Biochemistry

 Volume 29, Number 22 June 5, 1990

Accelerated Publications

A Nuclear Matrix Protein Binds Very Tightly to DNA in the Avian β-Globin Gene Enhancer†

David W. Zenk, Gordon D. Ginder, and Timothy W. Brotherton*
Department of Internal Medicine, University of Iowa College of Medicine, and VA Medical Center, Iowa City, Iowa 52242
Received February 28, 1990; Revised Manuscript Received April 11, 1990

ABSTRACT: Current evidence suggests that DNA is covalently attached to proteins in the nuclear matrix of eukaryotic cells and that specific DNA sequences are tightly associated with the nuclear matrix. However, it has not been documented that specific DNA sequences can become covalently attached to nuclear matrix protein. We have examined the binding of cloned DNA sequences that contain the avian β-globin gene enhancer, a region previously shown to be matrix associated in erythroid cells in vivo, with nuclear matrices from several avian tissue sources to determine if covalent DNA–protein bonds are formed. Our results indicate that sequence-specific DNA–protein complexes that are resistant to denaturation by SDS, boiling, and phenol and disulfide reduction are formed. Excess protein, capable of forming very tight bonds with DNA that contains the β-globin gene enhancer, is present in cells in which matrix attachment of this DNA sequence is not detected in vivo. Evidence is presented that suggests that the protein to which DNA forms very tight bonds is not topoisomerase II. These results are discussed in relation to current models of the nuclear matrix and the utility of in vitro assays of matrix attachment regions using cloned DNA.

Eukaryotic chromatin is organized into large loops of DNA that are associated with histones and a wide variety of nonhistone proteins, which together are attached to a salt-resistance structure collectively referred to as the nuclear matrix or scaffold (Berezney & Coffey, 1974; Benyajati & Worcel, 1976; Cook & Brazell, 1975). Evidence suggests that both replication (Pardoll et al., 1980; Smith & Berezney, 1982; Buongiorno-Nardelli et al., 1982; Razin et al., 1986; Blow & Laskey, 1988) and transcription (Jackson et al., 1981; Ciejek et al., 1983; Robinson et al., 1983; Keppel, 1986; Buttyan & Olsson, 1986; Roberge et al., 1988) occur at the interface of DNA with the nuclear matrix. Although there is convincing evidence that DNA is covalently bound to proteins in the nuclear matrix (Razin et al., 1981, 1988; Bodnar et al., 1983; Neuer & Werner, 1985; Werner & Rest, 1987; Avramova & Tsanev, 1987; Neuer-Nitsche et al., 1988; Cress & Kurath, 1988; Werner & Neuer-Nitsche, 1989), it has not yet been established that DNA sequences that have been identified as matrix attachment regions (MARs)[1] are covalently bound to protein. Furthermore, the identity of the nuclear matrix proteins covalently bound to DNA remains controversial. Proteins identified as being very tightly bound to eukaryotic DNA have been reported to range in size from being less than 20 kDa (Razin et al., 1981) to being too large to enter SDS stacking gels (Avramova & Tsanev, 1987). In particular, it has been suggested that topoisomerase II may be involved in some aspect of DNA binding to the nuclear matrix as this protein is a major constituent of the matrix (Berrios et al., 1985; Gasser et al., 1986), has a consensus sequence for cleavage that is similar to elements within reported nuclear matrix attachment sequences (Cockerill & Garrard, 1986; Spitzner & Muller, 1988), and can bind covalently to DNA (Wang, 1985).

We have recently mapped several nuclear matrix attachment sites within the avian β-globin cluster (Brotherton et al., 1989; Bennett et al., 1989) using the lithium diiodosalicylate method (Mirkovitch et al., 1984). One MAR of particular interest has been located within a 2200-bp fragment that contains the

† This work was supported by a Merit Award from the Veterans Administration to T.W.B., who is a Research Associate of the VA.
* Address correspondence to this author at the Department of Internal Medicine, University of Iowa.

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; PMSF, phenylmethanesulfonylfluoride; kDa, kilodalton; bp, base pair; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; MAR, matrix attachment region.

