[CANCER RESEARCH 52, 3807–3810, July 1, 1992]

*Advances in Brief*

# Partial Characterization of Nuclear Matrix Attachment Regions from Human Fibroblast DNA Using Alu-Polymerase Chain Reaction[1]

**Gregory J. Tsongalis, William B. Coleman, Gary J. Smith, and David G. Kaufman[2]**

*Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27599-7525*

## Abstract

**The proteinaceous nuclear matrix of mammalian cell nuclei has been suggested to be involved in the regulation of chromatin structure, DNA replication, and gene expression. Interaction between cellular DNA and the nuclear matrix is mediated by putative DNA binding sequences, matrix attachment regions (MARs), which may become altered during early events in cellular transformation. Among the cellular changes occurring during the development of neoplasia, all of which may potentially involve the nuclear matrix, are alterations in nuclear structure, loss of control of DNA replication, and significant modifications of cellular gene expression. Therefore, a better understanding of the interaction between DNA and the nuclear matrix is needed. Isolated matrix associated DNA from pulse labeled SV40 transformed human fibroblasts was shown to be enriched in newly replicated DNA, confirming the association of DNA replication with the nuclear matrix as observed by others. Subgenomic fractions of matrix associated DNA enriched in putative MARs sites were prepared from quiescent and logarithmically growing normal human fibroblasts and SV40 transformed human fibroblasts. These fractions of DNA were analyzed by Alu-polymerase chain reaction and agarose gel electrophoresis, revealing complex and unique patterns of DNA products for each cell type investigated. A number of prominent DNA fragments with similar molecular size were found to be present in the amplified DNA products of each DNA source, suggesting that these DNA fragments may represent common DNA sequences which contain MARs sites or which are associated with MARs sites. The application of Alu-polymerase chain reaction to the molecular analysis of nuclear matrix associated DNA may facilitate the isolation and characterization of potentially new human MARs sequences.**

## Introduction

Eukaryotic chromatin is organized into domains or loops which are generated by the attachment of chromatin to the proteinaceous nuclear matrix network (1–4). The constitutive proteins of the nuclear matrix have been partially characterized; however, little is known about the DNA sequences associated with the matrix (1–5). Nuclear MARs[3] in genomic DNA are thought to be involved in the regulation of chromatin organization, DNA replication, and RNA transcription and processing (2–9). Specific MARs sequences have been identified in tandemly repeated histone gene cluster, *Drosophila* heat shock genes, mouse immunoglobulin genes, the HL-60 cell c-*myc* protooncogene, and the Chinese hamster ovary cell *DHFR* gene (7, 8, 10, 11). MARs are A-T rich sequences that are several hundred base pairs long and have significant homology to the topoisomerase II consensus sequence (8). DNA replication and chromatin organization are modulated structurally by the nuclear matrix and are altered in the process of transformation, suggesting that functional alteration of MARs sites following transformation may represent either a cellular manifestation of transformation or a mechanistic involvement of MARs in the process of malignant transformation of normal cells.

In this report, we examine specific highly represented MARs sequences of normal and transformed human fibroblasts, to determine their association with DNA replication and their alteration in the transformation process. During MARs isolation, the bulk of the genomic DNA is removed by restriction enzyme digestion resulting in a DNA subfraction of matrix associated DNA enriched in MARs. In an effort to isolate families of consensus attachment regions from this DNA subfraction, we utilized the Alu-polymerase chain reaction primed with an Alu repetitive DNA sequence because repetitive sequences have been shown to be associated with the nuclear matrix (12–14). We hypothesized that at least some of the MARs sequences should have Alu repetitive sites with the proper orientation and sufficiently close together to permit amplification by the PCR technique. Alu sequences are the most abundant repetitive sequence in the human genome, may account for 3–6% of the total genomic DNA, and have been shown to have possible roles as origins of replication in an *in vitro* system (15). These sequences are characteristically 300 base pairs long, consist of two directly repeating units, and are located preferentially in A-T rich regions with a nonrandom distribution with respect to MARs (12, 16–20). We report here that Alu-PCR represents a valuable method for the molecular analysis of nuclear matrix associated DNA enriched in putative MARs sites in normal and transformed human cells.

