

Spatial Distribution of DNA Loop Attachment and Replicational Sites in the Nuclear Matrix

HAROLD C. SMITH,* EDMOND PUVION,† LINDA A. BUCHHOLTZ,* and RONALD BEREZNEY*

**Division of Cell and Molecular Biology, Department of Biological Sciences, State University of New York, Buffalo, New York 14260 and †Groupe de Microscopie Electronique, Institut de Recherches Scientifiques sur le cancer, F-94800 Villejuif, France. Dr. Smith's present address is Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030*

ABSTRACT Biochemical fractionation was combined with high resolution electron microscopic autoradiography to study the localization in rat liver nuclear matrix of attached DNA fragments, in vivo replicated DNA, and in vitro synthesized DNA. In particular, we determined the distribution of these DNA components with the peripheral nuclear lamina versus more internally localized structural elements of isolated nuclear matrix. Autoradiography demonstrated that the bulk of in vivo newly replicated DNA associated with the nuclear matrix (71%) was found within internal matrix regions. A similar interior localization was observed in isolated nuclei and in situ in whole liver tissue. Likewise, isolated nuclear lamina contained only a small amount (12%) of the total matrix-bound, newly replicated DNA. The structural localization of matrix-bound DNA fragments was examined following long-term in vivo labeling of the DNA. The radioactive DNA fragments were found predominantly within interior regions of the matrix structure (77%), and isolated nuclear lamina contained <15% of the total nuclear matrix-associated DNA. Most of the endogenous DNA template sites for the replicative enzyme DNA polymerase alpha (~70%) were also sequestered within interior regions of the matrix. In contrast, a majority of the endogenous DNA template sites for DNA polymerase beta (a presumptive repair enzyme) were closely associated with the peripheral nuclear lamina. A similar spatial distribution for both polymerase activities was measured in isolated nuclei before matrix fractionation. Furthermore, isolated nuclear lamina contained only a small proportion of total matrix-bound DNA polymerase alpha endogenous and exogenous template activities (3–12%), but a considerable amount of the corresponding beta polymerase activities (47–52%). Our results support the hypothesis that DNA loops are both anchored and replicated at nuclear matrix-bound sites that are predominantly but not exclusively associated with interior components of the matrix structure. Our results also suggest that the sites of nuclear DNA polymerase beta-driven DNA synthesis are uniquely sequestered within the characteristic peripheral heterochromatin shell and associated nuclear envelope structure, where they may potentially participate in DNA repair and/or replicative functions.

A number of investigators have shown that eucaryotic chromosomal DNA is organized into repeating supercoiled domains or loops (7, 22, 30, 35, 46, 50). Recent results suggest that the DNA loops are constrained by attachment to components of the interphase nuclear matrix (4, 11, 12, 20, 21, 24, 51, 59) or its mitotic counterpart, the chromosome scaffold (5, 25, 48, 51). While the precise relationships of isolated

nuclear matrices to in situ nuclear structure have yet to be elucidated (9), a number of studies have demonstrated, depending on the conditions of isolation, a considerable degree of structural correspondence to in situ structure (15, 19, 31, 40, 53, 62). In a particularly striking example, LaFond et al. (42) performed cell fusion and demonstrated the initial assembly of an internal matrix structure following the functional

reactivation of the erythrocyte nucleus. This and other studies (8, 9, 41, 53) suggest a relationship between the presence of internal matrix structure and nuclear function.

With respect to this proposed relationship, a large number of functionally related properties copurify with isolated nuclear matrix (for reviews see 2, 8, 9, 28, 54). Although the actual significance of many of these associations remains to be established, considerable progress has been made in elucidating the association of DNA replication with the nuclear matrix. The data suggest that DNA is replicated at or close to matrix-bound DNA loop attachment sites (12–14, 24, 34, 45, 47, 59). Based on these findings it has been proposed that during replication supercoiled DNA loops bound to matrix replicational complexes move bidirectionally and relative to one another at their points of association (12, 24, 45, 47).

In support of these structural models, isolated nuclear matrices contain the eucaryotic replicative enzyme DNA polymerase alpha, which is active in *in vitro* DNA synthesis on either matrix-bound endogenous DNA template (56) or exogenously provided templates (36, 55, 56). The *in vitro* synthesis of Okazaki-sized DNA fragments (56) and appropriate density shift experiments (Tubo, R., H. C. Smith, and R. Berezney, manuscript submitted for publication) strongly suggest that the matrix-bound *in vitro* DNA synthetic system continues synthesis along *in vivo*-initiated DNA strands. Moreover, the striking prereplicative induction of DNA polymerase alpha activity on the nuclear matrix (57) lends further support to the biological relevance of this matrix-bound activity in the replicative process.

Despite the above summarized progress, the precise location of DNA loop attachment sites relative to *in vivo* replicational and *in vitro* DNA synthetic sites remains unclear. In this study we have addressed this issue by combining biochemical and fractionation techniques with high resolution electron microscopic autoradiography.

MATERIALS AND METHODS

Nuclear Isolation: Nuclei were prepared from two-thirds partially hepatectomized rats (Sprague-Dawley, 230–280 g; King Animal Laboratories, Oregon, WI) as reported earlier (4, 12, 15) with slight modifications (56). After dense sucrose purification, nuclei were resuspended to 2.5 mg DNA/ml in 0.25 M sucrose, Tris-magnesium (TM)¹ buffer (0.25 M sucrose, 10 mM Tris-HCl, pH 7.4 (23°C), 5 mM MgCl₂).

Nuclear Matrix and Lamina Preparation: Nuclei in 0.25 M sucrose TM buffer were endogenously digested at 37°C for 45 min unless indicated otherwise, and divided into two aliquots. Nuclear matrices were prepared from one of the paired nuclear aliquots based on the procedure of Smith and Berezney (56). Briefly, endogenously digested nuclei were extracted once with low salt (LS) buffer (0.2 mM MgCl₂, 10 mM Tris-HCl, pH 7.4 (23°C), 1 mM phenylmethylsulfonyl fluoride), twice with high salt (HS) buffer (2 M NaCl, 0.2 mM MgCl₂, 10 mM Tris-HCl, pH 7.4 (23°C), 1 mM phenylmethylsulfonyl fluoride), once with 0.4% Triton X-100 in LS buffer and twice with LS buffer. Centrifugations were at 1,000 g for 15 min for all extractions except for HS buffer extractions, which were done at 6,000 g for 15 min. Final nuclear matrices were resuspended in LS buffer to 1/2–1/3 the original volume of nuclei.

Nuclear lamina was prepared from the second nuclear aliquot according to published procedures (3, 37). Endogenously digested nuclei were chilled to 0–4°C, and digested with 32 µg RNase A/mg nuclear DNA for 30 min at 0–4°C in the presence of 1 mM phenylmethylsulfonyl fluoride, and 5 mM dithiothreitol. All subsequent extractions were as described for the nuclear matrix preparation except for the inclusion of 5 mM dithiothreitol in all extraction buffers. When nuclear lamina was assayed for *in vitro* polymerase activity, an additional final wash with LS buffer without dithiothreitol was performed.

¹ Abbreviations used in this paper: HS, high salt; LS, low salt; TM, Tris-magnesium.