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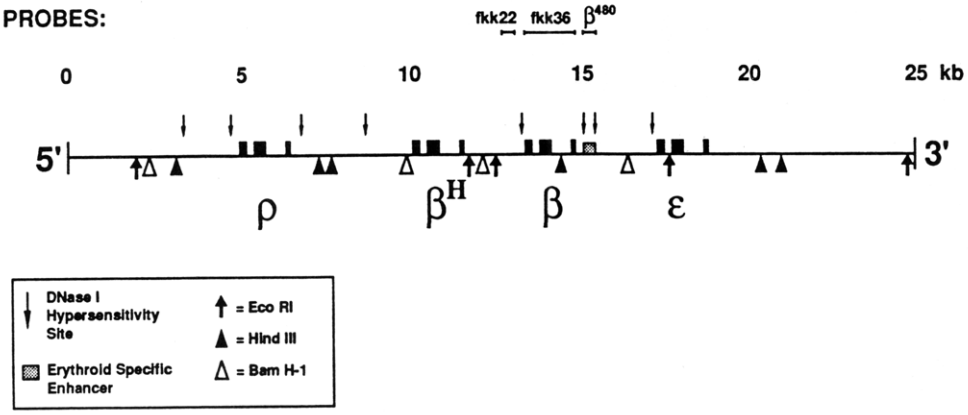

FIGURE 1: Map of avian β-globin gene cluster. Two embryonic globin genes (ρ and ε) flank two adult globin genes (β^H and β). The size and location of the cloned DNA probes used in this study are shown. The position of the erythroid-specific β-globin enhancer is indicated by the gray box and of known DNaseI-hypersensitive sites (Stalder et al., 1980; G. D. Ginder, unpublished results) near the β-globin gene by arrows above the line.

nuclease hypersensitive enhancer element (Choi & Engel, 1986; Hesse et al., 1986) that resides about 2000 bp downstream from the adult β-globin cap site (Bennett et al., 1989). This site is matrix attached in avian reticulocytes but not in avian brain cells (Bennett et al., submitted for publication). In this study we show that a cloned 480-bp DNA fragment that contains this 3′-enhancer element binds to nuclear matrices from avian erythrocytes and brain cells. Unlike other DNA fragments from the β-globin gene region that nonspecifically bind to nuclear matrices, this 480-bp fragment forms a very tight bond with the nuclear matrix and cannot be displaced by competitor DNA or protein denaturants such as the anionic detergent SDS. DNA strand cuts were not introduced by nuclear matrix binding, and binding occurred in the absence of divalent cations. These observations suggest that the nuclear matrix protein that is tightly bound to DNA containing the β-globin gene enhancer is not the previously described topoisomerase II.

Experimental Procedures

Nuclear Matrix Isolation. The method of Cockerill and Garrard (1986) was used to obtain matrices for the in vitro binding assay. The lithium diiodosalicylate method (Mirkovitch et al., 1984) was used to obtain nuclear matrices with largely intact DNA halos for restriction enzyme analysis. Nuclei were isolated from washed adult chicken reticulocytes (Brotherton & Ginder, 1986) or frozen adult chicken thymus or stripped brain tissue (Pel-Freeze) as previously described (Brotherton et al., 1980).

DNA Binding Assay. Matrices from approximately 1 mg of nuclei were washed three times in 40 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.25 M sucrose, and 0.25 mg/mL BSA and resuspended in 100 μL of this same buffer, with up to 20 mg/mL ^{32}P-end-labeled probe (see below) and up to 150 mg/mL sonicated (*Escherichia coli* or salmon sperm) DNA or unlabeled probe DNA. In some experiments, $MgCl_2$ (1 mM) replaced EDTA in the incubation buffer. The mixture of nuclear matrices and DNA was incubated for up to 2 h at 25 °C with constant mixing. After addition of 500 μL of incubation buffer, the nuclear matrices were pelleted by centrifugation at 12000*g* for 60 s at 4 °C. The first supernatant, containing DNA not associated with nuclear matrices, was saved. The pellet, containing DNA associated with the nuclear matrix, was washed three times with 500 μL of incubation buffer and then dissolved in 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 1% (w/v) SDS at 50 °C. Residual insoluble material was removed by centrifugation at 12000*g* for 10 s at 25 °C and the supernatant made 100 mM with KCl. SDS/K^+ complexes were precipitated by incubation on ice for 10 min followed by centrifugation at 12000*g* for 10 min at 4 °C. This second supernatant containing matrix-associated DNA not precipitated by SDS/K^+ was separated from the pellet and saved. The pellet was washed twice by heating to 50 °C in 500 μL of 100 mM KCl, 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 0.5% SDS. Proteinase K was added, and the solution was incubated overnight at 37 °C. The first and second supernatant fractions (see above) were made 0.5 M in NaCl and 0.5% in SDS and likewise incubated overnight at 37 °C with proteinase K. Carrier DNA (10 μg of sonicated *E. coli* DNA) was added to all fractions, followed by extraction with phenol and chloroform. The samples were precipitated and washed with ethanol and resuspended in 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA. Equal counts of each sample were subjected to electrophoresis in 1× TBE (0.089 M Tris, 0.089 boric acid, 2.5 mM EDTA, pH 8.4) on either polyacrylamide [5% acrylamide, 0.15% bis(acrylamide)] or 1–2% (w/v) agarose gels. After electrophoresis, acrylamide gels were air-dried between sheets of cellophane, and agarose gels were transferred by the alkaline Southern method to nylon (Reed & Mann, 1985) and autoradiographed.

