Frank-Kamenetskii, M. D. (1973) *Biopolymers 10*, 2623–2624.
Gray, D., Taylor, T., & Lang, D. (1978) *Biopolymers 17*, 145–157.
Hanlon, S., Brudno, S., Wu, T. T., & Wolf, B. (1975) *Biochemistry 14*, 1648–1660.
Hanlon, S., Glonek, T., & Chan, A. (1976) *Biochemistry 15*, 3869–3875.
Hanlon, S., Chan, A., & Berman, S. (1978) *Biochim. Biophys. Acta 519*, 526–536.
Maniatis, T., Venable, J., Jr., & Lerman, L. (1974) *J. Mol. Biol. 84*, 37–64.
Manning, G. (1969) *J. Chem. Phys. 51*, 924–933.
Manning, G. (1978) *Q. Rev. Biophys. 11*, 179–246.
McGhee, J. D., & von Hippel, P. H. (1975a) *Biochemistry 14*, 1281–1296.
McGhee, J. D., & von Hippel, P. H. (1975b) *Biochemistry 14*, 1297–1303.
McGhee, J. D., & von Hippel, P. H. (1977a) *Biochemistry 16*, 3267–3275.
McGhee, J. D., & von Hippel, P. H. (1977b) *Biochemistry 16*, 3276–3293.
Pohl, F., & Jovin, T. (1972) *J. Mol. Biol. 67*, 375–396.
Record, M. T., Jr. (1975) *Biopolymers 14*, 2137–2158.
Record, M. T., Jr., Woodbury, C., & Lohman, T. (1976) *Biopolymers 15*, 893–915.
Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.
Schumaker, V. N., & Schachman, H. K. (1957) *Biochim. Biophys. Acta 23*, 628–639.
Tunis-Schneider, M., & Maestre, M. (1970) *J. Mol. Biol. 52*, 521–541.
Wang, A., Quigley, G., Kolpak, F., Crawford, J., van Boom, J., van der Marel, G. & Rich, A. (1979) *Nature (London) 282*, 680–686.
Wolf, B., Berman, S., & Hanlon, S. (1977) *Biochemistry 16*, 3655–3662.
Zimmerman, S., & Pheiffer, B. (1980) *J. Mol. Biol. 142*, 315–330.

Isolation and Characterization of Rat Liver Nuclear Matrices Containing High Molecular Weight Deoxyribonucleic Acid†

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ABSTRACT: Rat liver nuclear matrices isolated by a method which limits DNA degradation contain a major portion of the total nuclear DNA. A majority of the DNA sediments at ≥100 S on alkaline sucrose gradients, which represents an estimated single strand size of ≥500 kilobases. These DNA-rich matrices were virtually identical with previously isolated DNA-depleted matrices in recovery of total nuclear protein and overall polypeptide composition on sodium dodecyl sulfate–acrylamide gels. Thin-sectioning electron microscopy revealed a structure similar to the DNA-depleted matrices with the addition of a prominent meshwork of DNA fibrils extended throughout the matrix interior. In vivo labeling of regenerating livers showed a continuous association of newly replicated DNA with DNA-rich matrices (≥80% of total labeled DNA) which is independent of the pulse period (1 min to 4 h). Moreover, the matrix-associated DNA is highly enriched in replicating intermediates after a 1-min in vivo pulse including a small amount of the primary Okazaki fragments. The matrix-associated replicating intermediates (4–50 S) are effectively chased into DNA of replicon size and larger (100 S) following a 1-h pulse. DNA-rich nuclear matrices may therefore provide a useful in vitro system for studying DNA replication in correlation with the higher order, intranuclear arrangement of eukaryotic DNA.

Interest has focused, recently, on the nuclear matrix as a structural milieu for the organization and integration of nuclear processess (Berezney & Coffey, 1976; Comings, 1978; Wunderlich, 1978; Shaper et al., 1979; Berezney, 1979a, 1981). A number of properties of isolated nuclear matrices support this possibility, including association of newly replicated DNA (Berezney & Coffey, 1975, 1976; Berezney, 1979b; Pardoll et al., 1980), newly transcribed RNA (Faiferman & Pogo, 1975; Miller et al., 1978; Herman et al., 1978; Herlan et al., 1979; Long et al., 1979; van Eekelen & van Venrooij, 1981), steroid receptor binding sites (Barrack et al., 1977, 1979; Barrack & Coffey, 1980; Agutter & Birchall, 1979), viral precursor proteins (Hodge et al., 1977; Chin & Maizel, 1977; Deppert, 1978; Buckler-White et al., 1980), and carcinogen binding sites (Hemminki & Vainio, 1979; Blazsek et al., 1979). With regard to DNA replication, it has been observed that a small proportion of the total nuclear DNA remains tightly bound to isolated nuclear matrices (Berezney & Coffey, 1975; Berezney, 1979a). This tightly bound or matrix-attached DNA represents 1–2% of the total DNA when isolated after controlled endogenous digestion of rat liver nuclei (Berezney & Coffey, 1975, 1976, 1977; Berezney, 1979a). The matrix-attached DNA fragments have an average size of 1–2 kilobases (kb)[1] and are highly enriched in 1-min-pulsed, in vivo

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[1] Abbreviations used: kb, kilobases; $NaDodSO_4$, sodium dodecyl sulfate; PMSF, phenylmethanesulfonyl fluoride; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid.

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replicated DNA (Berezney & Buchholtz, 1981). The rapid disappearance of in vivo replicated DNA from the matrix-attached fragments suggests that the DNA replicational sites are closely associated with the nuclear matrix (Berezney & Buchholtz, 1981). This conclusion is further supported by the remarkable nuclease sensitivity of the matrix-attached replicating DNA (Berezney & Buchholtz, 1981).

The aforementioned studies led us to consider a model for DNA replication in which the continuous DNA molecule is arranged in a series of topologically distinct matrix-attached DNA loops with an average size of ~80 kb (Berezney & Buchholtz, 1981). Replication then proceeds via specific replicational complexes associated with the matrix structure. Other investigators have come to similar conclusions from studies of nuclear matrices derived from either bovine liver cells (Dijkwel et al., 1979) or 3T3 fibroblasts grown in culture (Pardoll et al., 1980). In proposing these models, it is assumed that the total nuclear DNA is natively attached to the matrix structure, presumably at specific matrix-anchoring sites (Razin et al., 1979). However, the small size of the matrix-attached fragments precludes an evaluation of the hypothesized matrix-attached DNA loops, as well as meaningful analysis of potential DNA replicational intermediates. To circumvent these difficulties, we have developed a new method for isolating nuclear matrices containing high molecular weight DNA. It is demonstrated that a major portion of the total nuclear DNA is anchoring to the matrix. Moreover, early replicational intermediates including those of Okazaki fragment size are detected and can be chased into DNA of replicon size and larger.

