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Specific DNA sequences associated with the nuclear matrix in synchronized mouse 3T3 cells

(repetitive DNA/DNA organization/chromatin)

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ABSTRACT **Eukaryotic chromatin appears to be organized into arrays of supercoiled loops anchored to the scaffolding structure of the mitotic chromosome core or to the nuclear matrix of interphase nuclei. To reveal whether specific DNA sequences are involved in this level of chromatin organization, we isolated and cloned a population of DNA molecules [average length of 150 base pairs (bp)] closely associated with the nuclear matrix after exhaustive DNase digestion and subsequent extensive protease digestion. The nuclear matrix was obtained from murine BALB/c 3T3 cells synchronized at the G_1/S border of the cell cycle. We report the structure of two sequences, designated G4 and G5, which are highly enriched in the matrix DNA. Sequence G4, of 152 bp, contains three 31-bp direct head-to-tail repeats. An 11-bp sequence at the end of each repeat is homologous to the first large tumor antigen recognition site of human papova virus. Sequence G5, of 135 bp, consists of two well-defined domains, in which the first domain is a fragment of the B1 repetitive sequence. The results suggest the possibility that the loops of histone-depleted chromatin are connected to the scaffold of the nuclear matrix, with specific DNA sequences at the anchorage sites.**

The condensation of eukaryotic chromatin is achieved through an extensive hierarchical folding of the DNA, mediated by histones and nonhistone nuclear proteins (1). The first order of folding is defined by nucleosomes containing histone octamers with DNA coiled around them (2–5). At the next level of chromatin folding, nucleosomes are arranged into solenoids or superbeads engaging 6–10 nucleosomes (6–9). The linear condensation ratio of the DNA in this fiber is about 40. It is therefore clear that higher levels of chromatin packaging are necessary to achieve the condensation of the genome into the compact structures of chromosomes or even interphase chromatin. The most recent evidence suggests that at the highest level both interphase chromatin and metaphase chromosomes are organized into arrays of supercoiled loops, each loop engaging 50–100 kilobases of DNA (10–20). These loops are formed by attachment of the DNA to scaffolding structures composed of the chromosome core of mitotic chromosomes or the nuclear matrix (21–27) of the interphase nuclei. It is important to understand how this higher order of chromatin packaging is related to its functional state.

It has been suggested (28–30) that the attachment sites of the supercoiled loops to the nuclear matrix are the sites at or near which DNA replication takes place. It is important to note, in this regard, that DNA polymerase has been shown to be associated with the matrix (31). These and other studies (21, 32–34) have focused on the protein composition and structure of the matrix, while little is known about the primary structure of the DNA associated with it. In this communication we present evidence that DNA closely associated with the nuclear matrix from 3T3 cells synchronized at the G_1/S border is highly enriched in specific DNA sequences. This result suggests the possibility that, at least during a fraction of the cell cycle, DNA loops may be anchored to the nuclear matrix through interaction with a specific DNA sequence (or limited groups of sequences). We suggest that these DNA sequences are situated close to or compose part of the replication origins of the chromosome.

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MATERIALS AND METHODS

Synchronization of Mouse 3T3 Cells and Preparation of Matrix-Associated DNA. Murine BALB/c 3T3 cells were grown in 1,500-cm^2 roller bottles in Eagle's minimal essential (ME) medium supplemented with 10% newborn calf serum. When cells reached 1/8–1/4 confluency, 2 mM (final concentration) thymidine was added for 15 hr. After the 15-hr incubation, thymidine was removed and the cells were incubated for an additional 8 hr in fresh medium. At this time a large proportion of the cells were in mitosis. Mitotic cells were collected by rotating the roller bottles at ≈200 rpm for 5 min. The cells harvested in this fashion were plated in 30-mm dishes in fresh medium, and the rate of DNA synthesis was measured by [^{3}H]-thymidine incorporation (as shown in Fig. 1).

To prepare for the extraction of matrix DNA, mitotic cells were incubated in fresh medium in the presence of 2 mM thymidine for 8–9 hr (i.e., the average duration of G_1 phase). Cells were then chilled on ice and removed with a rubber policeman.