The cloned DNA probes used are described elsewhere (Brotherton & Ginder, 1986; Bennett et al., 1989) and shown in Figure 1. Probe fkk22 is a 500-bp fragment of the β-globin gene cluster that is located just upstream from the 5′-DNaseI hypersensitive site. Probe fkk36 is a 1300-bp fragment that spans the majority of the transcribed portion of the adult β-globin gene. β480 is a 480-bp fragment that spans the 350-bp region that is identified as a tissue-specific enhancer, and is situated about 2000 bp 3′ to the β-globin cap site. Of interest, major topoisomerase II cleavage sites have been mapped to the β-globin enhancer and to the region spanned by probe fkk36 both in vivo in avian erythroblast nuclei and in vitro with purified enzyme (Muller & Mehta, 1988). As fkk22 and β480 comigrate during electrophoresis, in some experiments, DNA was subjected to restriction enzyme digest with *Sac*I or *Bst*NI prior to electrophoresis.

Results

A MAR Is Located within the β-Globin Enhancer in Erythrocytes and Is Tightly Bound to Protein. The method for mapping MARs with DNaseI-treated nuclear matrices and ^{32}P-end-labeled cloned DNA fragments (Cockerill & Garrard, 1986) was used to study the interactions of avian tissue derived nuclear matrices with DNA sequences found in the region of

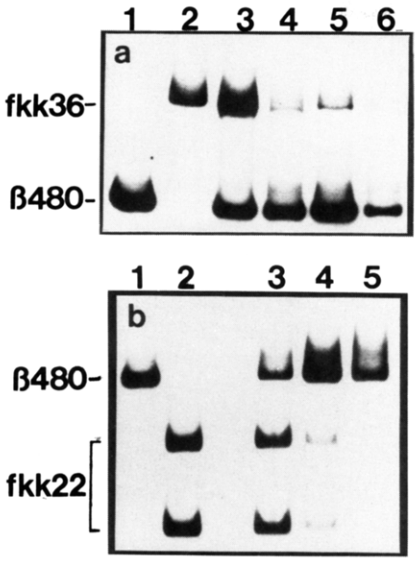

FIGURE 2: β480 DNA binds very tightly to reticulocyte nuclear matrix protein. Washed reticulocyte nuclear matrices were incubated with 5′-radiolabeled DNA fragments and cold competitor DNA as described under Experimental Procedures. After incubation, portions of the washed nuclear matrix pellet were subjected to SDS/K^+ precipitation. DNA was prepared from each sample by proteinase K digestion followed by phenol and chloroform extraction. Samples of equal counts were subjected to 5% acrylamide gel electrophoresis in 1× TBE. Shown are the autoradiograms of dried gels. (a) Nuclear matrices incubated with fkk36 and β480 DNA: Lane 1, β480 marker; lane 2, fkk36 marker; lane 3, DNA not associated with the nuclear matrix pellet after incubation; lane 4, DNA associated with the nuclear matrix pellet after incubation; lane 5, matrix-associated DNA soluble in SDS/K^+; lane 6, matrix-associated DNA precipitated by SDS/K^+. (b) Nuclear matrices incubated with fkk22 and β480 DNA. Samples were digested with *Sac*I prior to gel electrophoresis. Lane 1, β480 marker; lane 2, fkk22 marker; lane 3, DNA not associated with the nuclear matrix pellet after incubation; lane 4, matrix-associated DNA soluble in SDS/K^+; lane 5, matrix-associated DNA precipitated by SDS/K^+.