Materials and Methods

Normal and Regenerating Liver. Livers were obtained from 200–250-g Sprague-Dawley male rats (King Animal Labs). All regenerating liver studies were performed during the maximum period of in vivo DNA synthesis (18–22 h) after partial hepatectomy [see Berezney & Buchholtz (1981)]. Regenerating livers were pulsed in vivo with [*methyl*-^{3}H]-thymidine (50–80 Ci/mmol; New England Nuclear) according to Berezney & Buchholtz (1981).

Isolation of DNA-Depleted Matrix. Nuclei and DNA-depleted nuclear matrices were obtained from normal and regenerating rat liver as detailed elsewhere (Berezney & Buchholtz, 1981) with the following modifications: PMSF (1 mM) (Sigma Chemicals) and 0.1 mM sodium tetrathionate (ICN Pharmaceuticals) were added to all nuclear and matrix isolation solutions (Berezney, 1979b). The purified nuclei were then exogenously digested with DNase I (10 units/mg of DNA, Worthington Biochemicals) for 30 min at 0 °C in 0.25 M sucrose and TM buffer (5 mM $MgCl_2$ and 20 mM Tris, pH 7.4). This was followed by the standard protocol for matrix isolation (Berezney & Buchholtz, 1981). All centrifugations were performed in a Sorvall HS-4 rotor (Dupont-Sorvall, Inc.) at 1000*g* for 15 min. The DNase I digested nuclei were consecutively extracted 1 time with LM buffer (0.2 mM $MgCl_2$ and 10 mM Tris, pH 7.4), 3 times with HS buffer (2 M NaCl, 0.2 mM $MgCl_2$, and 10 mM Tris, pH 7.4), 1 time with 1% Triton X-100 in LM buffer, and 2 times with LM buffer. The DNA-depleted matrices contained <1% of the total nuclear DNA. DNA-free matrices were obtained from DNA-depleted matrices by exhaustive DNase I digestion (200 units of DNase I/mg of matrix protein, 60 min at 30 °C) in LM buffer containing 1 mM PMSF.

Isolation of DNA-Rich Nuclear Matrices. All solutions contained 1 mM PMSF and 0.1 mM sodium tetrathionate (Berezney, 1979b). Nuclei were isolated as previously reported (Berezney & Buchholtz, 1981) with the addition of 2 mM EGTA to the sucrose buffers. Forty volumes of EGTA–HS buffer (2 M NaCl, 2 mM EGTA, and 10 mM Tris, pH 7.4) were added to a 4 mg of DNA/mL nuclear suspension. A 35-mL sample of the suspension was dispersed with 40 strokes of a loose-fitting Potter-Elvehjem homogenizer (Arthur H. Thomas Co.), operating at ~1000 rpm, placed on top of a discontinuous sucrose gradient containing 10 mL each of 1.8 and 2.0 M sucrose–EGTA–HS buffer and centrifuged in the Beckman SW-25.2 rotor for 60 min at 20000 rpm (49200*g*). The matrix structures were removed from the 0.25–1.8 M sucrose interphase and washed and centrifuged 2 times with EGTA–HS buffer at 8000*g* for 20 min and 2 times at 4000*g* for 20 min in the HS-4 rotor. The matrices were then treated with 1% Triton X-100 in LM buffer and washed 2 times in LM buffer (4000*g*, 20 min).

Biochemical Analysis. RNA and DNA were separated according to the procedures of Munro & Fleck (1965). DNA in the 0.8 N perchloric acid hydrolysate was measured directly at 260 nm or by the diphenylamine reaction (Burton, 1968). Radioactivity was determined by scintillation counting of the DNA hydrolysates on a Delta 300 counter (Tracor-Analytic). Protein was determined by the Lowry assay (Lowry et al., 1951), and phospholipid was determined as previously reported (Berezney et al., 1972).

Alkaline Sucrose Gradient Analysis. Nuclei or final nuclear matrices containing 20–200 μg of DNA were resuspended in 0.2–0.5 mL of a dissolving solution containing 0.2 N NaOH and 10 mM EDTA and heated at 50 °C for 30 min. The sample was then carefully layered on top of a 5–20% (w/v) sucrose gradient containing 0.9 M NaCl, 1 mM EDTA, and 0.2 N NaOH and centrifuged in an SW-41 rotor (Beckman Instruments) at 20000 rpm for 16 h at 20 °C. Fractions (0.4 mL) were collected from the top of the gradient with an auto-densi-flow collector (Buchler Instruments). Bovine serum albumin (100 μg) (Sigma Chemicals) was added to each fraction, and the samples were precipitated with 0.3 N perchloric acid at 0 °C. The pellets were then digested with 0.8 N perchloric acid for 20 min at 70 °C, and the hydrolyzed DNA was read at 260 nm and counted by liquid scintillation. No measurable differences in the sedimentation profiles were found between 20 and 200 μg of DNA placed on each gradient. The gradients were calibrated with phage λ DNA obtained from Miles Biochemicals, and molecular weights were approximated according to Studier (1965).

Isolation of High Molecular Weight Liver DNA. Regenerating liver nuclei were prepared with sucrose–EGTA buffers as described above following a 1-h in vivo pulse with 200 μCi of [*methyl*-^{3}H]thymidine (50–80 Ci/mmol; New England Nuclear). DNA was isolated as previously described (Berezney & Buchholtz, 1981). The purified DNA had 260:280 and 260:230 absorbance ratios of 1.9 and 2.7, respectively, and a weighed average sedimentation coefficient of ~58 S (8×10^7 daltons).

$NaDodSO_4$–Acrylamide Gel Electrophoresis. Samples were prepared for $NaDodSo_4$–acrylamide gel electrophoresis as previously reported (Berezney & Coffey, 1977). Gradient slab gels (5–18%), 28 cm long, were run according to the procedures of Laemmli (1970). The gels were stained with Coomassie Blue R-250 and photographed. Individual slots were scanned on a Gilford Model 2520 gel scanner (Gilford Instruments, Inc.) at 550 nm with a fixed slit plate of 0.05 mm and a scanning speed of 1 cm/min. Areas under the polypeptide peaks were quantitated by weighting individual peaks. Standard proteins used for molecular weight calibration in-

