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

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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 nulcear 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

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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 $MgCl_2$).

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 $MgCl_2$, 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 $MgCl_2$, 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 ⅓–½ 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.

In Vivo DNA Synthesis: [^{3}H]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 [^{3}H]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 $MgCl_2$ (0.2 mM $MgCl_2$ 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 phenidon 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. 1*A*) 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. 1*B*) 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

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

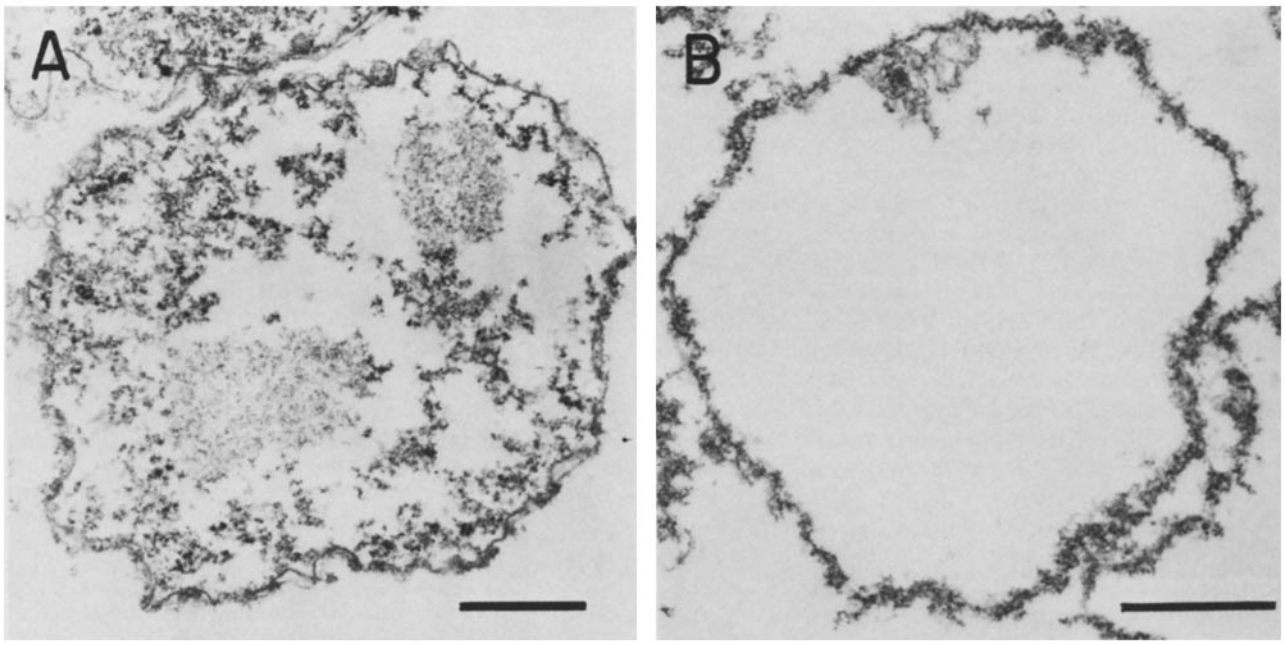

FIGURE 1 Thin-sectioned electron microscopy of the isolated total nuclear matrix (*A*) and nuclear lamina (*B*) fractions. Note the typical tripartite structure of the isolated nuclear matrix with the surrounding nuclear lamina and an internal structure consisting of residual nucleoli and an extensive extranucleolar fibrogranular matrix. This characteristic internal structure is lacking in the isolated nuclear lamina. Bars, 1 μm. (*A*) × 16,800; (*B*) × 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 ~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% ± 5.1 (SD) of the total silver grains were not in a peripheral shell having a thickness of 1/20th the diameter of the matrix structure. For a typical matrix structure with a diameter of 7 μm our definition of the "lamina region" would extend 3,500 Å 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-