Nuclear matrix DNA was prepared essentially as described by Pardoll *et al.* (28) with minor modifications. The 2 M NaCl extract of nuclei was fractionated by centrifugation at 12,000 × *g* for 40 min. The resulting pellet and the supernatant are referred to as the matrix and Sup 1, respectively. The matrix pellet at this step was digested with DNase I (deoxyribonuclease I, EC 3.1.21.1; Sigma) at 400 units/ml for 2 hr at room temperature and the supernatant (Sup 1) DNA was digested for a shorter time to produce DNA molecules with a size distribution similar to matrix DNA. Nuclease-treated matrix was pelleted again and further purified by successive washes with a buffer (10 mM Tris·HCl/0.2 mM $MgCl_2$/100 mM NaCl/1% Triton X-100). The supernatant (Sup 2) produced at this stage represents unprotected DNA released from the matrix by DNase digestion.

Construction of the DNA Libraries, Isolation of Clones, and *in Situ* Hybridization. Matrix and Sup 1 DNA were repaired *in vitro* with DNA polymerase I (DNA nucleotidyltransferase, deoxynucleoside triphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) before treatment with nuclease S1 (micrococcal endonuclease, EC 3.1.31.1) to produce DNA molecules with

Abbreviations: bp, base pair(s); T antigen, large tumor antigen.

blunt ends. Ligation of the *Bam*HI decamer linker onto the blunt-ended DNA was followed by exhaustive digestion with *Bam*HI endonuclease (endodeoxyribonuclease *Bam*HI, EC 3.1.23.6). The digested linker DNA was removed by electrophoresis in a 15% acrylamide gel. The DNA with attached linkers was electroeluted, concentrated, and ligated with an excess of pBR322 that had been digested with *Bam*HI and calf intestine alkaline phosphatase (EC 3.1.3.1). By this procedure, 100% of the obtained pBR322 transformants carried inserted DNA, as judged by antibiotic-resistance analysis of 150 of the obtained clones. The libraries of matrix and Sup 1 DNA were amplified by growing transformed cells in the presence of ampicillin. About 4,000 initial clones were obtained for each DNA. A computer simulation experiment showed that ≈32,000 colonies of the amplified library would include every original clone at the 95% confidence level.

To facilitate cross-hybridization and isolation of individual clones, both libraries were transferred into the mp8 vector (phage M13). Plasmid DNA isolated from both expanded libraries was digested with *Bam*HI. A total collection of inserts was purified from acrylamide gels and ligated into the *Bam*HI site of the mp8 vector. The ligation reaction was arranged to generate about 10^5 initial cloning events to keep the library representative (see above). Both libraries in M13 phage were plated on X-β-D-galactoside/isopropyl β-D-thiogalactoside indicator plates, and white plaques containing inserts were counted. The clones from these plates were "lifted" onto nitrocellulose filters and hybridized to an appropriate probe. Colony hybridization was performed by transferring colonies from the plate onto a nitrocellulose filter. From this "master filter," replica filters were made by applying fresh filters successively onto the original. Each replica filter was placed on a fresh agar plate and incubated overnight at 37°C. The cell mass was scraped from the filters before baking. Hybridization was performed in 0.9 M sodium chloride/0.09 M sodium citrate at 68°C for 16–20 hr.

The methods of partial chemical modification (35) and dideoxynucleotide chain termination (36) were used to determine the sequence of the clones in pBR322 and M13 phage mp8 vectors, respectively.

RESULTS

Two Types of DNA Sequence Are Highly Enriched in the Nuclear Matrix DNA from Synchronized Mouse Cells. It has been shown that the site of DNA replication is closely associated with the nuclear matrix (28–30). One intriguing possibility is that nuclear matrix DNA obtained from cells involved in the initiation of DNA synthesis might be enriched in sequences related to the origins of DNA replication. We therefore attempted a detailed analysis of the DNA sequences associated with the nuclear matrix obtained from cells synchronized at the G_1/S border of the cell cycle. Fig. 1 shows that synchronous incorporation of [^{3}H]thymidine into DNA can be achieved in mouse 3T3 cells by a combination of mitotic shaking and a thymidine block. The presence of thymidine during G_1 phase increases the degree of synchrony at the G_1/S border, and cells enter the S phase promptly upon removal of the drug. Nuclear matrix was prepared from these cells and residual matrix DNA (0.1% of the total DNA) was isolated after exhaustive protease digestion. Sup 2 DNA released during DNase digestion was subjected to size-distribution analysis along with the matrix-associated DNA. Polyacrylamide gel electrophoresis of both DNA samples shows (Fig. 2) that the size of matrix DNA [≈150 base pairs (bp)] is larger than that of Sup 2 DNA, indicating that at least a fraction of this DNA is protected from DNase digestion. The Sup 1 DNA remaining in solution after removal of the rapidly sedimenting matrix–DNA complex was subjected to limited DNase digestion to obtain a size distribution comparable to that of matrix-associated DNA.