***In Vivo* DNA Synthesis:** [³H]Thymidine (50–60 Ci/mmol, New England Nuclear, Boston, MA) was injected directly into the hepatic portal vein (100 µCi/250 g of body weight) as previously described (12). After a 1-, 5-, or 60-min pulse time, the livers were excised, minced in ice cold 0.25 M sucrose TM, and processed as described above. For long-term *in vivo* labeling, rats were injected intraperitoneally with 200 µCi of [³H]thymidine at 16, 18, 20, 22, and 24 h of liver regeneration. Livers were harvested 10 d later at which time normal liver mass had been regained.

***In Vitro* DNA Synthesis:** Nuclear and matrix alpha and beta *in vitro* DNA synthesis was performed as described previously (55, 56). Alpha and beta polymerase activities were distinguished by *N*-ethylmaleimide sensitivity in an alpha optimal buffer system (56). We have previously demonstrated that this means of discriminating between the two enzyme activities is reliable (55, 56), and that the relative distribution of beta polymerase activity in various subfractions is similar when the enzyme is assayed in beta optimal buffer systems.

Macromolecular Determinations: DNA, RNA, and protein, and the specific labeling of DNA were determined as previously reported (12). DNA was purified from isolated nuclear matrix and lamina as described by Basler et al. (4), and DNA fragment sizes were determined by neutral 0.7% agarose gel electrophoresis using the Tris-borate-EDTA buffer system (49).

Thin-section Electron Microscopy: Small pieces of liver tissue, isolated nuclei, or nuclear matrices prepared from *in vivo* labeling experiments were fixed in 2.5% glutaraldehyde, 100 mM sodium cacodylate, pH 7.4 (23°C), 5 mM MgCl₂ (0.2 mM MgCl₂ for matrices) for 2 h on ice. Nuclei and nuclear matrices from *in vitro* labeling experiments were centrifuged out of the reaction buffers (1,000 g for 10 min) and washed twice with excess 0.25 M sucrose TM buffer (nuclei) or LS buffer (matrices) before fixation. Isolated nuclear lamina was fixed directly in HS buffer as described by Kaufmann et al. (37). All samples were then rinsed with cacodylate buffer without glutaraldehyde and postfixed with 1% osmium tetroxide for 30 min on ice followed by dehydration (graded ethanol [30–100%] and 100% acetone) and infiltrated with Epon-Araldite resin (Electron Microscopy Sciences, Fort Washington, PA). Cured blocks (65°C/24 h) were sectioned with a diamond knife, stained with lead citrate and uranyl acetate, and examined with an Hitachi H-500 or Hu-11c electron microscope.

Electron Microscopic Autoradiography: Samples were prepared for electron microscopy as described above with the omission of osmium tetroxide postfixation. Ilford L 4 emulsion (Ilford Ltd., Ilford, Essex, England) was applied on thin sections mounted on Formvar-coated copper grids. After 6–8 mo. exposure, autoradiograms were developed with the gold latensification phenidol technique (18) and examined on a Siemens 101 electron microscope. Silver grain distribution in electron micrographs of thin sections of nuclei or matrices was analyzed by counting grains located over the total area, the internal area, and the peripheral area (a shell extending 1/20th of the nuclear diameter from the outermost boundary of the structure to its interior). The grain counts from 20 to 30 electron micrographs for each sample representing several sectioned blocks from two separate experiments were used for statistical analysis.

RESULTS

Isolation of Nuclear Matrix and Lamina

The rat liver nuclear matrix and lamina isolated in this study (see Materials and Methods) had structural and biochemical properties similar to those demonstrated in previous reports (15, 37). Briefly, the matrix preparations contained 8–14% of the protein, 1–5% of the DNA, and 20–30% of the RNA from isolated nuclei. The corresponding nuclear lamina fraction contained 1–3% of total nuclear protein, 0.2–1.0% of total nuclear DNA, and 1–2% of total nuclear RNA. Electron microscopy of thin sections from the nuclear matrix fraction (Fig. 1A) confirmed the characteristic tripartite configuration in which a pore complex lamina surrounded fibrogranular matrix structures and residual nucleoli (2, 8, 9, 21, 54). In contrast, the nuclear lamina preparation (Fig. 1B) consisted of a pore complex lamina surrounding an interior virtually devoid of structure (1, 37).

Spatial Distribution of DNA Attachment Sites

Nuclease digestion studies have shown that the average fragment size of the DNA remaining bound to the matrix

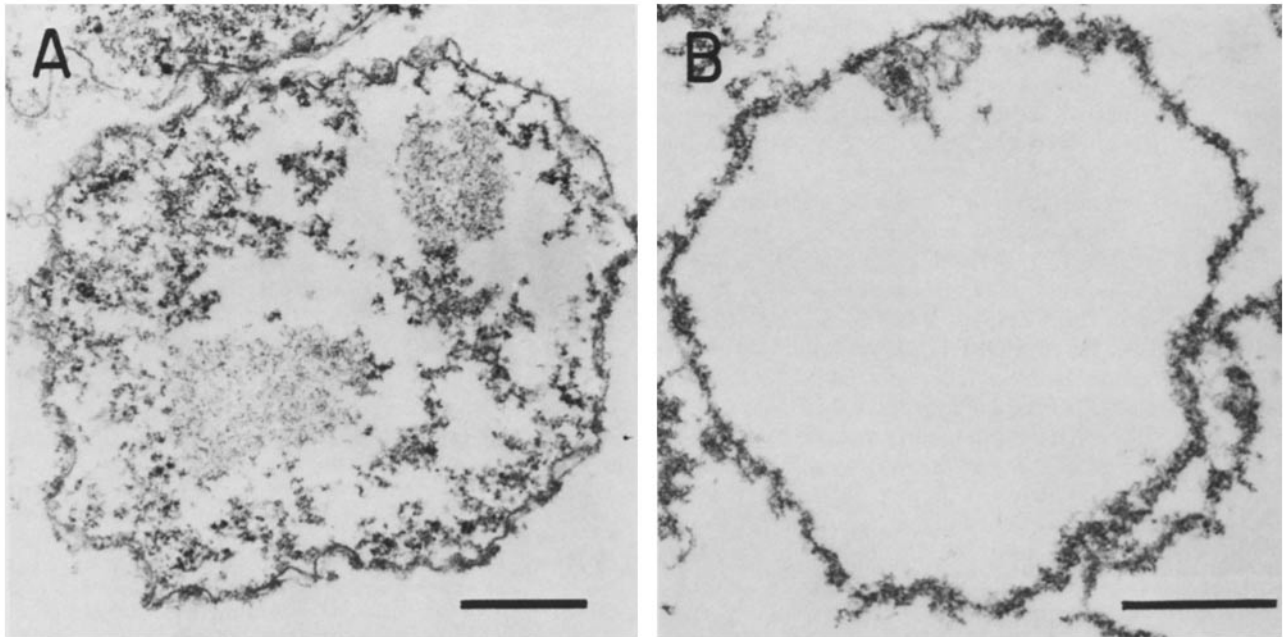


FIGURE 1 Thin-sectioned electron microscopy of the isolated total nuclear matrix (A) and nuclear lamina (B) fractions. Note the typical tripartite structure of the isolated nuclear matrix with the surrounding nuclear lamina and an internal structure consisting of residual nucleoli and an extensive extranucleolar fibrogranular matrix. This characteristic internal structure is lacking in the isolated nuclear lamina. Bars, 1 μm . (A) $\times 16,800$; (B) $\times 20,600$.

after high salt extraction is proportional to the amount of nuclear DNA recovered on the matrix (4, 10, 51). Significantly, the amount of bound DNA approaches zero as the average fragment size approached zero (10) and 100% when matrices are isolated under conditions that avoid cleavage or nicking of the DNA (11). These findings have led to the general view that the salt-resistant DNA that remains matrix-bound after nuclease digestion represents DNA at or near the attachment sites for the DNA "loops" (4, 10–12, 20, 21, 24, 45, 47, 51, 59; for recent reviews see references 9, 28). With these concepts in mind we have measured the distribution of DNA fragments in nuclear matrix and lamina prepared from a common batch of endogenously digested nuclei (see Materials and Methods). Isolated nuclear lamina contained only 0.25% of the total nuclear DNA, which represented $\sim 15\%$ of the DNA recovered in the total matrix (1.8% of total nuclear DNA). Since the average fragment size of matrix and lamina DNA were very similar (900–1,000 bp), these results suggest that most of the DNA loop attachment sites are localized in the nuclear matrix interior.