the adult β-globin gene. As shown in Figure 2, nuclear matrices prepared from reticulocytes bound fragment β480 in preference to fragments fkk36 (Figure 2a, lane 4) and fkk22 (Figure 2b, lane 4), in the presence of cold nonspecific competitor DNA. In addition, matrix-associated β480 was precipitated, in the presence of SDS/K^+ (see Experimental Procedures). SDS/K^+ precipitation has been shown to precipitate DNA covalently attached to proteins such as topoisomerase II (Liu et al., 1983), topoisomerase I, and histone H1 (Muller, 1984). Double- and single-stranded cuts were not introduced into SDS/K^+-precipitated β480 DNA (Figure 2 and data not shown). These results suggest that a MAR is located within the region of DNA spanned by probe β480 and that a protein constituent of the matrix preparation can form a complex with β480 DNA that is resistant to dissociation by SDS and heating to 50 °C. That the precipitation of β480 DNA is due to tight binding to protein is further supported by the observations that β480 DNA is not precipitated by SDS/K^+ prior to incubation with nuclear matrices. Furthermore, proteinase K digestion of β480 associated with nuclear matrices markedly reduces the quantity of radioactive β480 recovered by subsequent SDS/K^+ precipitation (data not shown). Finally, elimination of proteinase treatment prior to electrophoresis of isolated fragments results in the majority of the radiolabeled β480 DNA being retarded in the gel well (Figure 3). Similar results are obtained if samples were subjected to SDS gel electrophoresis (data not shown). Extraction with phenol (Figure 3, lane 5) or chloroform/isoamyl alcohol or boiling in the presence of disulfhydryl reducing

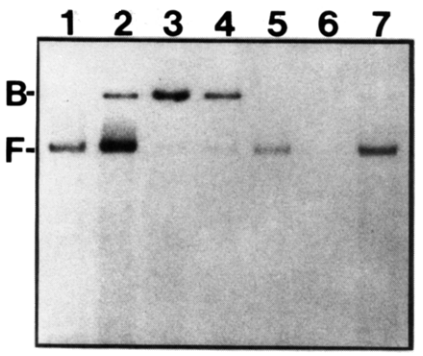

FIGURE 3: SDS/K^+-precipitated DNA is very tightly bound to nuclear matrix protein. Reticulocyte nuclear matrices were incubasted with 5′-radiolabeled β480 and cold competitor DNA as described under Experimental Procedures. Nuclear matrix associated DNA was fractionated by SDS/K^+ precipitation and either incubated with proteinase K (50 μg/sample, 2 h/37 °C) prior to phenol extraction, directly phenol extracted or directly subjected to 5% acrylamide gel electrophoresis. All samples were boiled in 1× TBE (see Experimental Procedures) and cooled on ice just prior to application to the gel. Lane 1, β480 DNA marker; lane 2, nuclear matrix associated DNA soluble in SDS/K^+. Lanes 3 to 7 are nuclear matrix-associated DNA precipitated by SDS/K^+: lane 3, sample directly submitted to gel electrophoresis; lane 4, phenol-extracted DNA sample, soluble in the phenol phase; lane 5, phenol-extracted DNA sample, soluble in the aqueous phase; lane 6, proteinase K digested DNA sample, soluble in the phenol phase; lane 7, proteinase K digested DNA sample, soluble in the aqueous phase.

agents (data not shown) does not dissociate the DNA–protein complex. Without prior proteinase treatment, up to 80% of the β480 DNA–portein complex is extracted into the phenol phase (Figure 3, lanes 5 and 6).