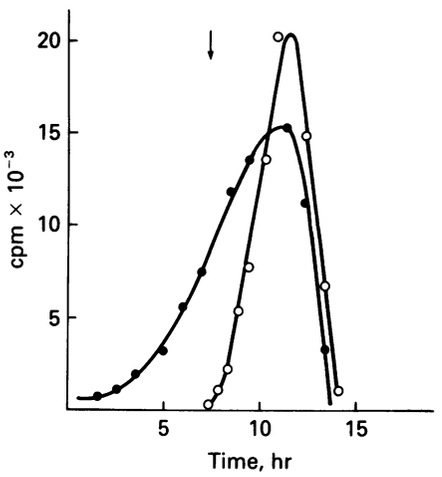

FIG. 1. Synchronization of BALB/c 3T3 mouse cells. 3T3 cells were grown in ME medium supplemented with 10% newborn calf serum and treated with thymidine. At time 0, mitotic cells were collected by shaking and replated on 30-mm dishes. The dishes were then divided into two groups, and the rate of DNA synthesis was measured by 10-min pulse incorporation of [^{3}H]thymidine (group 1; closed circles). Group 2 was incubated in the same medium containing 2 mM thymidine for 8 hr after mitosis. Thymidine was then removed (arrow), cells were washed with fresh medium containing deoxycytidine, and the rate of DNA synthesis was followed (open circles) as in the first group.

To analyze further the population of matrix DNA in comparison with Sup 1 DNA, random libraries in pBR322 were constructed from each DNA sample. Both libraries, each containing ≈4,000 initial clones, were amplified by growing the transformation mixture in broth supplemented with ampicillin. To facilitate cross-hybridization experiments and isolation of individual clones, a copy of each library was constructed using the mp8 vector.

To reveal clones containing DNA sequences highly represented in matrix-associated DNA, 40,000 colonies of the amplified matrix DNA library were hybridized to a nick-translated matrix DNA probe (10^5 cpm/ml). The clones that hybridized to the matrix DNA probe were isolated and their sequences were analyzed. Three types of clones were obtained—namely,

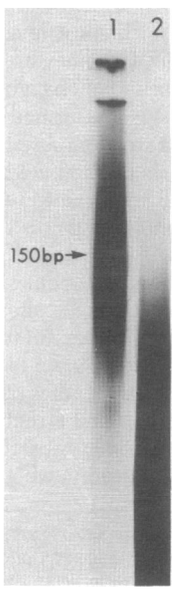

FIG. 2. Size distribution of matrix and Sup 2 DNA. Matrix (lane 1) and Sup 2 DNA (lane 2) were isolated after 2 hr of DNase digestion. Samples of both preparations were nick-translated and electrophoresed on a 15% polyacrylamide gel. The distribution shows that matrix-associated DNA is larger than DNA released after DNase digestion (Sup 2 fraction).

G35 (representing a fragment of the satellite DNA), G4 (Fig. 3A), and G5 (Fig. 3B).

A nick-translated probe was prepared from the DNA of these clones and hybridized to predetermined numbers of clones from matrix and supernatant DNA libraries in the mp8 vector. The results of this experiment (Table 1) show that although the relative content of clones hybridizing to the G35 satellite sequence is equal in both libraries, the matrix DNA library contains 10 and 1.3 times more clones hybridizable to sequences G4 and G5, respectively. The enrichment of sequence G5 in this experiment is underestimated, as will become clear from the data described below.