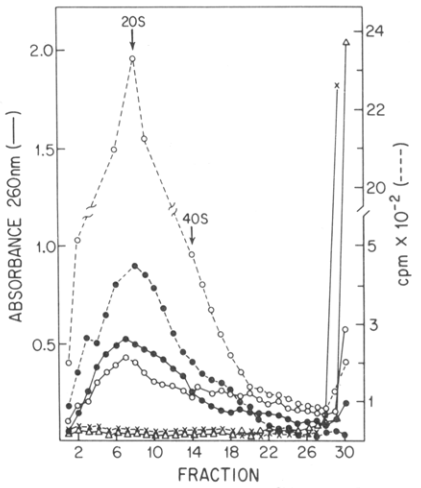

FIGURE 1: Alkaline sucrose gradient profiles of DNA from isolated nuclei and DNA-rich nuclear matrices. Regenerating livers were pulsed in vivo with [*methyl*-^{3}H]thymidine, and nuclei were isolated in the standard sucrose–TM buffers. Nuclei were processed immediately for DNA alkaline gradient analysis as described under Materials and Methods: (●) 1-min-pulsed nuclei (400 μCi of [^{3}H]-thymidine per liver); (○) 60-min-pulsed nuclei (100 μCi of [^{3}H]-thymidine per liver). DNAs from unlabeled nuclei isolated in the presence of 2 mM EGTA (△) and from DNA-rich nuclear matrices (×) were also run. The 5–20% alkaline sucrose gradients were calibrated with λ-DNA (40 S). The direction of sedimentation is from left to right.

cluded the following: thyroglobulin, 167 000; phosphorylase *a*, 94 000; bovine serum albumin, 68 000; pyruvate kinase, 57 000; ovalbumin, 43 000; lactate dehydrogenase, 36 000; and myoglobin, 17 000.

Phase-Contrast and Electron Microscopy. Nuclei and matrix suspensions were directly observed and photographed without fixation under a Zeiss Ultraphot II phase-contrast microscope. The procedures for thin-sectioning electron microscopy have been detailed elsewhere (Berezney & Coffey, 1977). Specimens were observed and photographed with Hitachi Hu-llc or H-500 electron microscope operating at 75 kV.

Results

Isolation and Morphology of Nuclear Matrices Containing High Molecular Weight DNA. Previous methods for nuclear matrix isolation have involved cleavage of the nuclear DNA by either endogenous or exogenously added nucleases [see, e.g., Berezney & Coffey (1975), Berezney (1979a,b), Berezney et al. (1979), Berezney & Buchholtz (1981), Herlan & Wunderlich (1976), Wunderlich & Herlan (1977), Hodge et al. (1977), Miller et al. (1978), and Pardoll et al. (1980)]. If the remaining matrix-attached DNA fragments represent a native association with the matrix structure, then it should be possible to demonstrate DNA–matrix attachments in the absence of significant DNA cleavage. This required a new approach to nuclear matrix isolation. As a first step, the nuclear isolation was modified to prevent rapid degradation of DNA by the active endonucleases present in both normal and regenerating rat liver nuclei (Berezney & Coffey, 1975; Hewish & Burgoyne, 1973; Keichline et al., 1976). The severity of this problem is illustrated in Figure 1, where the size distribution of total nuclear DNA from regenerating liver was measured on denaturing alkaline sucrose gradients immediately after nuclear isolation. The DNA is already highly degraded with a broad peak at 20 S or 8.6 kb. Moreover, the radioactivity and absorbance profiles of 1- and 60-min in vivo pulse-labeled DNA are virtually identical.

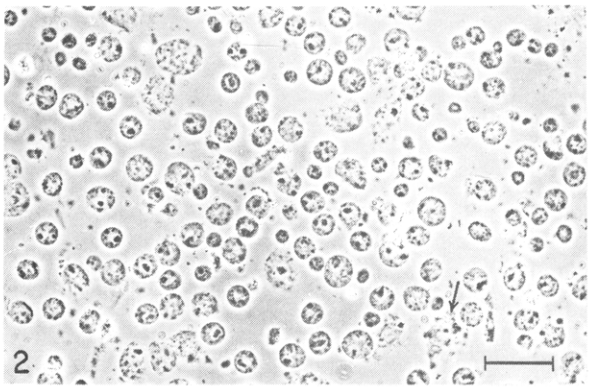

FIGURE 2: Phase-contrast micrograph of DNA-rich nuclear matrices. Rat liver nuclear matrices from regenerating liver were prepared as described under Materials and Methods and examined directly without fixation under the phase-contrast microscope. Arrow denotes a disrupted matrix structure. Bar indicates 40 μm.

We have overcome this degradation by adding 2 mM EGTA to our sucrose–TM isolation buffers. Under these conditions, most of the nuclear DNA now sediments at ≥100 S (Figure 1), which corresponds to an apparent single strand DNA size of ≥500 kb. Moreover, the presence of EGTA had no apparent effect on either the chemical composition or the morphology of the isolated nuclei as demonstrated by both phase-contrast and electron microscopy (data not shown). These results confirm the previous findings of Sanders (1978), who first used EGTA in a sucrose–Mg^{2+} buffer to stop exogenous digestion with micrococcal nuclease while maintaining nuclear integrity.

The protocol for isolating nuclear matrices containing high molecular weight DNA is presented under Materials and Methods. A phase-contrast micrograph of the final nuclear matrices is shown in Figure 2. The matrices appear intact and unaggregated with a well-developed internal nuclear structure including nucleoli and other dense regions interspersed with more diffuse regions. Note that many of the dense regions are in close proximity to the matrix periphery. Highly disrupted matrix structures were easily recognized (see arrow, Figure 2) but represented less than 15% of the total population. Most significantly, the matrix-associated DNA sedimented at ≥100 S on alkaline sucrose gradients similar to the sedimentation of total nuclear DNA from nuclei isolated in the presence of EGTA (Figure 1).

Thin-sectioning electron microscopy of the DNA-rich matrices confirmed the interior localization of at least the bulk of the matrix-attached DNA. A prominent electron-dense staining meshwork of DNA fibrils is distributed throughout the matrix structure (Figure 3). These fibrils were resistant to extensive digestion with RNase (Figure 3) but were completely removed by DNase I. Moreover, DNA-depleted matrices clearly lack this DNA meshwork (Figure 4). There is a remarkable clustering of DNA fibrils along the borders of the residual nucleoli, the periphery of the matrix, and in a number of interior regions (Figure 3). Since a similar pattern is characteristic of condensed chromatin in situ, the original deposition of DNA into tightly packed and diffuse regions is at least partially preserved. The DNA clusters may also correspond to the dense regions observed by phase microscopy of unfixed matrices (Figure 2). In this regard, no structural alterations were detected in the matrices under phase-contrast microscopy following the fixation and dehydration procedures for electron microscopy (results not shown).