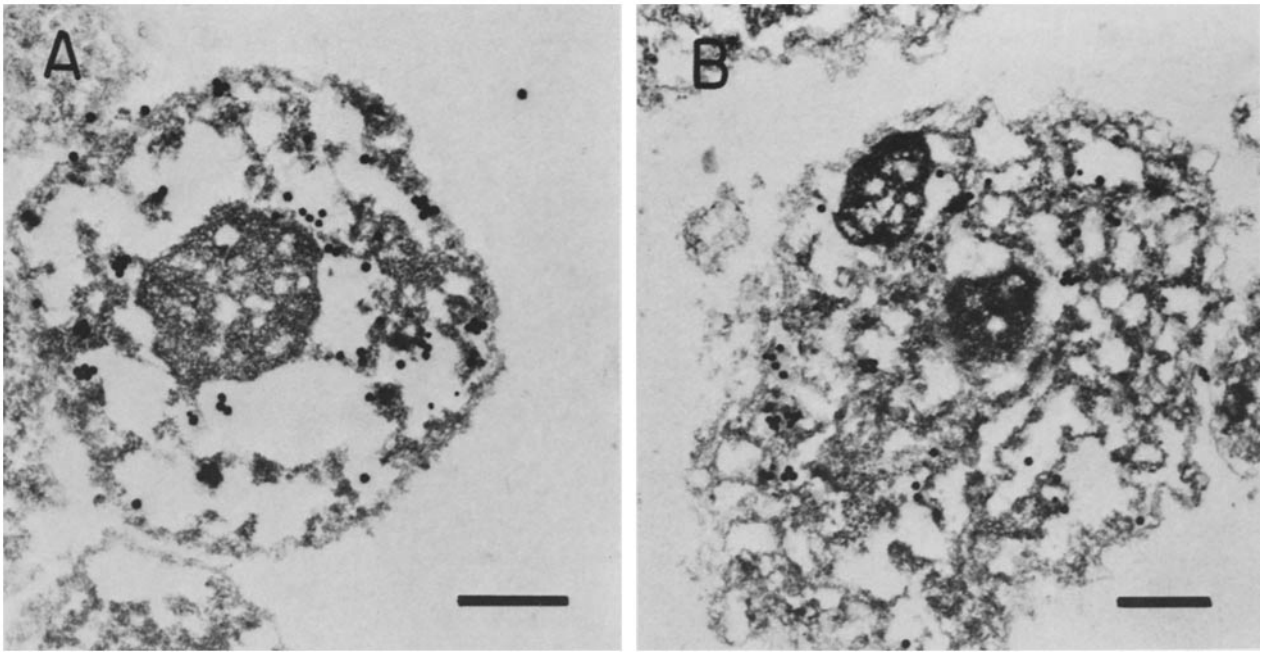

FIGURE 2 Spatial distribution of nuclear matrix DNA loop attachment sites. Regenerating rat liver nuclear DNA was long-term labeled, nuclei were purified, and the matrix was prepared as described in Materials and Methods. Sample preparation for thin-sectioning electron microscopy and autoradiography 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 μm. (*A*) × 14,400; (*B*) × 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 matrix-bound sites for in vitro DNA synthesis, we examined the distribution of alpha and beta polymerase activities in isolated nuclear matrix and lamina. Nuclear lamina was devoid of alpha polymerase endogenous and exogenous template-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-