The Consensus Sequence of the G5-Hybridizing Clones Is Different in Matrix-Associated and Supernatant DNA. To compare G5-hybridizing sequences present in the matrix DNA to those in the Sup 1 DNA, 14 G5-hybridizing clones were isolated from both matrix and supernatant mp8 libraries and their sequences were determined. Thirteen G5-hybridizable clones from the matrix DNA library, although inserted in different orientation, were identical in size (135 bp) and sequence to G5 (Fig. 3B). Sequence G5 contains a 63-bp homology to the ubiquitous repetitive B1 sequence (37) of mouse; the adjacent sequence does not show any homology to B1 or to the B1-flanking regions. One of 14 matrix clones contained only the part homologous to B1.

The sequences of the 14 G5-hybridizable clones from the Sup 1 library differ dramatically from the G5 sequence. These clones fall into two distinct classes (Fig. 4). Members of the first class contain the B1 homologous part of sequence G5 (domain I); members of the second class are homologous to the sequence adjacent to domain I of sequence G5 (domain II) and have no homology to the B1 sequence. None of the supernatant clone sequences has homology to both domains I and II. Comparison with the B1 sequence (37) shows that supernatant clones of the first class are essentially variants of this sequence admitting only small differences. Both classes of the supernatant clones vary in size within the limits of the cloned DNA and contain sequences of randomly variable length on both right- and left-hand flanks of their respective regions of homology to the

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A.                                    TAACAGCATCGCA          13
  TCAAAGTTAATAGTCAAGCCACCGCAGAAAA                            44
  TCACTTACAGTCATCCCCCTGCCTCAGAAAA                            75
                                      ----------
  TCACT  CAGTCATCCCaCTGCCTCAGAAAA                            104
                                      ----------
  TCACTcACAGTCATtCCCCTGCCTCAGAAAA                            135
                                      ----------
  TAAAACACAGCCATCCC                                          152

B.  CTGGCTGTCCTGGAACTCACTTTGTAGACCA                          31
    -ct----------------c--a------
    GGCTGGCCTCGAACTCAGAAATCCGCCTGCC                          62
    ---------------------------------
    TCTGCCCTCCAAACATGGAAAATGATAAAAA                          93
    ------
    CCACACTGTAGAACATTTTAGATGAGTGAGT                          124
    TACACTGAAAA                                              135
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FIG. 3. Sequence of matrix clones G4 and G5. Numbers indicate the position of the nucleotide at the end of each line. (*A*) Three 31-bp imperfect direct repeats are aligned (lines numbered 75, 104, and 135) as they appear in the sequence. Mismatches are indicated by lower-case letters. The 11-bp sequence homologous to BKV T-antigen recognition site is underlined in each repeat. (*B*) Sequence of matrix clone G5 and its homology to B1 mouse repetitive sequence. The region of homology to the B1 sequence is underlined, and where mismatches occur the corresponding nucleotide in B1 is shown.

Table 1. Quantitation of relative frequencies of satellite G4 and G5 sequences in matrix and supernatant DNA libraries

Probe	Number of hybridizable plaques: Matrix DNA*	Number of hybridizable plaques: Supernatant DNA†	Relative content, matrix/supernatant
G35	384	205	1
G4	79	4	10
G5	215	91	1.3
G5 DI	144	58	1.3
G5 DII	117	39	1.5
G5 DI and DII	50	3	8.4

The number of plaques hybridizable to both DI- and DII-specific probes was counted by overlaying two replica filters hybridized to each probe separately. The indicated number of mp8 clones from matrix and supernatant DNA were plated in duplicate on agar plates containing lactose indicator. Each plate was "lifted" on five separate nitrocellulose filters. Each filter was hybridized to a nick-translated probe. DI, domain I; DII, domain II.
*Total clones, 5.2×10^4.
†Total clones, 2.5×10^4.

matrix clones. The strongly delimited physical ends of the G5 homologous matrix DNA clones, compared to the more random length and totally different adjacent sequences of the supernatant clones, suggest that G5 is uniquely associated with the nuclear matrix at least at the start of S phase.