## Materials and Methods

**Cell Lines.** Normal human fibroblasts were cloned from human foreskin and used for these studies between passages 2 and 10. The cells were grown in Ham's F-10 medium supplemented with 10% fetal bovine serum and 5% bovine serum (GIBCO, Grand Island, NY). SV40 transformed human fibroblasts (21) were grown in basal medium (Eagle's) supplemented with 10% fetal bovine serum. All cells were maintained in a humidified atmosphere of 5% $CO_2$–95% air at 37°C.

**Isolation of Matrix Associated DNA Containing MARs.** Normal human fibroblasts were plated at $1 \times 10^6$ cells/100-mm tissue culture dish and kept at confluence for 5 days for quiescent cultures or harvested after 2 days in culture when in log phase. SV40 transformed human fibroblasts were maintained in continuous growth. All cells were uniformly labeled with 0.02 $\mu$Ci/ml [$^{14}$C]thymidine for 3 days in order to trace label the genomic DNA. DNA containing nuclear matrix attachment regions was isolated according to the method of Dijkwel and Hamlin (10). Briefly, human fibroblasts were harvested by trypsinization, resuspended in a hypotonic cell wash buffer (50 mM KCl, 0.5 mM EDTA-0.05 mM-spermine-0.125 mM spermidine-0.5% thiodiglycol-0.25 mM phenylmethylsulfonyl fluoride-5 mM Tris-HCl, pH 7.4) containing 0.01% digitonin, and lysed by passage through a 22-gauge hypodermic needle. Nuclei were isolated by centrifugation and stabilized using 0.5 mM $CuSO_4$ in cell wash buffer (10). The nuclei were extracted in 10 mM lithium 3,5-diiodosalicylate-100 mM lithium ace-

Received 4/29/92; accepted 5/21/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by NIH Grant CA42765. G. J. T. and W. B. C. received support under NIH Training Grant ES07017.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: MARs, matrix attachment regions; Alu-PCR, Alu-polymerase chain reaction.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/13/3807/2447369/cr0520133807.pdf by guest on 20 September 2024

tate-0.1% digitonin-0.05 mM spermine-0.125 mM spermidine-0.25 mM phenylmethylsulfonyl fluoride-20 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid)/KOH buffer (pH 7.4), and the extracted nuclei were harvested by centrifugation (10). Nuclear matrices were prepared by digestion with restriction enzymes (*Eco*RI, *Hind*III, *Hinf*I) (40 units/ml each enzyme) in buffers recommended by the supplier (Boehringer Mannheim) for 90 min, fresh enzyme (50 units/ml of each of the above mentioned enzymes) was added, and the nuclei were further digested overnight. DNA detached from the matrices by the restriction enzymes (loop DNA) was collected in the supernatant and ethanol precipitated. DNA containing MARs was isolated from the matrices by digestion of the matrix proteins with proteinase K followed by phenol:chloroform extraction and ethanol precipitation.

**Pulse Labeling.** Replicating DNA was pulse labeled with [$^3$H]thymidine in the human fibroblasts which were prelabeled with [$^{14}$C]thymidine. Cells were washed with phosphate buffered saline and fresh medium containing 50 μCi/ml [$^3$H]thymidine was added for the allotted pulse time. This medium was then removed and cells were washed as above before harvesting. Pulse-chase experiments were performed as described above except that the medium containing [$^3$H]thymidine was replaced with medium containing 1 mM unlabeled thymidine, and cells were incubated for 2 h longer.

**Alu-PCR Amplification.** Alu-PCR was carried out according to the protocol of Brooks-Wilson *et al.* (17). Briefly, genomic DNA fragments which contained MARs sequences were isolated following restriction digestion. This DNA (100 ng) was incubated in a total reaction volume of 100 μl containing Alu primer (0.5 μM), 0.25 μM concentrations of each deoxynucleotide triphosphate, 100 mM Tris-HCl (pH 8.3), 500 mM KCl, 1 mM $MgCl_2$, and 2.5 units *Thermus aquaticus* polymerase (Perkin-Elmer/Cetus) (17). The MARs containing DNA was denatured at 93°C for 8 min prior to PCR. PCR amplification was accomplished using 35 cycles consisting of a 2-min denaturation step (93°C), a 2-min primer annealing step (58°C), and a 3-min extension step (72°C). The final cycle included a 10-min extension step. PCR products were extracted with chloroform:isoamyl alcohol (24:1) and precipitated in 2 volumes of isopropyl alcohol at −20°C for at least 4 h. The resulting DNA pellets were dissolved in 10 mM Tris/1 mM EDTA (pH 7.6) buffer and analyzed electrophoretically on 2% agarose gels containing 40 mM Tris-acetate/1 mM EDTA (pH 7.6).