Reticulocyte DNA that contains the erythrocyte-specific enhancer is also very tightly bound to protein in nuclei. Nuclear matrices were prepared from reticulocytes by repeated 2 M NaCl washes and then subjected to *Eco*RI–*Hin*dIII digestion. The nuclear matrix associated DNA and matrix released DNA were then subjected to SDS/K^+ precipitation. About 30% of the nuclear matrix associated DNA was recovered after SDS/K^+ precipitation, and less than 1% of the matrix-released DNA was recovered by this method. A 3200-bp fragment that hybridized to β480 was both enriched in the matrix-associated fraction of DNA and present in SDS/K^+-precipitated DNA of this fraction. In contrast, the 1800-bp fragment that hybridized to fkk22 was greatly enriched in the matrix-released fraction of DNA and was not precipitated by SDS/K^+ (data not shown).

Nuclear Matrix Binding Sites for Probe β480 Are Present in Tissues That Do Not Express β-Globin Genes. The 2200-bp *Hin*dIII–*Bam*HI fragment of the β-globin gene cluster that contains the erythroid-specific enhancer (see Figure 1) is matrix associated in both primitive and definitive-stage avian reticulocytes, but not in brain nuclei. The 1800-bp *Eco*RI–*Hin*dIII fragment that contains the sequences found in fkk22 is not matrix associated in either brain or erythroid matrix preparations (Bennett et al., 1989; Bennet et al., submitted for publication). Therefore, avian brain nuclear matrices were assayed for binding of the β480 DNA fragment. As shown in Figure 4 brain nuclear matrices bind β480 (lane 6) and can form protein–β480 complexes that are precipitated by SDS/K^+ (lane 7). In addition, probe fkk22 can be associated with both adult reticulocyte (Figure 4, lane 3) and brain (Figure 4, lane 6) nuclear matrices when the ratio of cold competitor DNA to nuclear matrices is low (see below). Although this matrix-associated fkk22 DNA cannot be washed out by 2 M NaCl (Figure 4, lane 3), it is not precipitated by SDS/K^+ (Figure 4, lanes 4 and 7). Probe fkk36 did not become as-

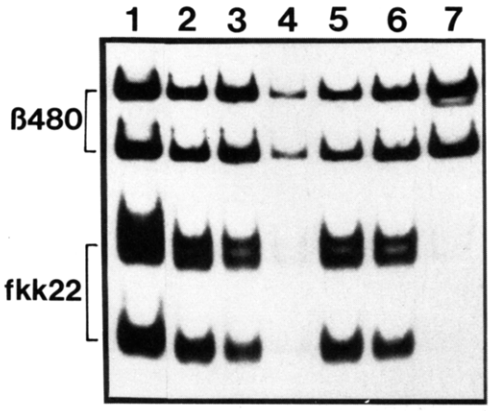

FIGURE 4: β480 DNA binds very tightly to brain nuclear matrix protein. Washed reticulocyte (lanes 2–4) and brain (lanes 5–7) nuclear matrices were incubated with 5′-radiolabeled β480 and fkk22 DNA in the absence of cold competitor DNA (see Experimental Procedures). After being washed three times with the incubation buffer, reticulocyte matrices were washed twice more with 2 M NaCl, 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA. The washed nuclear matrices were subjected to SDS/K^+ precipitation. DNA was prepared, and samples of equal counts were digested with *Bst*NI and *Sac*I prior to electrophoresis. Lane 1, digested β480 and fkk22 probe markers; lane 2, DNA not associated with reticulocyte nuclear matrices after incubation; lane 3, reticulocyte nuclear matrix associated DNA soluble in SDS/K^+; lane 4, reticulocyte nuclear matrix associated DNA precipitated by SDS/K^+; lane 5, DNA not associated with brain nuclear matrices after incubation; lane 6, brain nuclear matrix associated DNA soluble in SDS/K^+; lane 7, brain nuclear matrix associated DNA precipitated by SDS/K^+.

sociated with erythroid or brain nuclear matrices even in the complete absence of competitor DNA (data not shown). These findings indicate that nuclear matrices prepared from cells that do not express β-globin, and which do not bind the region of DNA that contains the erythroid-specific enhancer in vivo, have binding sites for the sequences found in the β480 fragment. In addition, the results suggest that SDS/K^+ precipitation may be used to confirm the specificity of binding by cloned DNA fragments to nuclear matrices.