The sequence comparison (Fig. 4) also suggests that the hybridization experiments using only a G5 probe can significantly underestimate the actual enrichment factor of sequence G5 in the matrix DNA because of cross-hybridization with related sequences. We therefore constructed probes specific to domain I and domain II to quantitate the relative content of clones containing both sequences in each library. Multiple nitrocellulose replicas of plates containing predetermined amounts of clones from matrix and supernatant DNA were hybridized to each of the probes. The clones hybridizing to both domains I and II were scored by overlaying the appropriate autoradiograms. The results show (Table 1) that the specific G5 sequence content of matrix DNA is ≈10-fold greater than that of the supernatant DNA.

G4 and G5 Sequences Contain Several Features Characteristic for Known Replication Origins. Although known replication origins generally do not share extensive sequence homologies, they contain several characteristic topological elements

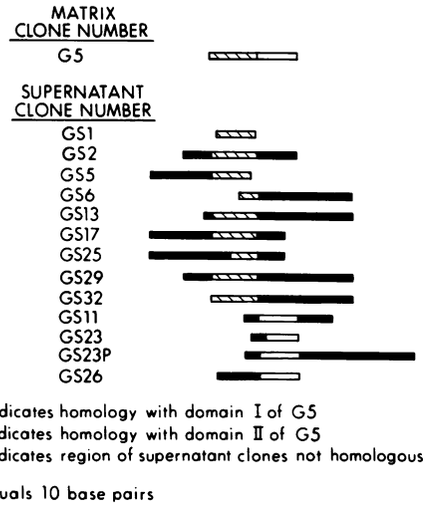

FIG. 4. Alignment of G5 with cross-hybridizing clones from supernatant library.

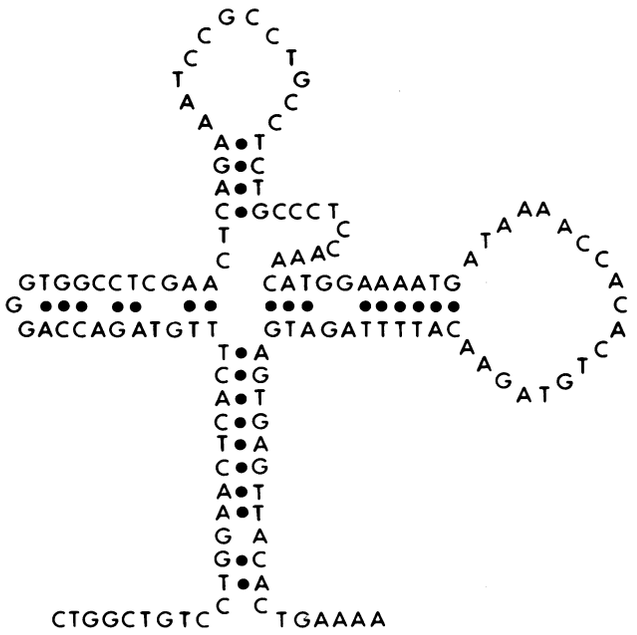

FIG. 5. Possible secondary structure of clone G5.

such as palindromes, direct repeats, and G·C/A·T switches with a purine bias (38). Additional guides to the structure of eukaryotic origins can presumably be found in the origins of small DNA viruses, such as simian virus 40 and human papova virus (BKV) (39, 40), which use cellular replication systems. As we described in the previous section, sequence G5 contains a unique combination of the fragment of the B1 sequence (domain I) adjacent to the domain II sequence. This particular combination is highly enriched in the nuclear matrix DNA. Fig. 5 shows a possible secondary structure of the G5 sequence in which the major stem consists of sequences contributed by both domains I and II. No such structure can be drawn for any of the supernatant clone sequences. In addition, the combination of domains I and II creates a G·C/A·T switch with a purine bias precisely at the border of the two domains. Both sequence G5 and the B1 homologous supernatant clones contain a 5-bp invariant element of viral large tumor antigen (T antigen) recognition sites repeated in the fashion, G-C-C-T-C-19bp-G-C-C-T-C-T-G-C-C-T-C. Alignment of the G5 sequence with the putative third T-antigen recognition site of the BKV (39) reveals a 13-bp homology with three mismatches adjacent to a G·C/A·T switch in both the G5 sequence and the viral DNA (Fig. 6). Sequence G4 of 152 bp contains three 31-bp direct head-to-tail repeats (Fig. 3A). An 11-bp sequence, present at the end of each repeat, is perfectly homologous to part of the first T-antigen recognition site of the BKV, including the same 5-bp invariant element.

