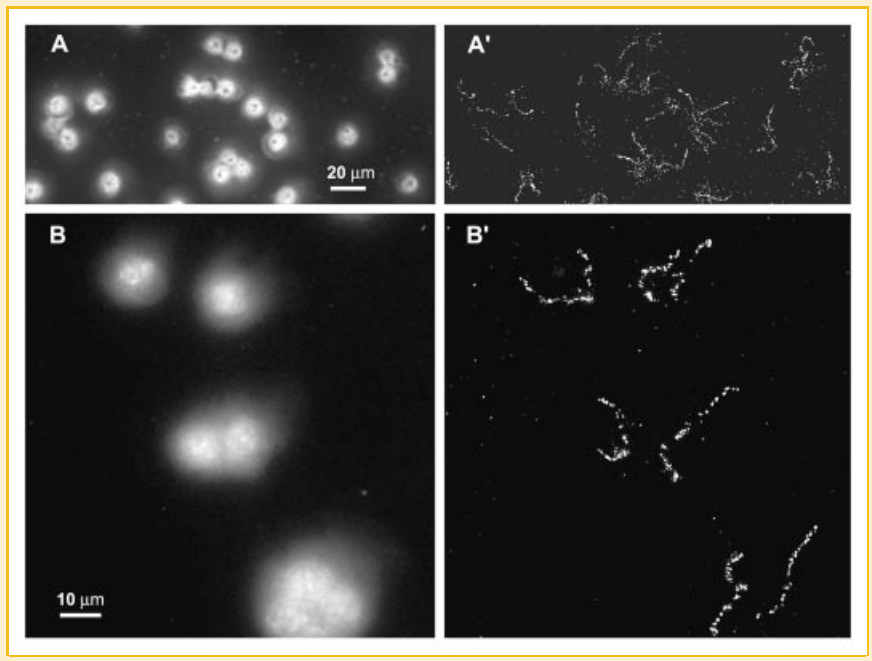

Fig. 5. Hybridization of the CH261-75C12 BAC DNA probe with nuclear halos from 10-day RBC. A,B: DAPI staining of nuclear halos. A′,B′: Results of hybridization.

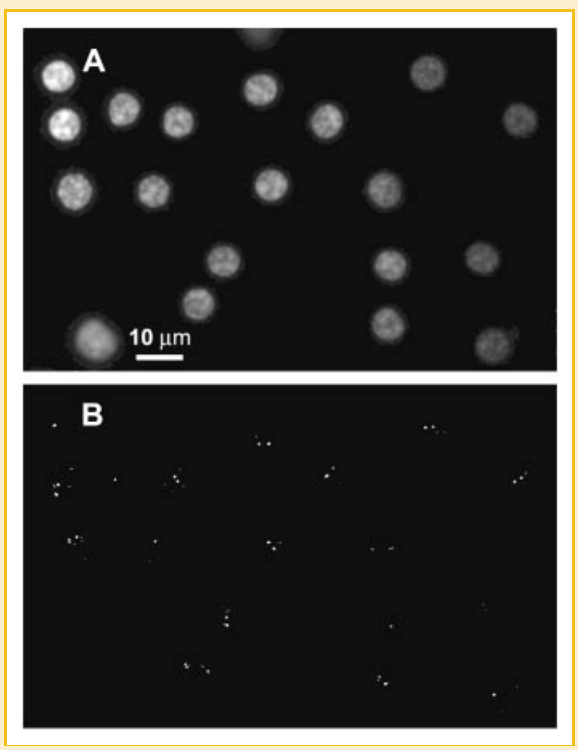

Fig. 6. Visualization of the sites of α^A gene transcription in 10-day RBC. A: DAPI staining of nuclei. B: Results of hybridization with a probe recognizing an intron of the α^A gene. Note that in the major portion of the nuclei the α^A gene is expressed.