To determine whether the low recovery of DNA fragments in the nuclear lamina was due to an artifact of selective loss of DNA during lamina preparation, we analyzed the distribution of radioactively tagged DNA fragments within intact matrix structures by electron microscopic autoradiography on thin sections. For these experiments, long term *in vivo*-labeled nuclear DNA (see Materials and Methods) was endogenously digested for 120 min in addition to our standard 45-min digestion. This reduced the average matrix-bound DNA fragment size to ~ 200 bp and provided a more rigorous evaluation for the structural localization of the DNA attachment sites. Measurements of silver grain distribution in electron micrographs such as in Fig. 2 demonstrated that the DNA attachment sites were predominantly distributed over the interior of intact matrices with no significant differences based on the

average size of the matrix-bound DNA fragments. We calculated that $77.2\% \pm 5.1$ (SD) of the total silver grains were not in a peripheral shell having a thickness of $1/20$ th the diameter of the matrix structure. For a typical matrix structure with a diameter of $7 \mu\text{m}$ our definition of the "lamina region" would extend $3,500 \text{ \AA}$ inward from the matrix periphery. Since this is a region considerably wider than the thickness of the nuclear lamina, it is possible that our analysis of the autoradiographs overestimates the number of lamina-associated DNA attachment sites. Likewise, we may be also overestimating the proportion of lamina-associated DNA replicational sites (see below).

Spatial Distribution of In Vivo Replicating DNA

To localize the sites of DNA replication, we determined the recovery of 1-min *in vivo* pulse-labeled DNA in total matrix and lamina (Table I). Newly replicated DNA was found in much greater amounts in nuclear matrix (27% of total nuclear-labeled DNA) compared with nuclear lamina (3% of total nuclear-labeled DNA). However, the specific activity of lamina DNA was approximately equivalent to that of total matrix DNA (Table I). These findings suggest that replication occurs in association with many regions of the matrix and that the replicational activity of the lamina (and presumably other structural domains of the matrix) is consistent with the estimated proportion of total DNA loop attachment sites in that domain (Fig. 2).

Electron microscopic autoradiography shown in Fig. 3 and compiled in Table II corroborated the biochemical analysis. 85% of the 1-min pulse-labeled DNA was located over the interior of *in situ* nuclei, whereas 77 and 71% of the autoradiographic grains were over the interior of isolated nuclei and nuclear matrices, respectively. A similar pattern of autoradiography grains was observed after 5-min (Table II) and 60-

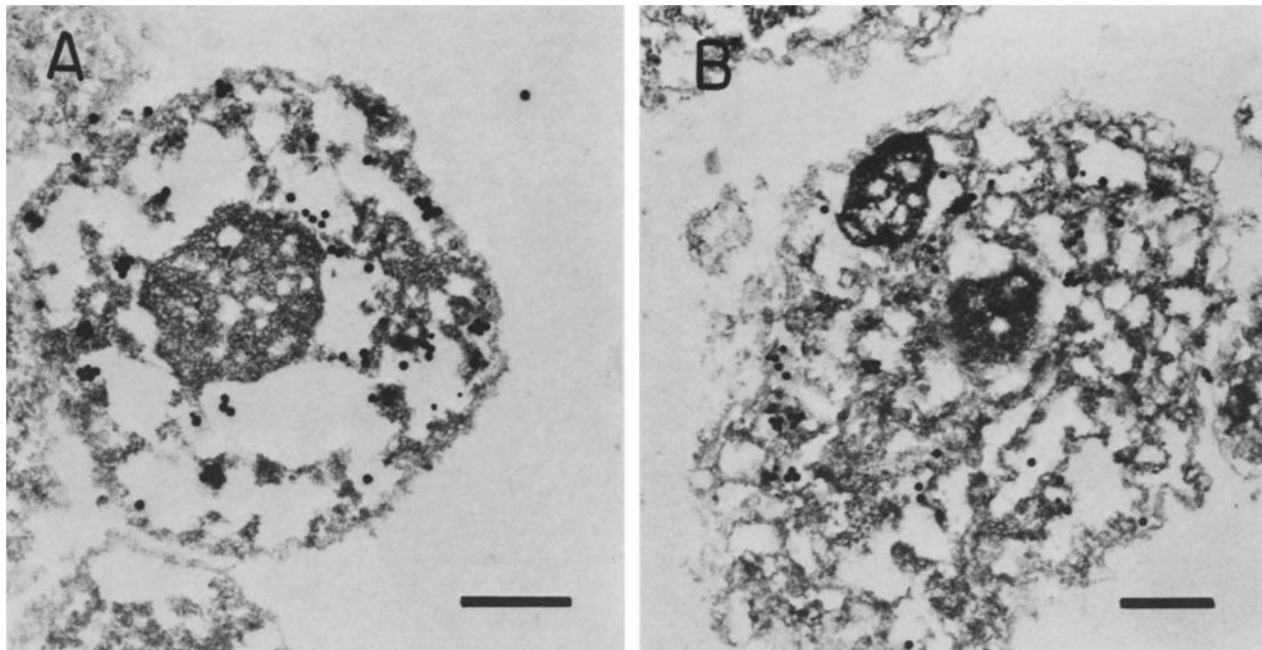


FIGURE 2 Spatial distribution of nuclear matrix DNA loop attachment sites. Regenerating rat liver nuclear DNA was long-term labeled, nuclei were purified, and the matrix was prepared as described in Materials and Methods. Sample preparation for thin-sectioning electron microscopy and autoradiography were also as described in Materials and Methods. (A) Nuclear matrix from 45-min endogenously digested nuclei with an average DNA fragment size of 900 bp. (B) Nuclear matrix from 120-min endogenously digested nuclei with an average DNA fragment size of 200 bp. Bars, 1 μ m. (A) \times 14,400; (B) \times 12,800.

TABLE I
Recovery of 1-min Pulse-labeled *In Vivo*-Replicated DNA in
Nuclear Matrix and Lamina

Fraction	Percent of total 1-min pulse-labeled DNA	Relative specific activity*
Nuclei	100	1.0
Nuclear matrix	27.2	16
Nuclear lamina	3.3	13.2

* Relative specific activities were determined as nuclear matrix or nuclear lamina specific activity (cpm/mg DNA) divided by the corresponding total nuclear specific activity.

min (Fig. 3 and Table II) *in vivo* pulses. This consistent distribution of autoradiographic grains for the three different pulse lengths suggests that the predominant internal localization of the replicational sites was not a result of differential uptake of DNA precursors in the interior regions compared with the peripheral lamina regions, but indeed represented the actual sites of synthesis.