Chemical Composition of DNA-Rich Matrices. By composition, the DNA-rich nuclear matrices are 64.2% protein,

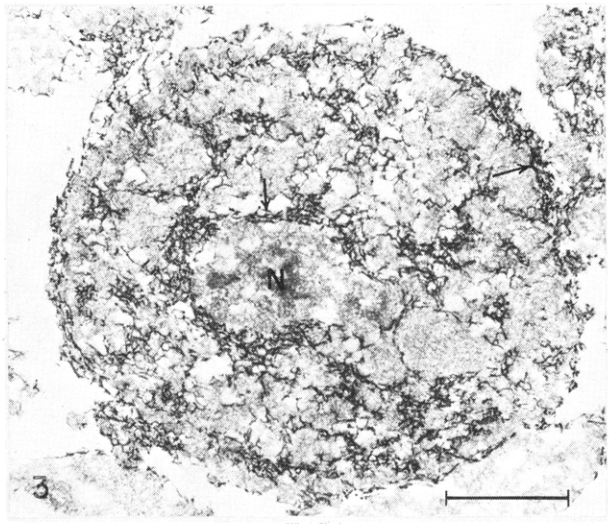

FIGURE 3: Thin-sectioning electron micrograph of DNA-rich matrix. The matrices were digested with RNase A (100 μg/mL, 30 min, 30 °C). Electron-dense staining DNA fibrils are distributed throughout the matrix interior. Note the perinucleolar and peripherally localized DNA clusters (arrows). Micrograph is from 20-h regenerating liver; N, residual nucleolus. Bar indicates 1.0 μm.

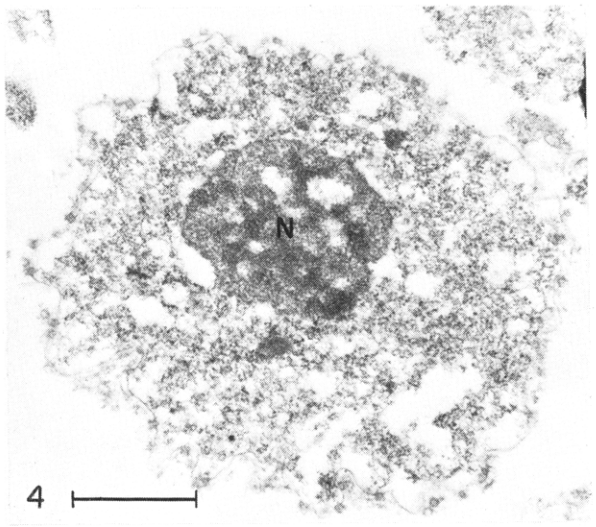

FIGURE 4: Thin-sectioning electron micrograph of DNA-depleted matrix. Note the absence of the electron-dense staining DNA fibrils observed in Figure 3. N, residual nucleolus. Bar indicates 1.0 μm.

27.0% DNA, and 8.8% RNA (Table I). Phospholipid was <1% and was not included in this analysis. We have estimated a recovery of approximately one-third of the total nuclei in the final matrix preparation based on the recovery of total nuclear phospholipid before Triton X-100 extraction (30.2 ± 1.8%) or by direct counting (33.6 ± 9.4%). Recovery of DNA, protein, and RNA per nuclear matrix structure was then evaluated by correcting for nuclei loss based on the more precise phospholipid measurements. An average of 37.4% of total nuclear protein, 64.9% of DNA, and 89.1% of RNA was recovered per matrix. Virtually identical compositions and recovery patterns were found for normal and 20-h-regenerating rat liver matrices. Significantly, a similar recovery of total nuclear protein (35.8%) was measured in DNA-depleted matrices (results not shown). Since the DNA recovery in some DNA-rich matrix preparations was as high as 90%, it is conceivable that less than complete recovery of DNA may be due to variable low levels of endogenous DNA cleavage during the isolation. This was further suggested by sizing total nuclear

Table I: Composition of DNA-Rich Nuclear Matrices[a]

	% of total	
	composition	recovery from nuclei
protein	64.2 ± 7.1	37.4 ± 3.7
DNA	27.0 ± 3.9	64.9 ± 7.9
RNA	8.8 ± 1.6	89.1 ± 6.3

[a] For percent composition, it is assumed that percent protein + percent DNA + percent RNA = 100%. Phospholipid in these matrices represented <1.0% of the total composition. The recovery from nuclei is corrected for nuclear loss during isolation (see text). Values represent the combined average ± one standard deviation for two preparations derived from normal liver and two preparations from regenerating liver. No significant differences were found between normal and regenerating liver DNA-rich matrices.

Table II: In Vitro Binding of High Molecular Weight DNA to DNA-Free Nuclear Matrices[a]

matrix protein (μg)	total bound cpm	μg of bound DNA
10	3	0.010
50	7	0.023
100	28	0.093
1000	7	0.023

[a] DNA was isolated from regenerating livers pulsed in vivo for 1 h. DNA (100 μg) was incubated in HS buffers (1 h, 0 °C) with different amounts of DNA-free nuclear matrices (see Materials and Methods). The nuclear matrices were then pelleted and washed 3 times with HS buffer. Matrix-bound DNA was determined by liquid scintillation after dissolving the final matrix pellets in 0.2 N NaOH. Specific activity of the labeled DNA was 3.0×10^5 cpm/mg of DNA.

DNA after incubation of isolated nuclei for 24 h at 0 °C in 2 mM EGTA–0.25 M sucrose–TM buffer. Under these conditions, there was a decrease in the overall sedimentation of the DNA on alkaline sucrose gradients from ≥100 S to a heterodisperse pattern with an average sedimentation of ~50 S (data not shown).

In Vitro Binding of High Molecular Weight DNA. ^{3}H-Labeled high molecular weight DNA prepared from regenerating liver nuclei was mixed with DNA-depleted matrices which were pretreated with DNase I to remove all traces of matrix-attached DNA fragments (see Materials and Methods). Residual DNase I activity was removed by four washes of the matrices with 1 mM EGTA and 10 mM Tris, pH 7.4. The final DNA-free matrices contained no measurable DNase activity. Different amounts of DNA-free matrices (10–1000 μg of protein) were then incubated with 100 μg of labeled DNA. The data summarized in Table II show no significant 2 M NaCl resistant binding of DNA. A similar lack of salt-resistant DNA binding was observed with matrices before DNase treatment or with DNA-rich matrices.