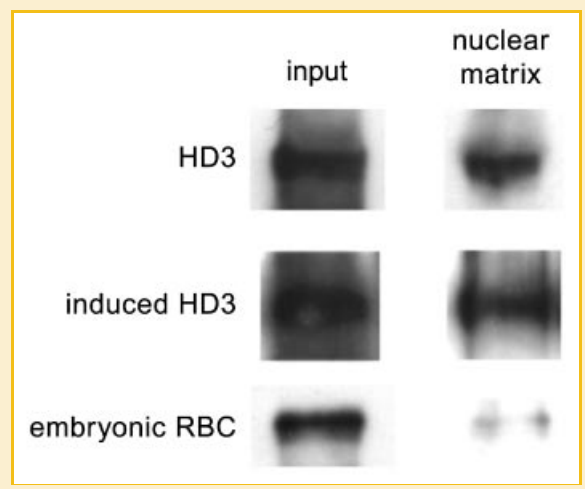

Fig. 7. Western-blot analysis of relative representation of RNA polymerase II in nuclear matrices isolated from HD3 cells and 10-day RBC. Chicken HD3 cells and 10-day RBC were subjected to DNase I/NaCl extraction, and after that the nuclear matrices were dissolved in the sample buffer for SDS–PAGE. Equal aliquots of the nuclear matrices prepared from proliferating HD3 cells, differentiated HD3 cells and 10-day RBC were separated by 10% SDS–PAGE and analyzed via immunoblotting using RNA polymerase II–specific antibodies. Equal aliquots of the whole cell extracts were used as input fraction in all immunoblotting experiments.

transcribed genes, was localized on the nuclear matrix, as demonstrated by FISH with nuclear halos. This result is similar to that obtained previously in experiments with a human genomic domain containing actively transcribed genes [Iarovaia et al., 2005]. Also, according to the previous data, the same 250 Kb DNA fragment was partially looped out from the nuclear matrix in DT40 cells where the central area of this fragment (including the domain of alpha-globin genes) is either not transcribed at all or transcribed at a very low level. As mentioned in the Introduction, association of transcribed sequences with the nuclear matrix was observed previously in a variety of different cells [for a review see Razin, 1987; Razin et al., 1995]. However, some data suggest that this may not be the case in embryonic cells. Thus, in chicken and amphibian oocytes RNA polymerases transcribing lampbrush chromosomes are not associated with any supporting structure [Doyle et al., 2002; Morgan, 2002]. Similarly, in amphibian oocytes the ribosomal genes transcribed by RNA polymerase I do not seem to be associated with the nuclear matrix [Miller and Beatty, 1969]. A drastic change has been shown to occur in the specificity of association of ribosomal genes with the nuclear matrix during early development and in somatic cells in amphibians [Vassetzky et al., 2000; Lemaitre et al., 2005; Hair and Vassetzky, 2007].

The data presented in this paper strongly support the supposition that in embryonic cells the spatial organization of transcription differs from that typical for cells of an adult organism idea. The large genomic fragment studied in our experiments was not associated with the nuclear matrix in most (90%) of immature erythrocytes present in blood of 10-day chick embryos in spite of active transcription of alpha-globin genes located in the central part of the 250 Kb long DNA region under study. Importantly, this spatial organization also can not be a consequence of active transcription of globin genes as in differentiated HD3 cells, where the α-globin genes are also actively transcribed, they remain associated with the nuclear matrix. The population of erythrocytes circulating in the blood of 10-day chick embryos is likely to be heterogenous [Landes et al., 1982]. That is why in ~10% of cases the region under study was associated with the nuclear matrix. In contrast, in HD3 cells derived from mature erythroblasts the same genomic region was associated with the nuclear matrix in all cells both before and after induction of terminal erythroid differentiation resulting in the beginning of globin gene expression. It would be interesting to check whether the observations made in our experiments with erythroid cells reflect the general situation, that is, whether in all embryonic cells transcription does not occur on the nuclear matrix. The reasons for the absence of an apparent association of transcribed genes with the nuclear matrix in embryonic erythrocytes are not clear at the moment. The simplest possibility, which can be eventually tested experimentally, is that in embryonic cells Pol II transcription complexes are not organized into transcription factories.

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