Spatial Distribution of the Sites for In Vitro DNA Synthesis

Previous studies in our laboratory (55, 56) have demonstrated that the nuclear matrix isolated from regenerating rat liver during active *in vivo* replication is highly enriched in the presumptive eucaryotic replicative enzyme DNA polymerase alpha, but contains only trace amounts of DNA polymerase beta (a presumptive DNA repair enzyme). To determine the distribution of alpha and beta polymerase activities in isolated nuclear matrix and lamina. Nuclear lamina was devoid of alpha polymerase endogenous and exogenous tem-

plate-driven activity (3–12% of the total activity in nuclear matrix preparations; Table III). In contrast, nuclear lamina was enriched compared with matrix in beta polymerase endogenous and exogenous template-driven activities and contained a large percentage of the total matrix beta polymerase activity (47–52%; Table III). Note that ~50 and 80% of total cellular DNA polymerase alpha and beta activities were recovered in the isolated regenerating liver nuclei used in these studies (56). Also, the recoveries of alpha and beta polymerase activities on the matrix were 30 and 5% of total nuclear and 15 and 4% of total cellular activities, respectively.

The differential localization of alpha and beta polymerase activities in nuclear matrices was confirmed by electron microscopic autoradiography on *in vitro* labeled nuclei and nuclear matrices. Most of the autoradiographic grains (81 and 72%) were distributed over interior regions of nuclei and matrices, respectively, after DNA synthesis in an alpha polymerase-optimized assay (Fig. 4 and Table IV). Consistent with the biochemical analysis, nuclear and matrix-bound beta polymerase showed a strikingly different distribution than the corresponding alpha polymerase-optimized *in vitro* synthesis. Most of the beta-specific autoradiographic grains (78 and 73%) were clustered around the periphery of nuclei and matrices, respectively (Fig. 4 and Table IV).

DISCUSSION

Numerous studies have shown that newly replicated DNA tends to copurify with the nuclear matrix (8, 9, 54), but none has yet fully addressed the question of the structural localization of these DNA fragments. In this paper we have presented evidence using combined biochemical techniques and high resolution electron microscopic autoradiography that sites of DNA attachment are distributed throughout the matrix struc-

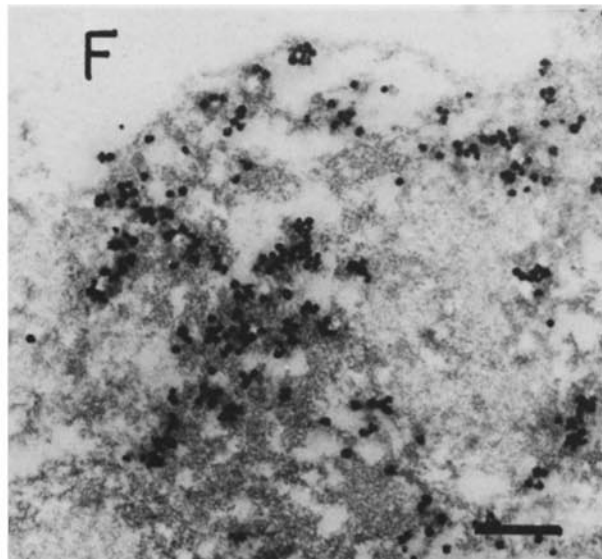
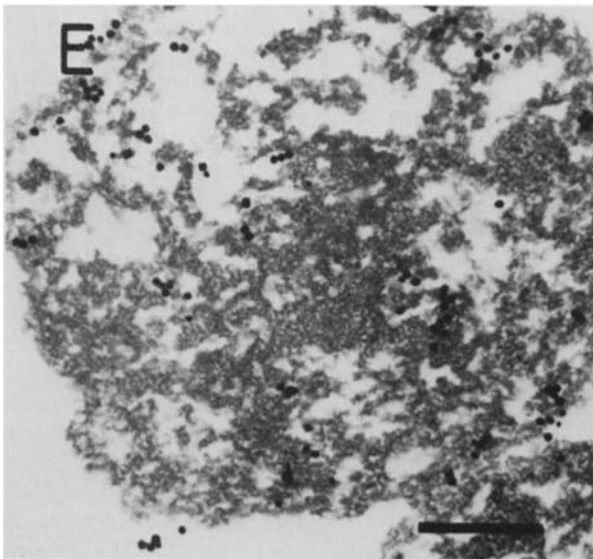
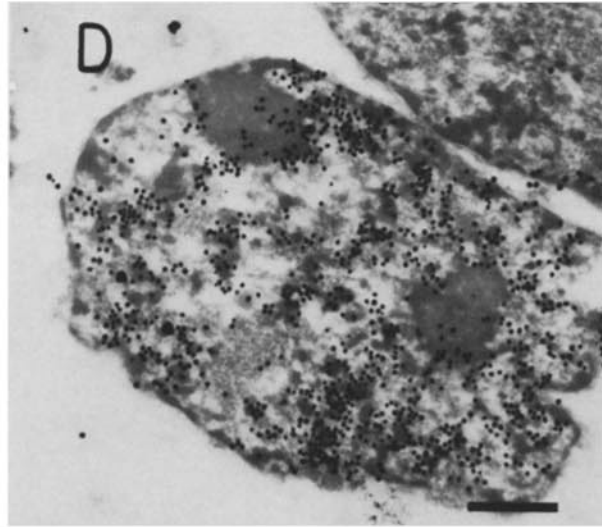
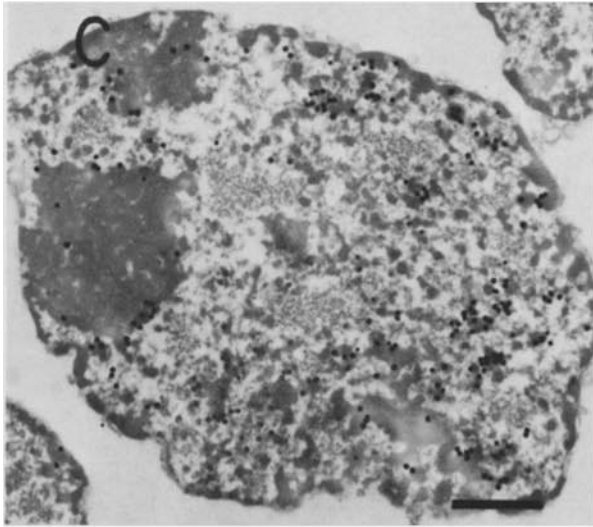
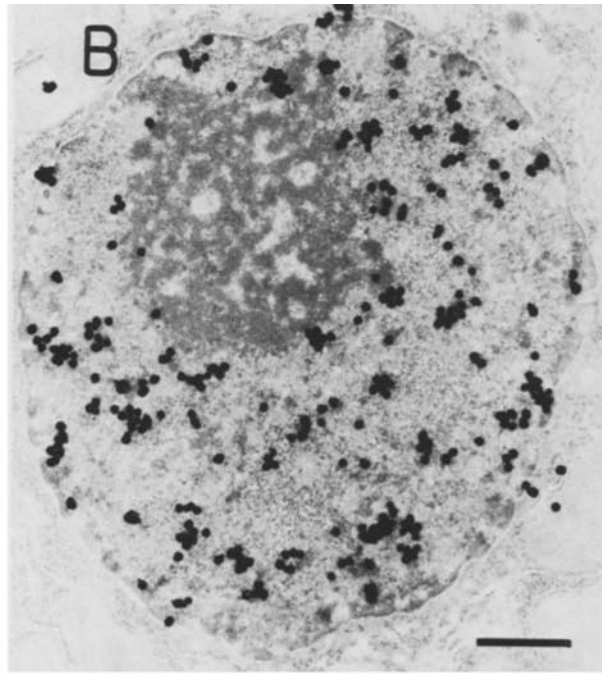
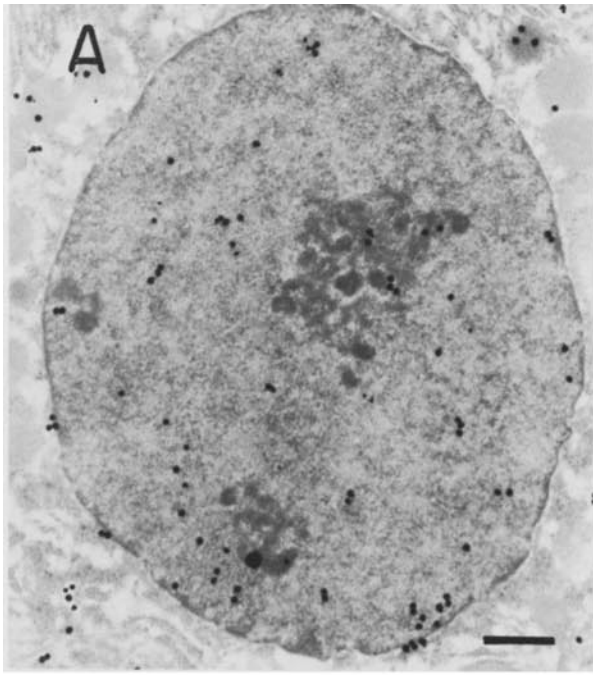