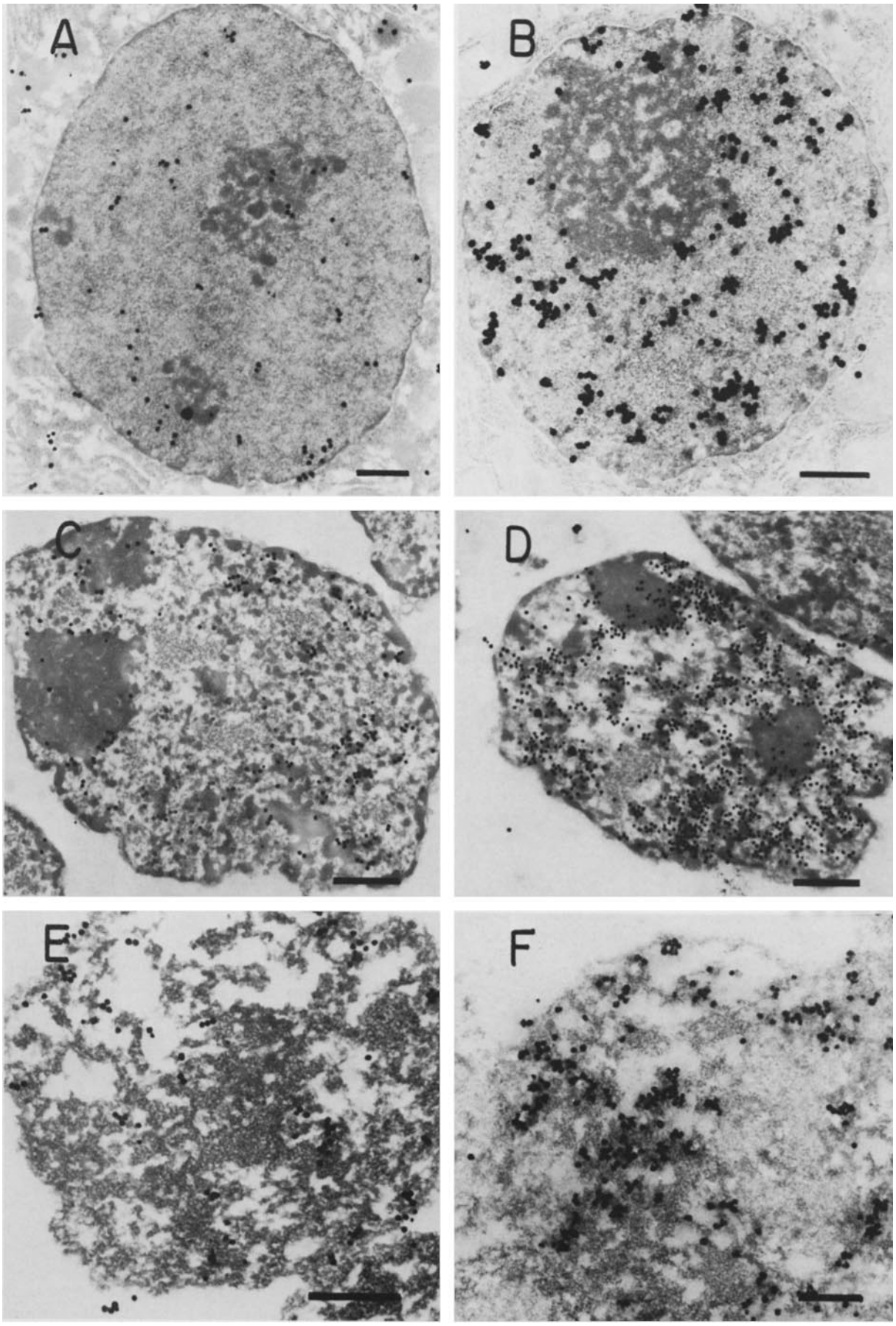
A
B
C
D
E
F

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 activity§	Relative specific activity‖	Percent of total matrix activity§	Relative specific activity‖
Endogenous	Nuclear matrix	100	1.0	100	1.0
	Nuclear lamina	3.0	0.1	52	1.6
Exdogenous	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 demonstrating 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 [^{3}H]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.