DNA Tightly Bound to Matrix Protein Cannot Be Displaced by Competitor DNA. To test the stability of the interaction between β480 DNA and nuclear matrix binding proteins, the effect of varying the concentration and time of addition of cold β480 containing DNA was examined. Brain nuclear matrices were incubated with a fixed quantity of either radiolabeled fkk22 or β480. Cold β480, either contained in the supercoiled 3600-bp plasmid pβ480-3′ or isolated from the plasmid following restriction enzyme digestion, was added in varying quantities to nuclear matrices either before, with, or after the addition of the radiolabeled probe. Supercoiled plasmid DNA containing β480 was tested as it has been reported that nuclear matrices preferentially bind supercoiled DNA (Muller et al., 1988). Nuclear matrices were incubated for 30 min at room temperature between additions of cold competitor or radiolabeled DNA. Following the last addition of DNA, samples were incubated an additional 30 min. Nuclear matrix pellets were isolated, washed in incubation buffer, and subjected to scintillation counting. The proportion of counts remaining in the nuclear matrix pellet relative to controls in which no cold competitor DNA was added was determined for each level of added competitor β480 DNA. As shown in Figure 5a, radiolabeled fkk22 could be displaced from the nuclear matrix pellet regardless of when the cold pβ480-3′ DNA was added. In contrast, radiolabeled β480 was only displaced from the matrix if cold competitor β480 was added prior to or with the radiolabeled probe (Figure 5b). Steric hindrance within the nuclear matrix protein cage may be an

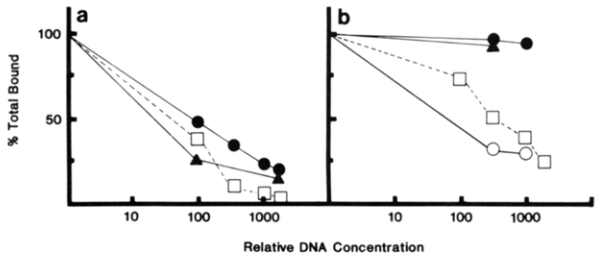

FIGURE 5: β480 DNA is not displaced from brain nuclear matrices by excess cold specific competitor DNA after binding to protein. Brain nuclear matrices were incubated with 5′-radiolabeled fkk22 (panel a) or β480 (panel b). Cold competitor β480 DNA, either contained in the 3600-bp supercoiled plasmid pβ480-3′ or released from the plasmid by restriction enzyme digestion, was added to the nuclear matrix incubation mixture before, concurrently with, or after the addition of radiolabeled DNA. When the incubation was finished, the matrix pellet was washed three times with incubation buffer, and radioactivity remaining in the pellet was determined. The percent total bound for each tested ratio (w/w) of cold β480 DNA to radiolabeled DNA is plotted with 100% taken as the counts bound in the absence of competitor DNA. The order of addition of DNA was as follows: preincubation with pβ480-3′ for 30 min prior to the addition of radiolabeled DNA and further incubation for 30 min (□), simultaneous incubation with pβ480-3′ and radiolabeled DNA for 1 h (●); simultaneous incubation with cold β480 and radiolabeled β480 for 1 h (○); preincubation with radiolabeled DNA for 30 min prior to the addition of pβ480-3′ (panel a) or cold β480 (panel b) and further incubation for 30 min (▲).

important determinant in these competition studies, as the supercoiled plasmid pβ480-3′ did not displace radiolabeled β480 when added concurrently with the radiolabeled probe but did block the binding of labeled probe when added prior to the probe. However, steric hindrance alone cannot completely explain the inability of competitor DNA to displace β480 from the nuclear matrix, as pβ480-3′ can displace fkk22 regardless of the sequence of addition. These results further support the hypothesis that a very stable bond is formed between DNA that contains the erythroid-specific enhancer and the nuclear matrix. Very stable bonds are also formed between endogenous cellular DNA and the nuclear matrix as the [^{3}H]thymidine-labeled DNA of K562 cells, a human erythroleukemic cell line, remaining associated with the nuclear matrix after brief DNaseI digestion cannot be displaced by incubation with up to a 50-fold excess of β480 DNA or a several hundred-fold excess of sheared genomic DNA (data not shown).

Discussion

The chief finding reported here is that a specific MAR, contained in the region of the β-globin gene enhancer, can become very tightly, perhaps covalently, bound to protein. In addition, we have presented evidence to suggest that the protein to which the β-globin enhancer is bound is not topoisomerase II (see below) and that the method of SDS/K^+ precipitation may be used to distinguish nonspecific from specific matrix association in the in vitro assay for MARs.