DISCUSSION

The nuclei of eukaryotic cells subjected to a series of extractions, including high salt, yield a fast-sedimenting complex of DNA and nonhistone proteins termed the nuclear matrix. Nuclease treatment removes most of the chromatin and leaves a residual fraction of the DNA that is resistant to further nuclease digestion. This fraction has been shown to be enriched in newly synthesized DNA (41–43).

We have shown that this residual DNA associated with the nuclear matrix of BALB/c 3T3 cells synchronized at the G_1/S border is highly enriched in at least two specific types of DNA sequence. Sequence G5 can be divided into two domains based on a comparison with the sequences of cross-hybridizing clones isolated from supernatant DNA. Domain I is homologous to a fragment of the repetitive sequence, B1. Domain II is adjacent to domain I and contributes to two topological features of the sequence: (*i*) a G·C/A·T switch with a purine bias that is created on the border of the two domains and (*ii*) an imperfect inverted repeat of 11 bp that contributes to a possible secondary structure in which (Fig. 5) the stem consists of sequences contributed by both domains. The enrichment of this particular sequence in matrix DNA is significantly greater than that estimated by hybridization with the total G5 DNA probe. Sequence G4 is also highly enriched in the matrix DNA and has a peculiar structure. Of the 152 bp in the sequence, 93 are involved in three head-to-tail direct repeats, each of which contains an 11-bp sequence homologous to the first T-antigen recognition site of the BKV. Domain II of sequences G5 and G4 represents elements of middle repetitive DNA (unpublished results). Only a small fraction of the B1 repetitive sequence is linked to the domain II sequence in the genome (unpublished results). The precise copy number and full genomic structure of these sequences remain to be determined.

Considerable controversy exists concerning the nonrandom distribution of the repetitive DNA between matrix-associated and random genomic DNA (44–49). Some of the discrepancies can be explained by differences in the methods used to isolate the matrix or by the limited specificity of the nucleases used in digestion of the chromatin. Our study shows that, at least in the case of the B1-related G5 sequence, cross-hybridization between portions of matrix-associated sequences and similar elements in genomic DNA contributes to an error in calculating the enrichment factor. Therefore, the identification of matrix-

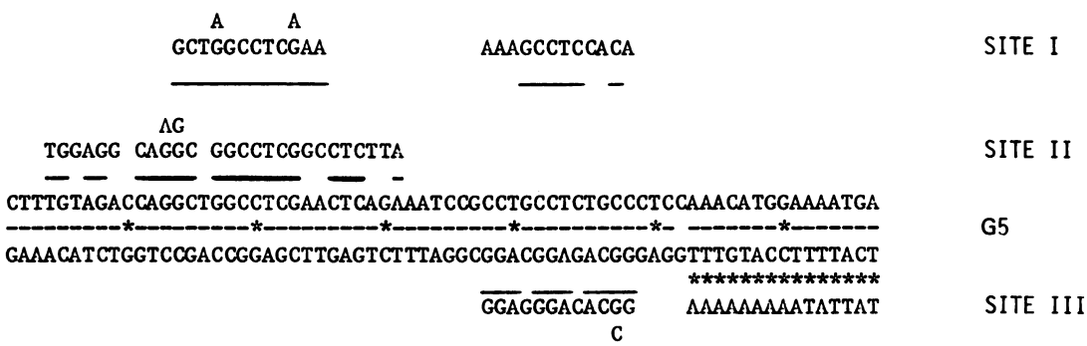

FIG. 6. Homology between G5 and BKV T-antigen binding sites. The BKV sequences (39) of the T-antigen binding sites I, II, and III are aligned with the G5 sequence (position 20–87). The border of domains I and II in the G5 sequence occurs at position 72. The sequence of each viral site is presented on a separate line as indicated. Homologous nucleotides are underlined. The insertions required for the alignment are shown above each sequence. Asterisks indicate the A·T side of the G·C/A·T switch in both G5 and BKV sequences. The viral (A+T)-rich sequence is shown adjacent to the third T-antigen binding site in the viral genome.

specific sequences may require a level of resolution greater than that attainable by C_0t analysis or DNA hybridization.