Polypeptide Profile of the DNA-Rich Matrix. DNA-depleted nuclear matrices have characteristic $NaDodSO_4$–acrylamide gel polypeptide profiles (Berezney & Coffey, 1974; Hodge et al., 1977; Wunderlich & Herlan, 1977; Berezney, 1979a; Berezney et al., 1979). In particular, matrices from rat liver have several prominent polypeptides between 60 000 and 70 000 daltons (Berezney & Coffey, 1974, 1976; Berezney, 1979a,b). It was, therefore, of interest to compare the polypeptide composition of DNA-depleted vs. DNA-rich matrices. As shown in Figure 5, the profiles of the DNA-rich matrices from both normal and regenerating liver (lanes 4 and 5) are remarkably similar to those of the corresponding DNA-depleted matrices (lanes 2 and 3). No qualitative differences were detected in several preparations by direct observation of

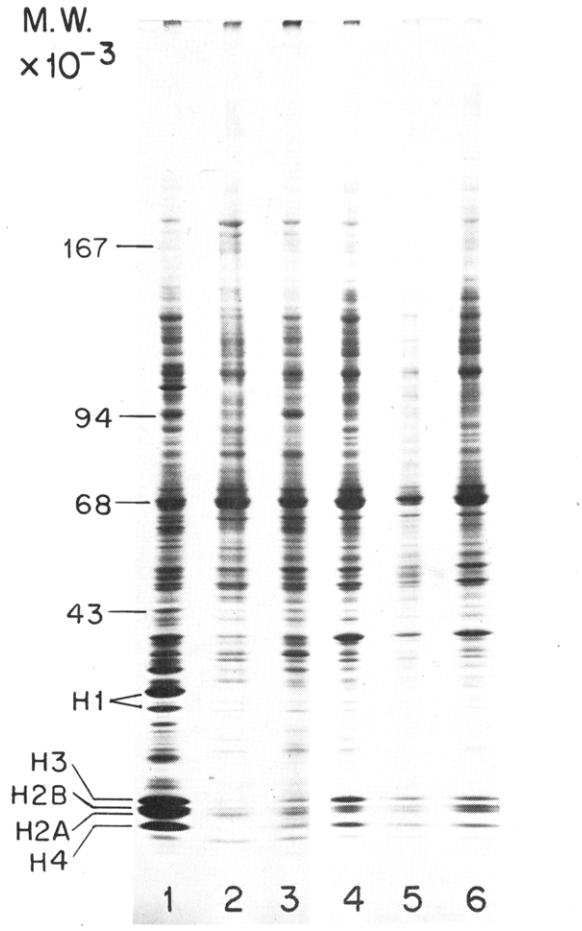

FIGURE 5: $NaDodSO_4$–acrylamide gel electrophoresis of total nuclear and matrix polypeptides. Samples were resolved on 5–18% acrylamide gradient gels described under Materials and Methods. (1) Total nuclear protein; (2) normal liver DNA-depleted matrix; (3) regenerating liver DNA-depleted matrix; (4) normal liver DNA-rich matrix; (5) regenerating liver DNA-rich matrix; (6) DNA–matrix aggregate (see text). No difference in polypeptide profiles was detected if the fractions were exhaustively treated with DNase I and RNase before electrophoresis. Approximately 40–80 μg of protein was run for each sample.

the stained gels or after high-resolution densitometric scanning. Moreover, the quantitative distribution of the protein stain among the polypeptide bands was also very similar. One possible exception is the ~40 000-dalton polypeptide band which appears in a severalfold higher concentration in the DNA-rich compared to the DNA-depleted matrix. An identical polypeptide profile was observed for nuclei extracted with the isolation solutions for DNA-rich matrices but not sheared to disrupt the nuclear gel (lane 6). Since these preparations contain large numbers of disrupted matrices, the much smaller population of disrupted structures observed in the DNA-rich matrices (<15%, Figure 2) is probably not enriched in specific subclasses of matrix proteins (e.g., the 40 000-dalton polypetide). The characteristic 60 000–70 000 matrix polypeptides are also prominent components of total nuclear protein where they constitute ~10% of the total protein stain as compared to 20–25% of the total stained matrix protein (see Figure 5).

Note that small but reproducible amounts of the core histones H2A, H2B, H3, and H4 were detected in both the DNA-depleted and DNA-rich matrices (Figure 5). Densitometric measurements indicated that approximately 2–5% of the total nuclear histone was recovered in the matrices. These tightly bound core histones are clearly resistant to removal by 2 M NaCl as demonstrated by repeated extractions (data not shown).

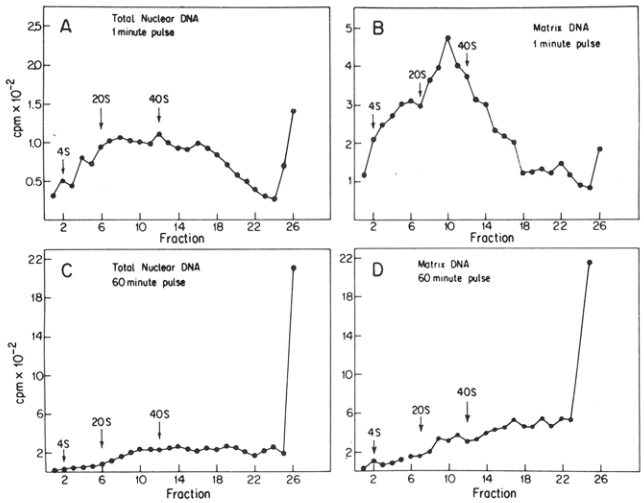

FIGURE 6: Alkaline sucrose gradient profiles of pulse-labeled total nuclear and matrix-attached DNA. Regenerating liver were pulsed in vivo for 1 and 60 min with [^{3}H]thymidine. The DNA was resolved on 5–20% alkaline sucrose gradients as described under Materials and Methods. (A) Total nuclear DNA, 1-min pulse; (B) matrix-attached DNA, 1-min pulse; (C) total nuclear DNA, 60-min pulse; (D) matrix-attached DNA, 60-min pulse. Direction of sedimentation is from left to right.

Table III: Replicating DNA Associated with DNA-Rich Nuclear Matrices[a]

pulse time	% of total labeled DNA recovered in nuclear matrix
1 min	96.1 ± 6.2
1 h	82.4 ± 7.1
4 h	83.0 ± 9.4

[a] Regenerating livers were pulsed in vivo with 50 μCi of [^{3}H]-thymidine for the indicated time periods. The percentage of total labeled nuclear DNA recovered in the DNA-rich matrices was corrected for nuclear breakage during matrix isolation as described in the text. Values represent the average ± one standard deviation for three different experiments.

Replicational Intermediates Associated with the DNA-Rich Matrices. It is possible to determine whether replicational intermediates are present in both the total nuclear and matrix-attached DNA, since the size of the bulk DNA in these fractions (≥500 kb) is considerably larger than that of the reported replicational intermediates (Edenberg & Huberman, 1975; Funderud et al., 1978). Regenerating rat livers were, therefore, pulsed in vivo for 1 and 60 min followed by isolation of nuclei and DNA-rich matrices. The chemical composition (see Table I), ultrastructure, and polypeptide profile (see Figure 5) of the regenerating liver matrices were virtually identical with those of their normal liver counterpart. As shown in Figure 6, a heterodisperse population of replicating intermediates including a small amount of 4–5S Okazaki fragments (Edenberg & Huberman, 1975; Funderud et al., 1978) was detected on the alkaline gradients of 1-min-pulsed nuclear and matrix-attached DNA. Significantly, the percent of labeled replicating intermediates which sedimented at less than 50 S was reduced from approximately 60% to 10% after an hour pulse with a corresponding increase in labeled DNA greater than 50 S. Table III reveals that essentially all the replicating DNA (>95%) is matrix attached after a 1-min pulse. Moveover, most of the replicated DNA (80–85%) remains matrix attached after pulse periods up to 4 h. This is indeed anticipated if both the replicational sites and high

molecular weight nuclear DNA are associated with matrix structure.