TABLE II
Autoradiographic Grain Distribution in Thin Sections from 1-, 5-, and 60-min In Vivo Pulse-labeled Regenerating Liver

Fraction	Percent of total grain distribution*	
	Peripheral	Internal
1-Min pulse		
In situ nuclei	14.8 ± 3.1	85.2 ± 3.1
Isolated nuclei	23.0 ± 3.7	77.0 ± 3.7
Nuclear matrix	29.4 ± 2.8	70.6 ± 2.8
5-Min pulse		
In situ nuclei	39.9 ± 6.6	60.1 ± 6.6
Isolated nuclei	31.0 ± 5.3	69.0 ± 5.3
Nuclear matrix	31.4 ± 3.4	68.6 ± 3.4
60-Min pulse		
In situ nuclei	18.6 ± 1.6	81.4 ± 1.6
Isolated nuclei	30.0 ± 3.7	70.0 ± 3.7
Nuclear matrix	40.1 ± 5.6	59.9 ± 5.6

* Autoradiography and calculations of percent total grain distribution were according to Materials and Methods. Values represent ± the standard deviation.

TABLE III
Distribution of DNA Polymerase Activities in the Nuclear Matrix and Lamina

Template*	Fraction†	Alpha polymerase		Beta polymerase	
		Percent of total matrix specific activity‡	Relative activity§	Percent of total matrix specific activity‡	Relative activity§
Endogenous	Nuclear matrix	100	1.0	100	1.0
	Nuclear lamina	3.0	0.1	52	1.6
Exogenous	Nuclear matrix	100	1.0	100	1.0
	Nuclear lamina	12	0.4	47	1.5

* In vitro DNA synthesis was carried out as described in Materials and Methods using the endogenous matrix-attached DNA as template or exogenous-activated calf thymus DNA as template.

† Nuclear lamina prepared as described in Materials and Methods contained 32% of the total matrix proteins.

‡ Matrix alpha polymerase activity represents ~15 and 30% of the total cellular and nuclear activity. Matrix beta polymerase activity represents ~4 and 5% of the total cellular and nuclear activity.

§ Relative specific activity is calculated as the specific activity (cpm/mg protein) of the nuclear lamina fraction divided by the specific activity of the total nuclear matrix.

ture. Moreover, the localization of in vivo replicational sites and DNA polymerase alpha-driven in vitro DNA synthetic sites in the matrix structure closely correlates with this overall distribution of DNA loop attachment sites. Taken together, our results are consistent with the hypothesis that the matrix-attached DNA loops represent the fundamental units of eucaryotic replication (9–12, 24, 45, 47, 59).

These findings also agree with previous results demonstrat-

ing the association of residual DNA fragments with both peripheral and interior components of the nuclear matrix (8, 14, 20, 21). They further demonstrate that the majority of the DNA loop attachment sites are sequestered in the matrix interior and are not found predominantly in the peripheral lamina as previously suggested (29, 58). Although Lebkowski and Laemmli (43) reported that DNA loops were attached to nuclear matrix structures irrespective of the presence of internal matrix components, these results are not inconsistent with our findings. Since DNA is presumably attached to the matrix in repeating loop structures, removal of internal DNA loop attachment sites would not necessarily abrogate the anchoring of the DNA via the remaining, albeit smaller, portion of lamina-associated attachment sites. This should result, however, in the formation of correspondingly larger DNA loops. Consistent with this prediction, Lebkowski and Laemmli (43) reported a substantial increase in the DNA halo size of nuclear matrix structures coincident with the removal of internal matrix components. These investigators also reported the release of a group of DNA-binding proteins during internal matrix removal that did not correspond in molecular weight to the major nuclear lamins (44). Some of these presumptive nonlamina DNA-binding proteins, at least, might represent proteins that anchor the DNA loops to internal nuclear matrix components. In this regard Bhorjee et al. (16) described several monoclonal antibodies to proteins tightly bound to DNA that discretely decorated the interior of the in situ nucleus by immunofluorescence and reacted with nuclear matrix proteins on western blots. Werner et al. (60) also demonstrated interior nuclear fluorescence using antibodies to proteins tightly bound to DNA, and Bodnar et al. (17) recently identified similar proteins in isolated nuclear matrices.

Our results further demonstrate that newly replicated DNA is distributed in situ throughout the entire nuclear structure and that the overall distribution of replicational sites is not significantly perturbed during nuclear and matrix preparation (Fig. 3). These results are in agreement with previous demonstrations that the eucaryotic sites of DNA initiation and replication are generally distributed throughout the nuclear interior and not preferentially associated with the nuclear periphery except possibly during late S phase (26, 27, 32, 39, 61, 63). Pardoll et al. (47) also demonstrated interior localization of newly replicated DNA in isolated nuclear matrix after a 5-min in vivo pulse.