Previous studies have shown that DNA is covalently bound to protein in eukaryotic nuclei (Neuer et al., 1983; Neuer & Werner, 1985; Avramova & Tsanev, 1987) and that this covalent DNA–protein complex is present in nuclear matrix preparations (Razin et al., 1981; Bodnar et al., 1983; Avramova & Tsanev, 1987; Cress & Kurath, 1988). Recently, by use of a filter-binding assay it has been reported that covalent DNA–polypeptide complexes are distributed throughout the chicken genome in a nonrandom fashion and are essentially identically located in oviduct and erythrocytes (Werner & Neuer-Nitsche, 1989). However, protein–DNA complexes were not found in the region of the ovalbumin gene previously

shown to be matrix associated in vivo (Ciejek et al., 1983; Werner & Neuer-Nitsche, 1989). In contrast, we have found evidence for the formation of a protein–DNA complex in a tissue-specific MAR, the erythroid-specific β-globin gene enhancer (Bennett et al., 1989; Bennett et al., submitted for publication). As this complex is resistant to dissociation by SDS and by phenol and disulfide reduction, the bond between DNA and protein is likely to be covalent. Taken together, our results using SDS/K^+ precipitation and the filter-binding studies cited above suggest that at least two types of MARs may exist. One type is tissue specific and, perhaps, functionally related to transcription. The other type is not tissue specific and is, apparently, unrelated to transcriptional activity. This conclusion is further supported by the previous report that MARs in transcriptionally active genes, but not those in inactive genes, can be eluted from the nuclear matrix by low salt (Razin et al., 1988).

The identity of the nuclear matrix proteins to which cellular DNA is attached is not yet established. Several studies have reported nuclear proteins in the molecular size range of 50 to 70 kDa to be covalently attached to DNA (Razin et al., 1981, 1983; Werner & Rest, 1987; Cress & Kurath, 1988). We have recently shown that DNaseI, which is commonly used in the preparation of nuclear matrices may become very tightly bound to oligonucleotide fragments and be confused for cellular protein–DNA complexes in the 40–100-kDa molecular size range (Brotherton & Zenk, 1990). Topoisomerase II has also been suggested as a candidate protein to mediate matrix–DNA interactions (Cockerill & Garrard, 1986; Razin et al., 1988). However, the results of the studies presented in this paper would suggest that the protein that is very tightly bound to β480 DNA and that mediates SDS/K^+ precipitation is not topoisomerase II. First, no double- or single-strand cuts were introduced into DNA by the binding protein. Topoisomerase II introduces staggered double-strand or single-strand cuts when covalently bound to the 5′-phosphoryl ends of each broken DNA strand (Liu et al., 1983; Muller et al., 1988). Therefore, short fragments of 5′-end-labeled DNA should have been released after the addition of SDS if topoisomerase II was the protein bound to β480 DNA, and these should have been detectable by acrylamide gel electrophoresis. Second, divalent cations are not needed for the association of β480 DNA with nuclear matrices and the formation of the SDS/K^+-precipitate complex. Topoisomerase II requires divalent cations to initiate strand cleavage and, thus, covalent attachment (Wang, 1985). Third, we (Brotherton & Zenk, 1990) and others (Avramova & Tsanev, 1987) have previously reported that radioiodination of purified protein–DNA complexes, prior to DNase I digestion, labels a large molecular size protein that fails to enter standard SDS–acrylamide gels. Topoisomerase II has a molecular size of 170–180 kDa (Wang, 1985). Moreover, the peptide map of the large radioiodinated protein differs from that of purified topoisomerase II (Avramova & Tsanev, 1987). Further work will be needed to positively identify the nuclear matrix protein(s) responsible for binding and precipitating β-globin enhancer DNA in the presence of SDS/K^+.