Discussion

Isolation of DNA-Rich Nuclear Matrices. Addition of EGTA to the nuclear isolation buffers and appropriate modifications in the matrix isolation enabled us to prevent extensive DNA cleavage and isolate nuclear matrices which contain an average of two-thirds of the total nuclear DNA. Most of this DNA has an apparent single strand size ≥500 kb. It is very likely that less than complete recovery of DNA in the matrices is a result of a small but significant degree of DNA cleavage which still occurs during the isolation.

It is important to demonstrate that the association of high molecular weight DNA with the nuclear matrices is not a preparative artifact. While previous reconstruction experiments demonstrated the absence of significant 2 M NaCl resistant binding of small DNA fragments to isolated matrix (Berezney & Buchholtz, 1981), it is conceivable that the high molecular weight DNA obtained in this study may nonspecifically aggregate with the matrix during isolation. Several criteria, however, argue against this possibility. First, phase-contrast microscopy (Figure 2) reveals relatively intact, individual matrix structures with no indication of aggregated DNA masses bound to the matrix surfaces. It might be further argued, however, that the large size of the DNA precludes its removal from the matrix interior. Thus, the DNA may be physically trapped rather than specifically attached to the matrix interior. While this is conceivable, it is unlikely, since DNA from disrupted matrix structures is still associated with the matrices as DNA–matrix aggregates. Moreover, the isolated matrices do not have intact nuclear envelopes, and the remaining residual nuclear envelope may not, therefore, constitute an effective barrier to macromolecular diffusion. Electron microscopy also demonstrated the presence of most of the nuclear DNA in the matrix interior (Figure 3). Localized clusters of fibrils are observed which resemble the normal distribution of chromatin in intact nuclei including the characteristic perinucleolar and peripherally localized condensed chromatin (Berezney & Coffey, 1977). This striking pattern of DNA fibrils suggests that the DNA maintains stable attachments during matrix isolation. Finally, DNA–matrix in vitro reconstruction experiments revealed no salt resistant binding of high molecular weight DNA to DNA-free, DNA-depleted, or DNA-rich nuclear matrices.

Matrix Proteins and DNA Attachment Sites. A nearly identical $NaDodSO_4$–acrylamide gel polypeptide profile was found for the DNA-rich and DNA-depleted matrices (Figure 5). As previously reported (Berezney & Coffey, 1974), several 60 000–70 000-dalton polypeptides represent the major components. The recovery of total nuclear protein in DNA-rich and DNA-depleted matrices is virtually identical (~35–37%). This suggests that the DNA-rich matrices are not significantly contaminated with chromosomal proteins which might be directly attached to the long DNA fibrils and, thus, not be directly attached to the matrix structure. It is interesting to consider the proportion of total matrix protein that is potentially involved in binding DNA at the putative matrix-attachment sites. We estimate a minimum of 10^8 copies of matrix protein per isolated rat liver nucleus, based on an average protein content per matrix of 10 pg and an average molecular weight of 60 000. The number of DNA binding sites associated with the matrix is estimated at ~125 000, based on an average DNA content of 10 pg per nucleus and an average of 80 kb per matrix-attached DNA loop (Berezney & Buchholtz, 1981). Thus, only 1 out of 1000 or 0.1% of the total matrix proteins may be directly involved in DNA binding, assuming one matrix protein per attachment site. If a complex of approximately ten matrix proteins is involved (M_r ~ 600 000), this still represents only a small proportion of the total matrix protein (~1%).

The identification of a full complement of the nucleosomal core histones (H2A, H2B, H3, and H4) raises the intriguing possibility that the DNA may be anchored to the matrix via a matrix-bound core nucleosomal structure. Approximately 2–5% of the total nuclear histone is recovered in the matrix fraction based on quantitative densitometric measurements. This provides ~10^6–10^7 copies of histones per matrix structure. Since each nucleosome consists of an octamer of the four core histones (Kornberg, 1977), there are clearly enough matrix-bound core histones to account for one or more nuclesomes at each of the ~10^5 putative matrix–DNA attachment sites. While it remains to be determined whether the matrix-bound histones are actually involved in the anchoring of DNA to the matrix structure, it is unlikely that the tight association of histones with the matrix is an "isolation artifact" since both the DNA-rich and DNA-depleted matrices contain similar levels of bound histones. Moreover, these histones are not removed by repeated extractions with 2 M NaCl and/or low ionic strength solutions (10 mM Tris, pH 7.4). In addition, we have not detected 2 M NaCl resistant binding of exogenously added histones to isolated matrices (R. Berezney, unpublished experiments). Finally, Grebanier & Pogo (1979) have demonstrated a specific association of histone H3 with a small proportion of matrix proteins after chemical cross-linking of intact nuclei. This strongly argues for a native interaction between histone H3 and certain matrix proteins. The inability to clearly identify histones in earlier nuclear matrix studies may have been due to several factors, such as high sensitivity of histones to proteolytic degradation and use of a $NaDodSO_4$–acrylamide gel system which does not completely resolve histones (Berezney & Coffey, 1977).

Matrix-Attached DNA and Replication. Previous studies have concluded that DNA replication is continuously associated with the nuclear matrix (Berezney & Coffey, 1975, 1976; Dijkwel et al., 1979; Berezney, 1979b; Pardoll et al., 1980; Berezney & Buchholtz, 1981). Hypothetical models were proposed to describe this association involving matrix-attached loops [Dijkwel et al., 1979; Pardoll et al., 1980; Berezney, 1981; Berezney & Buchholtz, 1981; see also Comings (1978)]. The possible identity of replicating DNA loops with the replicon units observed with DNA fiber autoradiography (Huberman & Riggs, 1968; Edenberg & Huberman, 1975; Hand, 1978) led us to further purpose (Berezney, 1981; Berezney & Buchholtz, 1981) that a matrix-attached organization for eukaryotic DNA might provide the basis for the orderly procession of replication as well as the observed phenomena of replicon clustering (Edenberg & Huberman, 1975; Funderud et al., 1978; Hand, 1975, 1978; Kowalski & Cheevers, 1976; Jasny & Tamm, 1979). In this regard, it is important to note that many previous investigations have also concluded that eukaryotic DNA is arranged in a looped or topologically constrained manner in the interphase nucleus (Cook & Brazell, 1975; Cook et al., 1976; Benyajati & Worcel, 1976; Igo-Kemenes & Zachau, 1978; Nakane et al., 1978; Wanka et al., 1977; Comings, 1978). A similar view has been suggested by Georgiev et al. (1978) for the higher order arrangement of chromatin. Moreover, a well-organized loop structure of DNA anchored to a central scaffold has been directly visualized in mitotic chromosomes following removal of histones (Paulson & Laemmli, 1977).