Using recently reported methods for selective solubilization of the internal matrix structure (3, 37), we have extended these studies to show that most of the in vivo replicational sites associated with the matrix are extracted when the internal matrix (the bulk of the total matrix protein) is solubilized (Table II and Fig. 1). The replicational sites that remain associated with the lamina, however, are just as active in DNA synthesis as those associated with the interior matrix domain(s) (Table II). This stresses the point that the nuclear lamina represents a quantitatively small but distinct structural domain of total nuclear DNA replication. For example, the strategically located peripheral heterochromatin shell which

FIGURE 3 Spatial distribution of DNA replicational sites. Regenerating rat liver (22 h posthepatectomy) was pulse-labeled in vivo for 1 min (A, C, and E) or 60 min (B, D, and F) with [³H]thymidine as described in Materials and Methods. Whole tissue sections (A and B), isolated nuclei (C and D), and nuclear matrices (E and F) were then prepared for electron microscopy and autoradiography. Bars, 1 μm. (A) × 9,600; (B) 12,800; (C and D) 12,000; (E) × 16,800; (F) × 11,200.

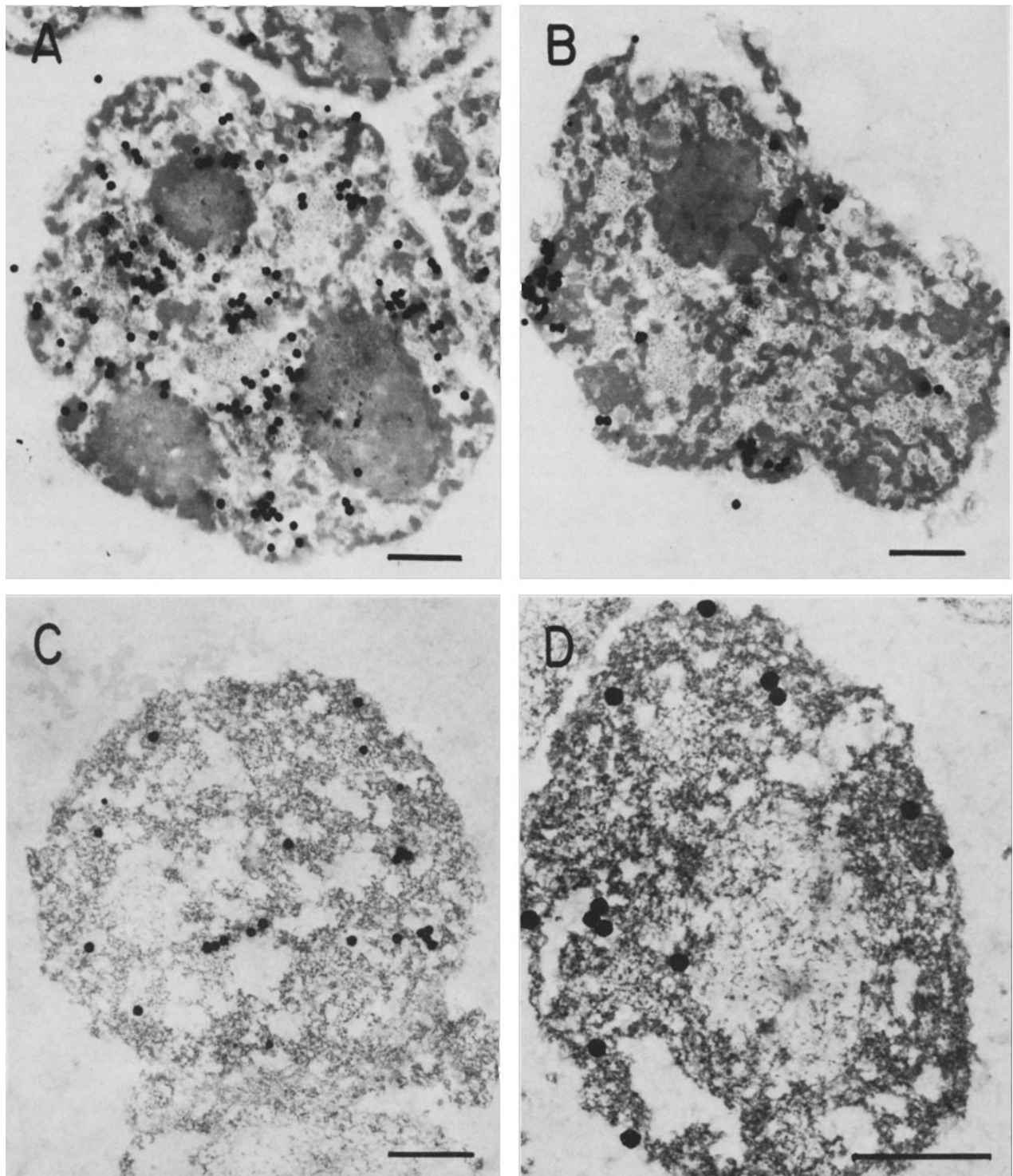


FIGURE 4 Spatial distribution of the sites of in vitro DNA synthesis for DNA polymerases alpha and beta. Nuclei and nuclear matrices were prepared from 22-h regenerating rat liver. DNA polymerase alpha and beta were assayed in vitro and distinguished by *N*-ethylmaleimide sensitive as described in Materials and Methods. (A and C) Nuclear and nuclear matrix alpha polymerase optimized-driven DNA synthesis, respectively. (B and D) Nuclear and nuclear matrix beta polymerase-driven DNA synthesis, respectively. Approximately 65 and 90% of total DNA polymerase activity in the alpha-optimized assay was due to alpha polymerase in total nuclei and matrix, respectively. Bars, 1 μ m. (A and B) \times 13,500; (C) \times 14,400; (D) \times 23,400.

is characteristic of the cell nucleus might potentially be replicated along nuclear lamina-associated DNA loops. This possibility is consistent with the increased peripheral location of DNA replication in late S phase when there is a correspondingly large increase in heterochromatin replication (32, 39,

61, 63) and the association of remnants of this heterochromatin shell with isolated nuclear lamina (33).

We have also examined DNA polymerase alpha (the presumptive replicative enzyme) and beta (the presumptive repair enzyme) activities in isolated nuclear matrix and lamina.

TABLE IV
Autoradiographic Grain Distribution in Thin Sections from In Vitro Labeled Nuclei and Nuclear Matrices

	Percent of total grain distribution*	
	Peripheral	Internal
Alpha polymerase [†]		
Isolated nuclei	19.2 ± 2.9	80.8 ± 2.9
Nuclear matrix	27.8 ± 6.5	72.2 ± 6.5
Beta polymerase [†]		
Isolated nuclei	78.1 ± 2.4	21.9 ± 2.4
Nuclear matrix	73.1 ± 3.1	26.9 ± 3.1

* Autoradiography and calculations of percent total distribution were according to Materials and Methods. Values represent ± SE.

[†] Alpha and beta polymerase activities were measured as described in Materials and Methods.

The matrix-bound alpha polymerase endogenous and exogenous template-driven activities were depleted in the nuclear lamina when expressed as a percent of total matrix activity or on a specific activity basis (Table III). These results agree with earlier reports that suggested the absence of DNA polymerase activity in isolated nuclear envelopes (23, 38). In contrast, the small amount of nuclear DNA polymerase beta activity associated with the isolated nuclear matrix (~5% of total nuclear activity; see also references 55–57) had a strikingly different distribution. Total beta polymerase endogenous and exogenous template-driven activities were approximately equally divided in the lamina and internal matrix and were considerably enriched in the lamina when expressed on a protein basis (Table III).