The results contained in this paper present an apparent paradox: although there are many binding sites available for the β480 DNA sequences in brain nuclear matrix preparations, endogenous brain DNA that contains the β-globin enhancer DNA is not matrix associated in brain nuclei in vivo. We have been able to show by SDS/K^+ precipitation that brain nuclei chromatin containing the β-globin enhancer is not protein bound in vivo (Zenk et al., unpublished results). Therefore, it would seem that the β-globin enhancer DNA does not become protein bound in brain nuclei in vivo, because of other factors, absent in the in vitro assay, that block the β-globin enhancer binding to nuclear matrix protein(s) in intact nuclei. Factors known to be missing in the in vitro assay include CpG methylation, salt-soluble nuclear proteins such as histones that bind this DNA region in brain cells (Zenk et al., unpublished results), and the *trans*-acting regulatory proteins that bind this region in erythroid cells (Emerson et al., 1987). Additional studies will be needed to identify the factors that regulate tissue-specific matrix attachment of the β-globin enhancer DNA region and the functional role of matrix attachment in erythroid cells.

Acknowledgments

We thank Dr. J. Bennett for his helpful discussions and reading of the manuscript, J. Reneker for laboratory assistance, and S. Brown for her help in putting the manuscript together.

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Hydrolysis of 3′-Terminal Mispairs in Vitro by the 3′ → 5′ Exonuclease of DNA Polymerase δ Permits Subsequent Extension by DNA Polymerase α†

Fred W. Perrino‡ and Lawrence A. Loeb*

The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology, University of Washington, Seattle, Washington 98195

Received March 2, 1990; Revised Manuscript Received March 30, 1990

ABSTRACT: Purified DNA polymerase α, the major replicating enzyme found in mammalian cells, lacks an associated 3′ → 5′ proofreading exonuclease that, in bacteria, contributes significantly to the accuracy of DNA replication. Calf thymus DNA polymerase α cannot remove mispaired 3′-termini, nor can it extend them efficiently. We designed a biochemical assay to search in cell extracts for a putative proofreading exonuclease that might function in concert with DNA polymerase α in vivo but dissociates from it during purification. Using this assay, we purified a 3′ → 5′ exonuclease from calf thymus that preferentially hydrolyzes mispaired 3′-termini, permitting subsequent extension of the correctly paired 3′-terminus by DNA polymerase α. This exonuclease copurifies with a DNA polymerase activity that is biochemically distinct from DNA polymerase α and exhibits characteristics described for a second replicative DNA polymerase, DNA polymerase δ. In related studies, we showed that the 3′ → 5′ exonuclease of authentic DNA polymerase δ, like the purified exonuclease, removes terminal mispairs, allowing extension by DNA polymerase α. These data suggest that a single proofreading exonuclease could be shared by DNA polymerases α and δ, functioning at the site of DNA replication in mammalian cells.

DNA polymerase α is thought to be one of the principal enzymes that replicates the mammalian genome (Fry & Loeb, 1986; Lehman & Kaguni, 1989). Purified DNA polymerase α is most frequently, but not invariably (Chen et al., 1979; Skarnes et al., 1986; Ottiger et al., 1987; Cotterill et al., 1987; Bialek et al., 1989), devoid of exonucleolytic proofreading activity (Chang et al., 1984; Wahl et al., 1984; Wang et al., 1984; Reyland & Loeb, 1987; Nasheuer & Grosse, 1987). In procaryotes and bacteriophage, a 3′ → 5′ exonuclease activity is a major contributor to the fidelity of DNA replication. The 3′ → 5′ exonuclease either is an integral part of the DNA polymerase polypeptide or is a separate subunit of the DNA replication complex (Brutlag & Kornberg, 1972; Scheuermann & Echols, 1984). It has been estimated that exonucleolytic proofreading enhances the fidelity of DNA synthesis in vitro 10-1000-fold (Kunkel, 1988). Considering the evolutionary conservation of enzymes involved in DNA replication (Wang et al., 1989), it seems possible that an exonuclease is associated with DNA polymerase α in mammalian cells but is separated during purification. However, the identity of such an exonuclease has not been clearly established.

We designed a biochemical assay to detect a putative proofreading exonuclease that functions in concert with DNA polymerase α in vitro and searched for this activity in calf thymus tissue. Our results show that a 3′ → 5′ exonuclease activity that preferentially removes terminally mispaired nu-

† This work was supported by National Institutes of Health Grants T32-CA-09437 (F.W.P.) and R35-CA-39903 (L.A.L.).
* Address correspondence to this author.
‡ Present address: Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

0006-2960/90/0429-5226$02.50/0