## Results

For isolation of MARs, we utilized normal human foreskin fibroblasts and a SV40 transformed human fibroblast cell line. Simultaneous digestion with the three restriction enzymes (*Eco*RI, *Hind*III, *Hinf*I) released most of the fraction of genomic DNA not associated with the nuclear matrix (loop DNA, approximately 95% of total genomic DNA) from the fragments of DNA which contained MARs sites associated with the nuclear matrix (approximately 5% of the total genomic DNA). The fraction of total DNA associated with the nuclear matrix could be varied (5–20%) by using different restriction enzymes (Table 1).

Table 1 *Recovery of nuclear matrix associated DNA after digestion with various numbers of restriction enzymes*

| Restriction enzyme[a] | MARs[b] | *n* |
|---|---|---|
| *Eco*RI | 21.6 | 1 |
| *Eco*RI<br>*Hind*III | 10.9 ± 3.0 | 4 |
| *Eco*RI<br>*Hind*III<br>*Hinf*I | 4.0 ± 0.5 | 3 |

[a] All restriction enzymes were purchased from Boehringer Mannheim.
[b] MARs sites expressed as percentage of total DNA isolated (±SE).

To ascertain whether the isolation protocol did produce a DNA fraction enriched in matrix associated DNA sequences, the MARs fraction was evaluated for newly replicated DNA which is thought to occur in association with the nuclear matrix. Pulse labeling experiments were performed using SV40 transformed human fibroblasts. Cells uniformly prelabeled with [$^{14}$C] thymidine were pulsed labeled with [$^3$H]thymidine, and the nuclei were isolated. Matrix associated DNA was isolated and specific activities of newly replicated DNA relative to total DNA ($^3$H/$^{14}$C) were determined for the DNA fraction enriched in MARs (Fig. 1*A*). We observed a 4-fold increase in the specific activity of the MARs fraction at the earliest pulse times indicating that the matrix associated DNA is enriched in newly replicated DNA. After longer pulse times, the specific activity of the total DNA approached that of the MARs fraction, suggesting that as DNA is being synthesized, previously labeled DNA loses its association with the nuclear matrix by passing through an association site. As predicted by this hypothesis, if the pulse is followed by a chase of cold thymidine in the absence of label, the [$^3$H]thymidine labeled DNA disappears from the MARs fraction and the specific activity of this DNA approaches that of the total DNA (Fig. 1*B*). These pulse chase experiments demonstrate the association of the isolated DNA fraction with the nuclear matrix, and confirm that there is a transient association of DNA with the nuclear matrix when it is replicating (9, 22–31).

To determine if there are specific consensus DNA sequences which are highly represented within the nuclear matrix associated DNA fraction, we used the polymerase chain reaction with primers directed against the Alu repetitive sequences (Alu-PCR) (16, 17) (Fig. 2). Alu-PCR was performed using nuclear matrix associated DNA prepared from normal human fibroblasts (logarithmically growing or quiescent) and SV40 transformed human fibroblasts as template DNA sources (Fig. 2). This Alu-PCR technique utilizes a single primer directed against the 3′-end of the human Alu consensus sequence to amplify segments of DNA that are present between two closely associated (0.5–3 kilobases apart) Alu sequences that are in opposite orientations with respect to each other (Fig. 2) (16, 17). Thus, only a small subset of the nuclear matrix associated DNA template will be amplified by this technique using the specified primer. Agarose gel electrophoresis of the Alu-PCR amplified DNA products revealed a number of distinct DNA fragments ranging in size from approximately 400 to 3000 base pairs as shown in Fig. 3. Several common prominent DNA fragments appeared to be represented in the amplified DNA from each of the three sources of DNA template based on molecular size. In addition, apparently unique amplification fragments were present in the Alu-PCR products from each of the three nuclear matrix associated DNA template sources.