These biochemical findings were confirmed by electron microscopic autoradiography of in vitro-labeled nuclei and matrix (Fig. 4). Importantly, the grain distribution from in vitro DNA synthesis under alpha polymerase-optimized conditions was similar to autoradiographs of in vivo-labeled nuclei and matrix in its predominantly interior localization and in the high incidence of grains at the border of condensed and diffuse structures (Fig. 3). Inhibition of alpha polymerase with *N*-ethylmaleimide further demonstrated that most of the peripheral grains in nuclei and matrix were due to DNA polymerase beta activity. The predominant association of DNA polymerase alpha activity with internal matrix components is also consistent with the immunochemical localization studies of Bensch et al. (6), who demonstrated that DNA polymerase alpha is largely found in the diffuse interchromatinic domains of the nucleus interior. It is from these in situ interchromatinic regions that the internal matrix structures are generally believed to be derived (8, 9, 15, 40, 41, 53).

To our knowledge, this is the first report that DNA polymerase beta activity is sequestered at the nuclear periphery. These results are clearly representative of the cellular distribution of this enzyme since ~80% of total cellular beta polymerase activity is recovered in our isolated regenerating liver nuclei (56). While the biological significance of this peripheral localization is beyond the scope of this current study, we wish to briefly point out possible functions as a basis for further experimentation. Keeping with the presumptive role of DNA polymerase beta in DNA repair processes (52), we propose that the peripherally located heterochromatin and associated components such as the nuclear lamina and envelope may represent a major site of DNA repair in

the cell nucleus. Additionally or alternatively, the beta polymerase may play some as yet undefined role in DNA replication within the peripheral heterochromatin shell.

The authors thank A. J. Siegel for his assistance with electron microscopy and Gladys Martin for typing this manuscript.

This work was supported by National Institutes of Health grant GM 23922 to R. Berezney and a Centre National de la Recherche Scientifique, Organization et Expression du Genome grant to E. Puvion. Reprint requests should be addressed to Dr. Berezney at the Department of Biological Sciences, State University of New York, Buffalo, NY 14260.

Received for publication 17 January 1984, and in revised form 10 July 1984.

REFERENCES

- Adolph, K. W. 1980. Organization of chromosomes in HeLa cells: isolation of histone-depleted nuclei and nuclear scaffolds. *J. Cell Sci.* 42:291–304.
- Agutter, P. S., and J. C. W. Richardson. 1980. Nuclear non-chromatin proteinaceous structures: their role in the organization and function of the interphase nucleus. *J. Cell Sci.* 44:395–435.
- Barrack, E. R., and D. S. Coffey. 1980. The specific binding of estrogens and androgens to the nuclear matrix of sex hormone responsive tissues. *J. Biol. Chem.* 255:7265–7275.
- Basler, J. N., D. Hastie, D. Pietras, S. -I. Matsui, A. A. Sandberg, and R. Berezney. 1981. Hybridization of nuclear matrix attached deoxyribonucleic acid fragments. *Biochemistry*. 20:6921–6929.
- Bekers, A. G. M., H. J. Gijzen, R. D. F. M. Taalman, and F. Wanka. 1981. Ultrastructure of the nuclear matrix from *Physarum polycephalum* during the mitotic cycle. *J. Ultrastruct. Res.* 75:352–362.
- Bensch, K. G., S. Tanaka, S. Z. Hu, T. S.-F. Wang, and D. Korn. 1982. Intracellular localization of human DNA polymerase α with monoclonal antibodies. *J. Biol. Chem.* 257:8391–8396.
- Benyajati, C., and A. Worcel. 1976. Isolation, characterization and structure of the folded interphase genome of *Drosophila melanogaster*. *Cell*. 9:393–407.
- Berezney, R. 1979. Dynamic properties of the nuclear matrix. In *The Cell Nucleus*. H. Busch, editor. Academic Press, Inc., New York. 7:413–456.
- Berezney, R. 1984. Organization and functions of the nuclear matrix. In *Chromosomal Nonhistone Proteins*. L. S. Hnilica, editor. CRC Press Inc., Boca Raton. 4:119–180.
- Berezney, R., J. Basler, L. A. Buchholtz, H. C. Smith, and A. J. Siegel. 1982. Nuclear matrix organization and DNA replication. In *The Nuclear Envelope and the Nuclear Matrix*. G. G. Maul, editor. Alan R. Liss, Inc., New York. 183–197.
- Berezney, R., and L. A. Buchholtz. 1981. Isolation and characterization of rat liver nuclear matrices containing high molecular weight deoxyribonucleic acid. *Biochemistry*. 20:4995–5002.
- Berezney, R., and L. A. Buchholtz. 1981. Dynamic association of replicating DNA fragments with the nuclear matrix of regenerating liver. *Exp. Cell Res.* 132:1–13.
- Berezney, R., and D. S. Coffey. 1975. Nuclear protein matrix: association with newly synthesized DNA. *Science (Wash. DC)*. 189:291–293.
- Berezney, R., and D. S. Coffey. 1976. The nuclear protein matrix: isolation, structure and functions. *Adv. Enzyme Regul.* 14:63–100.
- Berezney, R., and D. S. Coffey. 1977. Nuclear matrix. Isolation and characterization of a framework structure from rat liver nuclei. *J. Cell Biol.* 73:616–637.
- Bhorjee, J. S., S. L. Barclay, A. Wedrychowski, and A. M. Smith. 1983. Monoclonal antibodies specific for tight-binding human chromatin antigens reveal structural rearrangements within the nucleus during the cell cycle. *J. Cell Biol.* 978:389–396.
- Bodnar, J. W., C. J. Jones, D. H. Coombs, G. D. Pearson, and D. C. Ward. 1983. Proteins tightly bound to HeLa cell DNA at nuclear matrix attachment sites. *Mol. Cell Biol.* 3:1567–1579.
- Boutelle, M. 1976. The "Ligop" method for routine ultrastructural autoradiography. A combination of single grid coating, gold latensification, and phenidon development. *J. Microsc. Biol. Cell.* 27:121–127.
- Brasch, K. 1982. Fine structure and localization of the nuclear matrix in situ. *Exp. Cell Res.* 140:161–171.
- Comings, D. 1978. Compartmentalization of nuclear and chromatin proteins. In *The Cell Nucleus*. H. Busch, editor. Academic Press, Inc., New York. 4:345–371.
- Comings, D. E., and T. A. Okada. 1976. Nuclear proteins. III. The fibrillar nature of the nuclear matrix. *Exp. Cell Res.* 103:341–360.
- Cook, P. R., and I. A. Brazell. 1975. Supercoils in human DNA. *J. Cell Sci.* 19:261–279.
- Deumling, B., and W. W. Franke. 1972. Nuclear membranes from mammalian liver. V. On the question of DNA polymerase activities associated with the nuclear envelope. *Hoppe-Seyler's Z. Physiol. Chem.* 353:287–297.
- Dijkwel, P. A., L. H. F. Mullenders, and F. Wanka. 1979. Analysis of the attachment of replicating DNA to a nuclear matrix in mammalian interphase nuclei. *Nucleic Acids Res.* 6:219–230.
- Earnshaw, W. C., and U. K. Laemmli. 1983. Architecture of metaphase/chromosomes and chromosome scaffolds. *J. Cell Biol.* 96:84–93.
- Fakan, S. 1978. High resolution autoradiography studies on chromatin functions. In *The Cell Nucleus*. H. Busch, editor. Academic Press, Inc., New York. 5:3–53.
- Fakan, S., and R. Hancock. 1974. Localization of newly-synthesized DNA in a mammalian cell as visualized by high resolution autoradiography. *Exp. Cell Res.* 83:95–102.
- Hancock, R. 1983. Topological organization of interphase DNA: the nuclear matrix and other skeletal structures. *Biol. Cell.* 46:105–121.
- Hancock, R., and M. E. Hughes. 1982. Organization of DNA in the interphase nucleus. *Biol. Cell.* 44:201–212.
- Hartwig, M. 1978. Organization of mammalian chromosomal DNA: supercoiled and folded circular DNA subunits from interphase cell nuclei. *Acta Biol. Med. Germ.* 37:421–432.
- Herlan, G., R. Quevedo, and F. Wunderlich. 1978. Structural transformation of mam-