It is important to emphasize that the findings to date are consistent with but do not directly demonstate that DNA replication occurs along matrix-attached DNA loops. Indeed, the existence of specific matrix-attached DNA loops remains to be clarified. It is our hope, however, that the DNA-rich matrices isolated in this study will be a valuable new approach to these important issues. In this regard, we have recently observed the association of highly folded DNA loop structures with isolated DNA-rich nuclear matrices (R. Berezney and A. J. Siegel, unpublished experiments). Moreover, while this manuscript was in preparation, Volgelstein et al. (1980) reported that intact DNA associated with 3T3 fibroblast nuclear matrix is highly supercoiled.

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References

Agutter, P., & Birchall, K. (1979) *Exp. Cell Res. 124*, 453–460.

Barrack, E. R., & Coffey, D. S. (1980) *J. Biol. Chem. 255*, 7265–7275.

Barrack, E. R., Hawkins, E. F., Allen, S. L., Hicks, L. L., & Coffey, D. S. (1977) *Biochem. Biophys. Res. Commun. 79*, 829–836.

Barrack, E. R., Hawkins, E. F., & Coffey, D. S. (1979) in *Steroid Hormone Receptor Systems* (Leavitt, W. W., & Clark, J. H., Eds.) pp 47–70, Plenum Press, New York.

Benyajati, C., & Worcel, A. (1976) *Cell (Cambridge, Mass.) 9*, 393–407.

Berezney, R. (1979a) *Cell Nucl. 7*, 413–456.

Berezney, R. (1979b) *Exp. Cell Res. 123*, 411–414.

Berezney, R. (1981) in *International Cell Biology 1980–1981* (Schweiger, H. G., Ed.) pp 214–224, Springer-Verlag, Heidelberg.

Berezney, R., & Coffey, D. S. (1974) *Biochem. Biophys. Res. Commun. 60*, 1410–1419.

Berezney, R., & Coffey, D. S. (1975) *Science (Washington, D. C.) 189*, 291–293.

Berezney, R., & Coffey, D. S. (1976) *Adv. Enzyme Regul. 14*, 63–100.

Berezney, R., & Coffey, D. S. (1977) *J. Cell Biol. 73*, 616–637.

Berezney, R., & Buchholtz, L. A. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 2008.

Berezney, R., & Buchholtz, L. A. (1981) *Exp. Cell Res. 132*, 1–13.

Berezney, R., Macaulay, L. K., & Crane, F. L. (1972) *J. Biol. Chem. 247*, 5549–5561.

Berezney, R., Basler, J., Hughes, B. B., & Kaplan, S. C. (1979) *Cancer Res. 39*, 3031–3039.

Berezney, R., & Buchholtz, L. A., & Siegel, A. J. (1980) *Eur. J. Cell Biol. 22*, 115.

Blazsek, I., Vauhkonen, M., & Hemminki, K. (1979) *Res. Commun. Chem. Pathol. Pharmacol. 23*, 611–626.

Buckler-White, A. J., Humphrey, G. W., & Pigiet, V. (1980) *Cell (Cambridge, Mass.) 22*, 37–46.

Burton, K. (1968) *Methods Enzymol. 12B*, 163–166.

Chin, W. W., & Maizel, J. V., Jr. (1977) *Virology 76*, 79–89.

Comings, D. E. (1978) *Cell Nucl. 4*, 345–371.

Cook, P. R., & Brazell, I. A. (1975) *J. Cell Sci. 19*, 261–279.

Cook, P. R., Brazell, I. A., & Jost, E. (1976) *J. Cell Sci. 22*, 303–324.

Deppert, W. (1978) *J. Virol. 26*, 165–178.

Dijkwel, P. A., Mullenders, L. H. F., & Wanka, K. (1979) *Nucleic Acids Res. 6*, 219–230.

Edenberg, H. J., & Huberman, J. A. (1975) *Annu. Rev. Genet. 9*, 245–284.

Faiferman, I., & Pogo, A. O. (1975) *Biochemistry 14*, 3808–3816.

Funderud, S., Andreassen, R., & Haugli, F. (1978) *Cell (Cambridge, Mass.) 15*, 1519–1526.

Georgiev, G. P., Nedospasov, S. A., & Bakayev, V. V. (1978) *Cell Nucl. 6*, 3–34.

Grebanier, A. E., & Pogo, A. O. (1979) *Cell (Cambridge, Mass.) 18*, 1091–1099.

Hand, R. (1975) *J. Cell Biol. 64*, 89–97.

Hand, R. (1978) *Cell (Cambridge, Mass.) 15*, 317–325.

Hemminki, K., & Vainio, H. (1979) *Cancer Lett. (Shannon, Irel.) 6*, 167–173.

Herlan, G., & Wunderlich, F. (1976) *Cytobiologie 13*, 291–296.

Herlan, G., Eckert, W. A., Kaffenberger, W., & Wunderlich, F. (1979) *Biochemistry 18*, 1782–1788.

Herman, R., Weymouth, L., & Penman, S. (1978) *J. Cell Biol. 78*, 663–674.

Hewish, D. R., & Burgoyne, L. A. (1973) *Biochem. Biophys. Res. Commun. 52*, 504–510.

Hodge, L. D., Mancini, P., Davis, F. M., & Heywood, P. (1977) *J. Cell Biol. 72*, 194–208.

Huberman, J. H., & Riggs, A.D. (1968) *J. Mol. Biol. 32*, 327–341.

Igo-Kemenes, T., & Zachau, H. G. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 109–118.

Jasny, B. R., & Tamm, I. (1979) *J. Cell Biol. 81*, 692–697.

Keichline, L. D., Villee, C. A., & Wasserman, P. M. (1976) *Biochim. Biophys. Acta 425*, 84–89.

Kornberg, R. D. (1977) *Annu. Rev. Biochem. 46*, 931–954.

Kowalski, J., & Cheevers, W. P. (1976) *J. Mol. Biol. 104*, 603–615.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Long, B. H., Huang, C. Y., & Pogo, A. O. (1979) *Cell (Cambridge, Mass.) 18*, 1079–1090.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. . (1951) *J. Biol. Chem. 193* 265–275.