## Discussion

Association of cellular genomic DNA with the proteinaceous nuclear matrix (3) is important in the maintenance of nuclear chromatin structure (2) and may play significant roles in the cellular processes of DNA replication (6, 9, 10, 22–31) and gene expression (2, 4, 7, 8, 11). Although numerous hypotheses exist as to the role of the nuclear matrix in these cellular processes, very few DNA binding sequences have been identified as nuclear matrix attachment regions. Endonucleolytic digestion of nuclei for isolation of matrix associated DNA results in the isolation of short segments of DNA containing nuclear matrix protein binding sequences, or MARs, with flanking regions of DNA which may constitute up to several kilobases. This nuclear matrix associated DNA fraction is thought to



Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/13/3807/2447369/cr0520133807.pdf by guest on 20 September 2024

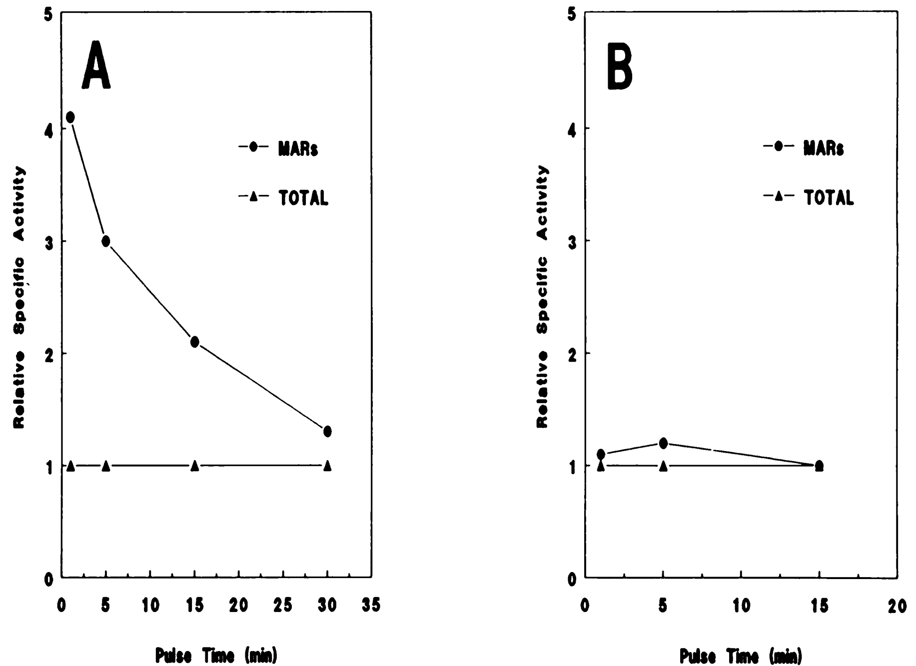


Fig. 1. Association of newly replicated DNA with the nuclear matrix in SV40 transformed human fibroblasts. Cells were uniformly labeled with [$^{14}$C]thymidine and then either pulse labeled with [$^{3}$H]thymidine for the given time intervals (*A*) or pulse labeled and chased with 1 mM thymidine for 2 h (*B*). Nuclear matrix associated DNA was isolated by extraction with a hypotonic lithium salt buffer and restriction enzyme digestion (*Eco*RI, *Hind*III, *Hin*fI). The specific activities of the MARs and the loop DNA fractions were calculated, and results are expressed as specific activity relative to total DNA isolated.