- malian cells. *Nature (Lond.)* 241:32-36.
32. Huberman, J. A., A. Tsai, and R. A. Deich. 1973. DNA replication sites within nuclei of mammalian cells. *Nature (Lond.)* 241:32-36.
 33. Hubert, J., D. Bover, J. Arnoult, and M. Bouteille. 1981. Isolation and partial characterization of the nuclear shell of HeLa cells. *Exp. Cell Res.* 131:446-452.
 34. Hunt, B. F., and B. Vogelstein. 1981. Association of newly replicated DNA with the nuclear matrix of *Physarum polycephalum*. *Nucleic Acids Res.* 9:349-363.
 35. Igo-Kemenes, T., and H. G. Zachau. 1978. Domains in chromatin structure. *Cold Spring Harbor Symp. Quant. Biol.* 42:109-118.
 36. Jones, C., and R. T. Su. 1982. DNA polymerase alpha from the nuclear matrix of cells infected with simian virus 40. *Nucleic Acids Res.* 10:5517-5532.
 37. Kaufmann, S. H., D. S. Coffey, and J. H. Shaper. 1981. Considerations in the isolation of rat liver nuclear matrix, nuclear envelope and pore complex lamina. *Exp. Cell Res.* 132:105-123.
 38. Kay, R. R., D. Fraser, and I. R. Johnston. 1972. A method for the rapid isolation of nuclear membranes from rat liver. Characterization of the membrane preparation and its associated DNA polymerase. *Eur. J. Biochem.* 30:145-154.
 39. Kay, R. R., M. E. Haines, and I. R. Johnston. 1971. Late replication of the DNA associated with the nuclear membrane. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 16:233-236.
 40. Kizyzowska-Gruca, S., A. Zborek, and S. Gruca. 1983. Distribution of interchromatin granules in nuclear matrices obtained from nuclei exhibiting different degrees of chromatin condensation. *Cell Tissue Res.* 231:427-437.
 41. LaFond, R. E., and C. L. F. Woodcock. 1983. Status of the nuclear matrix in mature and embryonic chick erythrocyte nuclei. *Exp. Cell Res.* 147:31-39.
 42. LaFond, R. E., H. Woodcock, C. L. F. Woodcock, E. R. Kundahl, and J. J. Lucas. 1983. Generation of an internal matrix in mature avian erythrocyte nuclei during reactivation in cytoplasts. *J. Cell Biol.* 96:1815-1819.
 43. Lebkowski, J. S., and U. K. Laemmli. 1982. Evidence for two levels of DNA folding in histone-depleted HeLa interphase nuclei. *J. Mol. Biol.* 156:309-324.
 44. Lebkowski, J. S., and U. K. Laemmli. 1982. Non-histone proteins and long-range organization of HeLa interphase DNA. *J. Mol. Biol.* 156:325-344.
 45. McCready, S. J., J. Godwin, D. W. Mason, I. A. Brazell, and P. R. Cook. 1980. DNA is replicated at the nuclear cage. *J. Cell Sci.* 46:365-386.
 46. Nakane, M., T. Ide, K. Anzai, S. Ohara and T. Andoh. 1978. Supercoiled DNA folded by nonhistone proteins in cultured mouse carcinoma cells. *J. Biochem. Tokyo.* 84:145-157.
 47. Pardoll, D. M., B. Vogelstein, and D. S. Coffey. 1980. A fixed site of DNA replication in eucaryotic cells. *Cell.* 19:527-536.
 48. Paulson, J. R., and U. K. Laemmli. 1977. The structure of histone-depleted metaphase chromosomes. *Cell.* 12:817-828.
 49. Peacock, A. C., and C. W. Dingman. 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gels. *Biochemistry.* 7:668-674.
 50. Pinon, R., and Y. Salts. 1977. Isolation of folded chromosomes from the yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* 74:2850-2854.
 51. Razin, S. V., V. L. Mantieva, and G. P. Georgiev. 1979. The similarity of DNA sequences remaining bound to scaffold upon nuclease treatment of interphase nuclei and metaphase chromosomes. *Nucleic Acids Res.* 7:1713-1735.
 52. Scovassi, A. I., P. Plevani, and U. Bertazzoni. 1980. Eukaryotic DNA polymerases. *Trends Biochem. Sci.* 5:335-337.
 53. Setterfield, G., R. Hall, T. Bladon, J. Little, and J. G. Kaplan. 1983. Changes in structure and composition of lymphocyte nuclei during mitogenic stimulation. *J. Ultrastruct. Res.* 82:264-282.
 54. Shaper, J. H., D. M. Pardoll, S. H. Kaufmann, E. R. Barrack, B. Vogelstein, and D. S. Coffey. 1979. The relationship of the nuclear matrix to cellular structure and function. *Adv. Enzyme Regul.* 17:213-248.
 55. Smith, H. C., and R. Berezney. 1980. DNA polymerase alpha is tightly bound to the nuclear matrix of actively replicating liver. *Biochem. Biophys. Res. Commun.* 97:1541-1547.
 56. Smith, H. C., and R. Berezney. 1982. Nuclear matrix-bound deoxyribonucleic acid synthesis: an in vitro system. *Biochemistry.* 21:6751-6761.
 57. Smith, H. C., and R. Berezney. 1983. Dynamic domains of DNA polymerase alpha in regenerating rat liver. *Biochemistry.* 22:3042-3046.
 58. Stück, R., and H. Schwarz. 1983. Disappearance and reformation of the nuclear lamina structure during specific stages of meiosis in oocytes. *Cell.* 33:949-958.
 59. Vogelstein, D., D. M. Pardoll, and D. S. Coffey. 1980. Supercoiled loops and eucaryotic DNA replication. *Cell.* 22:79-85.
 60. Werner, D., H.-P. Zimmermann, E. Rauterberg, and J. Spalinger. 1981. Antibodies to the most tightly bound proteins in eucaryotic DNA. *Exp. Cell Res.* 133:149-157.
 61. Williams, C. A., and C. H. Ockey. 1970. Distribution of DNA replicator sites in mammalian nuclei after different methods of cell synchronization. *Exp. Cell Res.* 63:365-372.
 62. Wolfe, J. 1980. A possible skeletal substructure of the macronucleus of *Tetrahymena*. *J. Cell Biol.* 84:160-171.
 63. Wunderlich, F., R. Berezney, and H. Kleining. 1976. Nuclear envelope: an interdisciplinary analysis of its morphology, composition and functions. In *Biological Membranes*. D. Chapman and D. F. H. Wallach, editors. Academic Press, Inc., New York. 3:241-333.