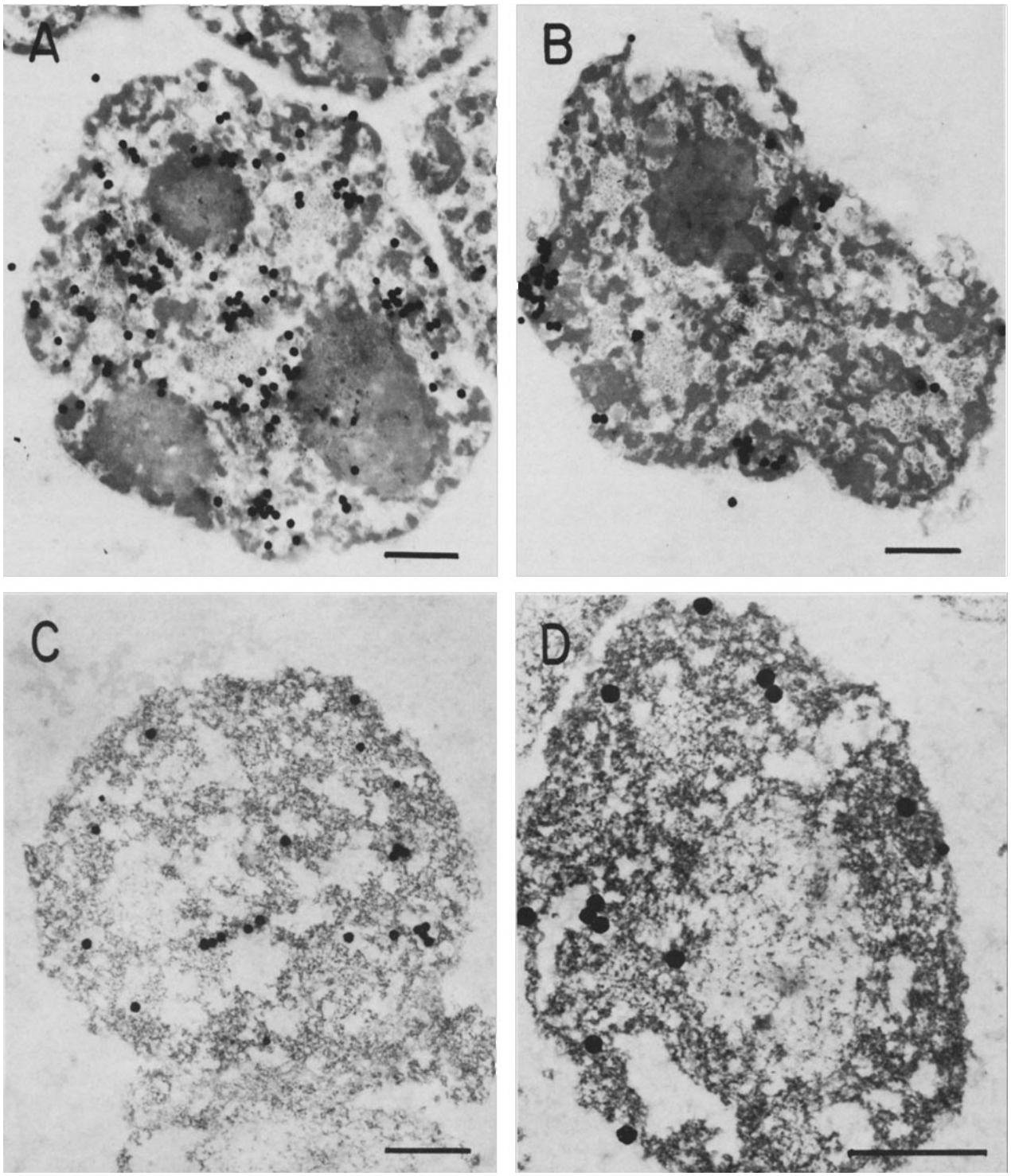

FIGURE 4 Spatial distribution of the sites of in vitro DNA synthesis for DNA polymerases alpha and beta. Nuclei and nuclear matrices were prepared from 22-h regenerating rat liver. DNA polymerase alpha and beta were assayed in vitro and distinguished by *N*-ethylmaleimide sensitive as described in Materials and Methods. (*A* and *C*) Nuclear and nuclear matrix alpha polymerase optimized-driven DNA synthesis, respectively. (*B* and *D*) Nuclear and nuclear marix 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 μm. (*A* and *B*) × 13,500; (C) × 14,400; (*D*) × 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.

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