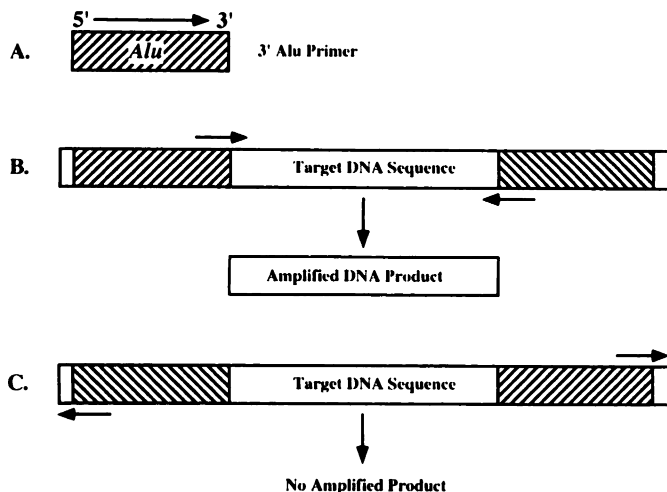


Fig. 2. Scheme for Alu-PCR amplification of target DNA sequences within nuclear matrix associated DNA fragments. (*A*) The consensus Alu repetitive DNA sequence is approximately 300 base pairs long consisting of two directly repeating monomer units. The orientation of the Alu repetitive DNA is given. The primer is directed against the sequence of nucleotides 268–285 of the 3′ end of the Alu repetitive DNA sequence (18). (*B*) Since Alu-PCR amplification reactions utilize a single primer specific for the 3′ end of the Alu repetitive sequence, amplification of a putative target DNA fragment is accomplished only when this target is flanked by two Alu sequences in the correct orientation as shown. PCR primers are indicated by *arrowheads* to distinguish 5′ to 3′ orientation relative to the Alu sequence. (*C*) When Alu sequences are incorrectly oriented with respect to each other, no amplification product can be made using the primer directed against the 3′ portion of the Alu sequence.

consist of sequence specific sites for the attachment to nuclear matrix proteins which serve both functional and structural roles.

In this report, we describe the molecular characterization of DNA fragments within the subset of matrix associated DNA which are putative consensus MARs. Nuclear matrix associated DNA was prepared by endonucleolytic digestion of nuclei isolated from normal human fibroblasts (logarithmically growing or quiescent) and SV40 transformed human fibroblasts (10). Despite the enrichment of MARs DNA afforded by this method (10), further characterization of this DNA fraction has been extremely difficult using standard approaches and techniques. Thus, a novel approach was needed to further enrich this DNA fraction for putative MARs sites or DNA sequences which are physically associated with MARs sites. It has been reported previously that MARs DNA sequences are sometimes in close association with repetitive DNA sequences (12–14, 32). Therefore, we utilized Alu repetitive DNA sequences as boundaries for amplification of DNA sequences that may contain MARs sites or be associated with MARs sites (as shown in Fig. 2). Using primers directed against the 3′-end of the Alu consensus sequence (18), we were able to amplify DNA sequences positioned between two Alu sequences in opposite orientations, while amplifying only a 15-base pair portion of the Alu sequence itself. Utilizing this technique (16, 17), we observed that Alu-

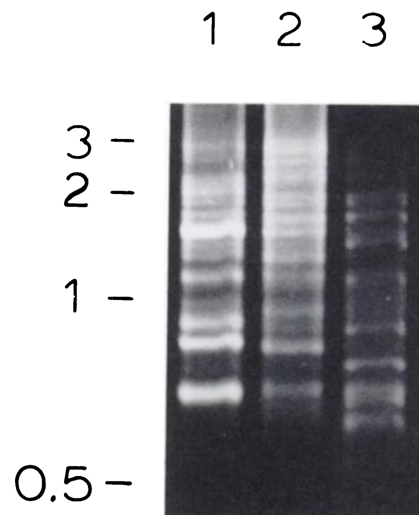


Fig. 3. Gel electrophoresis of Alu-PCR amplified products from logarithmically growing (*Lane 1*), SV40 transformed (*Lane 2*), and quiescent (*Lane 3*) human fibroblasts. Cells were plated 1 × 10$^{6}$ cells/100-mm dish and kept at confluence for 5 days as quiescent cultures or harvested after 2 days for logarithmically growing or transformed human fibroblast cultures. All cells were uniformly labeled with [$^{14}$C]thymidine. Nuclear matrix associated DNA was isolated using a lithium salt buffer and restriction enzyme digestion. Alu-PCR was performed using specific primers to the 3′ end of the Alu consensus sequence. Amplified DNA samples were electrophoresed on 2% agarose gels.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/13/3807/2447369/cr0520133807.pdf by guest on 20 September 2024