The most intriguing implication of the results showing high enrichment of the specific sequences in matrix DNA is that some of those sequences may be involved in anchoring the chromatin loops to the nuclear matrix. These anchorage sites have been implicated as fixed sites of DNA replication in eukaryotic cells (28). Whole-mount autoradiography of pulse-labeled interphase cells shows that nucleotides are incorporated into DNA near the chromatin–matrix attachment site at the base of the loops and are displaced toward the periphery of the loops as replication proceeds (14). These results imply two structural possibilities, which cannot be discriminated on the basis of these data. (*i*) Specific DNA sequences are associated with the nuclear matrix only during the initiation of replication. Elongation of the nascent strand results in their displacement to the periphery of the chromatin loops as DNA is reeled through the replication complex. These sequences are then transiently associated with the matrix. The best chance to identify these sequences is to analyze the DNA composition of the nuclear matrix obtained from cells involved in the initiation of DNA replication. (*ii*) Alternatively, specific DNA sequences are permanently bound to the matrix and initiation of replication occurs through assembly of two replication complexes adjacent to the anchoring sites. The DNA then would be reeled in from both sides, with replication occurring in a bidirectional fashion. This view is in agreement with the replication model proposed by Dingman (50) to reconcile the membrane-bound replication postulated by Jacob *et al.* (51) with the bidirectional replication of the bacterial chromosome.

The latter model predicts that the spectrum of the specific matrix-bound DNA sequences will remain largely constant through the S phase. Analysis of the DNA sequence distribution in the matrix-associated DNA throughout the cell cycle can yield results favoring one of the possibilities. A recent study of [^{3}H]thymidine incorporation into matrix-associated DNA of *Physarum polycephalum* suggests that replication origins remain permanently attached to the nuclear scaffold through the G_2 phase (52).

The organization of the cellular chromatin into supercoiled loops persists through the cell cycle, including the mitotic chromosome. The scaffolding structure supporting this organization, however, undergoes dramatic changes. In late G_2 phase, a part of the interphase scaffold dissolves and recent studies (15, 16, 20) suggest that the remnant of the interphase scaffold is involved in driving the condensation of the mitotic chromosome. The role of the DNA sequences and their arrangement in this process is not clear and, therefore, the topological relationship between the chromatin loops in the interphase nuclei and those in the metaphase chromosome remains to be elucidated.