PCR produced unique and reproducible patterns of amplified DNA fragments using nuclear matrix associated DNA isolated from quiescent, logarithmically growing, and transformed human fibroblasts as the template for amplification (Fig. 3). These complex patterns of DNA fragments produced by Alu-PCR demonstrate both qualitative similarities and differences between samples produced using as templates the nuclear matrix associated DNA prepared from these three sources (Fig. 3). We hypothesize that the amplified DNA bands present in samples from all sources represent likely candidates to contain or be linked to MARs sequences. It is highly likely that some proportion of MARs sequences should be functional in all of the different cell types. Amplified DNA fragments that appear to be unique between these nuclear matrix DNA sources may reflect the use of different MARs sites in the maintenance of nuclear structure and/or DNA replication in the cells, reflecting different physiological conditions (normal *versus* transformed cell types, quiescent *versus* logarithmically growing cells). Additionally, it has been suggested that gene expression requires association of the genomic DNA containing the gene with the nuclear matrix (2, 7, 8, 11). Therefore, differences in gene expression between these different cell types may also contribute to the differences observed in the Alu-PCR patterns produced.

## Acknowledgments

We wish to thank Dr. B. P. Brylawski, C. J. Civalier, L. D. Coumeau, D. G. Gioeli, and G. L. Murray for their technical assistance and valuable comments.

## References


1. Cupo, J. F. Electrophoretic analysis of nuclear matrix proteins and the potential clinical applications. J. Chromatog., *569:* 389–406, 1991.
2. Nelson, W. G., Pienta, K. J., Barrack, E. R., and Coffey, D. S. The role of the nuclear matrix in the organization and function of DNA. Annu. Rev. Biophys. Chem., *15:* 457–475, 1986.
3. Berezney, R., and Coffey, D. S. Identification of a nuclear protein matrix. Biochem. Biophys. Res. Commun., *60:* 1410–1419, 1974.
4. Fey, E. G., and Penman, S. Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc. Natl. Acad. Sci. USA, *85:* 121–125, 1988.
5. Stuurman, N., Meijne, A. M. L., van der Pol, A. J., de Jong, L., van Driel, R., and van Renswoude, J. The nuclear matrix from cells of different origin. J. Biol. Chem., *265:* 5460–5465, 1990.
6. Tubo, R. A., Martelli, A. M., and Berezney, R. Enhanced processivity of nuclear matrix bound DNA polymerase $\alpha$ from regenerating rat liver. Biochemistry, *26:* 5710–5718, 1987.
7. Mirkovitch, J., Mirault, M., and Laemmli, U. K. Organization of higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. Cell, *39:* 223–232, 1984.
8. Cockerill, P. N., and Garrard, W. T. Chromosomal loop anchorage of the $\kappa$ immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell, *44:* 273–282, 1986.
9. Vaughn, J. P., Dijkwel, P. A., Mullenders, L. H. F., and Hamlin, J. L. Replication forks are associated with the nuclear matrix. Nucleic Acids Res., *18:* 1965–1969, 1990.
10. Dijkwel, P. A., and Hamlin, J. L. Matrix attachment regions are positioned near replication initiation sites, genes, and an interamplicon junction in the amplified dihydrofolate reductase domain of Chinese hamster ovary cells. Mol. Cell. Biol., *8:* 5398–5409, 1988.
11. Chou, R. H., Churchill, J. R., Flubacher, M. M., Mapstone, D. E., and Jones, J. Identification of a nuclear matrix-associated region of the c-*myc* protooncogene and its recognition by a nuclear protein in the human leukemia HL-60 cell line. Cancer Res., *50:* 3199–3206, 1990.
12. Small, D., Nelkin, B., and Vogelstein, B. Nonrandom distribution of repeated DNA sequences with respect to supercoiled loops and the nuclear matrix. Proc. Natl. Acad. Sci. USA, *79:* 5911–5915, 1982.
13. Razin, S. V., Mantieva, V. L., and Georgiev, G. P. The similarity of DNA sequences remaining bound to scaffold upon nuclease treatment of interphase nuclei and metaphase chromosomes. Nucleic Acids Res., *7:* 1713–1735, 1979.
14. Matsumoto, L. H. Enrichment of satellite DNA on the nuclear matrix of bovine cells. Nature (Lond.), *294:* 481–482, 1981.
15. Ariga, H. Replication of cloned DNA containing the Alu family sequence during cell extract promoting simian virus 40 DNA synthesis. Mol. Cell. Biol., *4:* 1476–1482, 1984
16. Nelson, D. L., Ledbetter, S. A., Corbo, L., Victoria, M. F., Solis, R., Webster, T. D., Ledbetter, D. H., and Caskey, C. T. Alu polymerase chain reaction: a method for rapid isolation of human specific sequences from complex DNA sources. Proc. Natl. Acad. Sci. USA, *86:* 6686–6690, 1989.
17. Brooks-Wilson, A. R., Goodfellow, P. N., Povey, S., Nevanlinna, H. A., de Jong, P. J., and Goodfellow, P. J. Rapid cloning and characterization of new chromosome 10 DNA markers by Alu element mediated PCR. Genomics, *7:* 614–620, 1990.
18. Kariya, Y., Kato, K., Hayashizaki, Y., Himeno, S., Tarui, S., and Matsubara, K. Revision of consensus sequence of human Alu repeats: a review. Gene, *53:* 1–10, 1987.
19. Jelinek, W. R. Repetitive sequences in eukaryotic DNA and their expression. Annu. Rev. Biochem., *51:* 813–844, 1982.
20. Daniels, G. R., and Deininger, P. L. Integration site preferences of the Alu family and similar repetitive DNA sequences. Nucleic Acids Res., *13:* 8939–8953, 1985.
21. Todaro, G. J., Green, H., and Swift, M. R.. Susceptibility of human diploid fibroblast strains to transformation by SV40 virus. Science (Washington DC), *153:* 1252–1254, 1966.
22. Berezney, T., and Coffey, D. S. Nuclear protein matrix: association with newly synthesized DNA. Science (Washington DC), *189:* 291–292, 1975.
23. Dijkwel, P. A., Wenink, P. W., and Poddighe, J. Permanent attachment of replication origins to the nuclear matrix in BHK cells. Nucleic Acids Res., *14:* 3241–3247, 1986.
24. Van der Velden, H. M. W., van Willigen, G., Wetzels, R. H. W., and Wanaka, F. Attachment of origins of replication to the nuclear matrix and chromosomal scaffold. Fed. Proc., *171:* 13–16, 1984.
25. Pardoll, D. M., Vogelstein, B., and Coffey, D. S. A fixed site of DNA replication in eukaryotic cells. Cell, *19:* 527–536, 1980.
26. Aelen, J. M. A., Opstelten, R. J. G., and Wanaka, F. Organization of DNA replication in *Physarum polycephalum*. Attachment of origins of replicons and replication forks to the nuclear matrix. Nucleic Acids Res., *11:* 1181–1195, 1983.
27. Kalandadze, A. G., Bushara, S. A., Vassetzky, Y. S., and Razin, S. V. Characterization of DNA pattern in the site of permanent attachment to the nuclear matrix located in the vicinity of replication origin. Biochem. Biophys. Res. Commun., *168:* 9–15, 1990.
28. Vogelstein, B., Pardoll, D. M., and Coffey, D. S. Supercoiled loops and eukaryotic DNA replication. Cell, *22:* 79–85, 1980.
29. Razin, S. V., Kekelidze, M. G., Lukanidin, E. M., Schee, K., and Georgiev, G. P. Replication origins are attached to the nuclear skeleton. Nucleic Acids Res., *14:* 8189–8207, 1986.
30. Hamlin, J. L., Vaughn, J. P., Dijkwel, P. A., Leu, T., and Ma, C. Origins of replication: timing and chromosomal position. Curr. Opinion Cell Biol., *3:* 414–421, 1991.
31. Dijkwel, P. A., Vaughn, J. P., and Hamlin, J. L. Mapping of replication initiation sites in mammalian genomes by two-dimensional gel analysis: stabilization and enrichment of replication intermediates by isolation on the nuclear matrix. Mol. Cell. Biol., *11:* 3850–3859, 1991.
32. Opstelten, R. J. G., Clement, J. M. E., and Wanka, F. Direct repeats at nuclear matrix-associated DNA regions and their putative control function in the replicating eukaryotic genome. Chromosome (Berl.), *98:* 422–427, 1989.


Downloaded from http://aacrjournals.org/cancerres/article-pdf/52/13/3807/2447369/cr0520133807.pdf by guest on 20 September 2024