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1. Georgiev, G. P., Nedospasov, S. A. & Bakayev, V. V. (1978) in *The Cell Nucleus*, ed. Busch, H. (Academic, New York), Vol. 6, pp. 1–34.
2. Olins, A. L. & Olins, D. E. (1974) *Science* **183**, 330–332.
3. Kornberg, R. D. (1974) *Science* **184**, 868–871.
4. Oudet, P. M., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4**, 281–299.
5. Kornberg, R. D. (1977) *Annu. Rev. Biochem.* **46**, 931–954.
6. Woreel, A. & Benyajati, C. (1977) *Cell* **12**, 83–120.
7. Ris, H. (1975) in *CIBA Foundation Symposium*, eds. Fitzsimons, D. W. & Wolstenholme, G. E. F. (American Elsevier, New York), Vol. 28, pp. 7–22.
8. Finch, J. T. & Klug, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1897–1901.
9. Hozier, J., Renz, M. & Nehls, P. (1977) *Chromosoma* **62**, 356–364.
10. Benyajati, C. & Worcel, A. (1976) *Cell* **9**, 393–407.
11. Cook, P. R., Brazell, I. A. & Jost, E. (1976) *J. Cell Sci.* **22**, 303–324.
12. Igo-Kemenez, T. & Zachau, H. G. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 109–118.
13. Adolph, K. W. (1980) *J. Cell Sci.* **42**, 231–291.
14. Vogelstein, B., Pardoll, D. M. & Coffey, D. S. (1980) *Cell* **22**, 79–85.
15. Lebkowski, J. S. & Laemmli, U. K. (1982) *J. Mol. Biol.* **156**, 309–324.
16. Lebkowski, J. S. & Laemmli, U. K. (1982) *J. Mol. Biol.* **156**, 325–344.
17. Adolph, K. W., Cheng, S. M. & Laemmli, U. K. (1977) *Cell* **12**, 805–816.
18. Adolph, K. W., Cheng, S. M. & Laemmli, U. K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4937–4961.
19. Paulson, J. R. & Laemmli, U. K. (1977) *Cell* **12**, 817–828.
20. Lewis, C. D. & Laemmli, U. K. (1982) *Cell* **29**, 171–181.
21. Berezney, R. & Coffey, D. S. (1974) *Biochem. Biophys. Res. Commun.* **60**, 1410–1417.
22. Comings, D. E. & Okada, T. A. (1976) *Exp. Cell. Res.* **103**, 341–360.
23. Merlan, G. & Wunderlich, F. (1976) *Cytobiologie* **13**, 291–301.
24. Keller, J. M. & Rilley, D. C. (1976) *Science* **193**, 399–401.
25. Hodge, L. D., Mancini, P., Davis, F. M. & Heywood, P. (1977) *J. Cell Biol.* **72**, 192–208.
26. Herman, R., Weymouth, L. & Penman, S. (1978) *J. Cell Biol.* **78**, 663–674.
27. Miller, T. E., Huang, C. & Pogo, A. O. (1978) *J. Cell Biol.* **76**, 692–704.
28. Pardoll, D. M., Vogelstein, B. & Coffey, D. S. (1980) *Cell* **19**, 527–536.
29. Berezney, R. & Coffey, D. S. (1975) *Science* **189**, 291–293.
30. Dijkwel, P. A., Mullenders, L. H. S. & Wanka, F. (1979) *Nucleic Acids Res.* **6**, 219–230.
31. Smith, H. C. & Berezney, R. (1980) *Biochem. Biophys. Res. Commun.* **97**, 1541–1547.
32. Capco, D. G., Wan, K. M. & Penman, S. (1982) *Cell* **29**, 847–858.
33. Wunderlich, F. & Herlan, G. (1977) *J. Cell Biol.* **73**, 271–278.
34. Berezney, R. & Coffey, D. S. (1977) *J. Cell Biol.* **73**, 616–637.
35. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
36. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
37. Kraev, A., Kramerov, D., Scryabin, K., Ryskov, A., Bayer, A. & Georgiev, G. (1980) *Nucleic Acids Res.* **8**, 1201–1215.
38. Kornberg, A. (1980) in *DNA Replication* (Freeman, San Francisco), pp. 443–466.
39. Yang, R. A. & Wu, R. (1979) *Science* **206**, 456–462.
40. Soeda, E., Arrand, J., Smolar, N. & Griffin, B. E. (1979) *Cell* **17**, 357–370.
41. Nelkin, B. D., Pardoll, D. & Vogelstein, B. (1980) *Nucleic Acids Res.* **8**, 5623–5633.
42. Cook, P. R. & Brazell, I. A. (1980) *Nucleic Acids Res.* **8**, 2895–2908.
43. Robinson, S. I., Nelkin, B. D. & Vogelstein, B. (1982) *Cell* **28**, 99–106.
44. Pardoll, D. & Vogelstein, B. (1980) *Exp. Cell. Res.* **128**, 466–470.
45. Matsumoto, L. H. (1981) *Nature (London)* **294**, 481–482.
46. Jeppesen, P. G. N. & Bankier, A. T. (1979) *Nucleic Acids Res.* **7**, 49–67.
47. Razin, S. V., Mantieva, V. L. & Georgiev, G. P. (1979) *Nucleic Acids Res.* **7**, 1713–1735.
48. Basler, J., Hastie, N. D., Pietras, D., Matsui, S. I., Sandberg, A. A. & Berezney, R. (1981) *Biochemistry* **20**, 6921–6929.
49. Small, D., Nelkin, B. & Vogelstein, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5911–5915.
50. Dingman, C. W. (1974) *J. Theor. Biol.* **43**, 187–195.
51. Jacob, F., Brenner, S. & Cuzin, F. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 329–348.
52. Aelen, J. M. A., Opstelten, R. M. G. & Wanka, F. (1983) *Nucleic Acids Res.* **11**, 1181–1195.