Miller, T. E., Huang, C. Y., & Pogo, A. O. (1978) *J. Cell Biol. 76*, 675–691.

Munro, H. N., & Fleck, A. (1965) *Methods Biochem. Anal. 14*, 113–176.

Nakane, M., Ide, T., Anzai, K., Ohara, S., & Andoh, T. (1978) *J. Biochem. (Tokyo) 84*, 145–157.

Pardoll, D. M., Volgelstein, B., & Coffey, D. S. (1980) *Cell (Cambridge, Mass.) 19*, 527–536.

Paulson, J. R., & Laemmli, U. K. (1977) *Cell (Cambridge, Mass.) 12*, 817–828.

Razin, S. V., Mantieva, V. L., & Georgiev, G. P. (1979) *Nucleic Acids Res. 7*, 1713–1735.

Sanders, M. M. (1978) *J. Cell Biol. 79*, 97–109.

Shaper, J. H., Pardoll, D. M., Kaufmann, S. H., Barrack, E. R., Volgelstein, B., Coffey, D. S. (1979) *Adv. Enzyme Regul. 17*, 213–248.

Studier, F. W. (1965) *J. Mol. Biol. 11*, 373–390.

van Eekelen, C. A. G., & van Venrooij, W. J. (1981) *J. Cell Biol. 88*, 554–563.

Vogelstein, B., Pardoll, D. M., & Coffey, D. S. (1980) *Cell (Cambridge, Mass.) 22*, 79–85.
Wanka, F., Mullenders, L. H. F., Bekers, A. G. M., Pennings, L. J., Aelen, J. M. A., & Eygensteyn, J. (1977) *Biochem. Biophys. Res. Commun. 74*, 739–747.
Wunderlich, F. (1978) *Naturwiss. Rundsch. 31*, 282–288.
Wunderlich, F., & Herlan, G. (1977) *J. Cell Biol. 73*, 271–278.

Left Handed Double Helices: Effect of Sequence on the Spatial Configuration of High Anti Nucleic Acids†

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ABSTRACT: The conformational properties of purine–pyrimidine and pyrimidine–purine dinucleoside monophosphates in which the glycosidic torsion is fixed to $\simeq 120°$ by the formation of a covalent link between the base and the sugar ring are explored by ^1H NMR spectroscopy in order to obtain information about the spatial configuration of high anti nucleic acids. The intramolecular stack of the high anti dimers were found to be left handed, in contrast to that (right handed) for natural oligomers, which are low anti. Even though both the high anti pyrimidine–purine and purine–pyrimidine dimers have similar backbone torsion angles, they display widely different relative geometry between the bases; thus in the former there is extensive base–base overlap in the stack, and in the latter there is negligible intramolecular base–base overlap. In addition it was found that purine–pyrimidine systems form miniature double helices in which there is substantial interstrand purine–purine interaction; on the other hand the pyridine–purine high anti dinucleosides have no proclivity to form such base-paired complexes in solution. Mathematical polymerization of the conformation of the high anti purine–pyrimidine dinucleoside monophosphates generates a left handed helix for high anti polynucleotides. This also means that the double helix for high anti nucleic acids containing purine–pyrimidine repeated units may also be left handed, as had been suggested [Sundaralingam, M., & Yathindra, N. (1977) *Int. J. Quantum Chem., Quantum Biol. Symp. 4*, 285]. It is suggested that the plasticity in the structure of genomic DNA is such that, if under certain conditions of interactions the sugar–base torsion of certain domains assume high anti values, that domain will become left handed, and this in turn can be a mechanism for the control of expression by genomic DNA.

It is well recognized that the variety in nucleic acid structures is accomplished primarily by the variations in the phosphodiester torsion angles and to a lesser extent by changes in other torsion angles (Sundaralingam, 1973, 1975; Sundaralingam & Westhof, 1979). Out of a total of nine staggered conformations possible by rotation about the phosphodieser bonds, only one conformation, having gauche–gauche (g^-g^-, $\omega' \simeq 290°$, $\omega \simeq 290°$; see Figure 1 for nomenclature) orientation, leads to a right handed helical organization (Yathindra & Sundaralingam, 1974; Pullman & Saran, 1976; Govil & Saran, 1971; Kim et al., 1973). The preferred ranges of the remaining torsion angles are $\phi \simeq 180°$, $\psi \simeq 60°$; the sugar could be either ^2E or ^3E; and the glycosidic torsion angle (χ) is in the anti range. Furthermore, the right handed helical organization is maintained even if the glycosidic torsion is toggled to syn orientation by introducing bulky substituents on C-8 of purines (Govil et al., 1977). However, recent single-crystal studies of self-complementary d-CGCGCG (Wang et al., 1979) and d-CGCG (Drew et al., 1980), fiber diffraction studies of poly(dG-dC)·poly(dG-dC) (Arnott et al., 1980), and NMR studies of poly(dG-dC)·(poly(dG-dC) (Mitra et al., 1981) have clearly shown that the various torsion angles can assume values so that a left handed double helix can be generated. Extensive theoretical investigations in several laboratories (Yathindra & Sundaralingam, 1976; Sundaralingam & Yathindra, 1977; Fujii & Tomita, 1976; Olson & Dasika, 1976; Olson, 1977) showed that the sense of base stack in high anti systems is left handed, but these calculations reach different conclusions with respect to the helical organization of the backbone.

The unusual CD spectra observed for model dimers (Ikehara et al., 1970; 1974) A^spA^s and A^opA^o (in which the glycosidic torsion is fixed in high anti orientation, $\simeq 120°$, by covalent linkage between the sugar and the base moieties; see Figure 1 and legend for abbreviations) are approximate mirror images of the unmodified ApA in which the glycosidic torsion is in the anti domain. The inverted CD spectra of these high anti systems indicate that the sense of base stack is left handed in contrast to the right handed stacking observed for ApA. This means that the ratio of the base stacking parameters (Olson, 1976) Z/θ (Z = vertical displacement of the stacked bases, θ = the base stacking angle) is positive for ApA and negative for cyclodimers A^spA^s and A^opA^o. The complete chemical shift and (Dhingra et al., 1978) proton dimerization shifts observed for A^spA^s in aqueous solution indicate that A^spA^s adopts a left handed base-stacked geometry. The backbone torsion angles and base-stacking parameters determined by Dhingra et al. (1978) for A^spA^s are in agreement with those predicted by theoretical calculations (Fujii & Tomita, 1976).

Olson & Dasika (1976) have claimed that the left handed stacking does not necessarily mean that the helical sense is left handed. From their geometrical calculations, they found that single- and double-stranded polycyclonucleotides with glycosidic torsion in the high anti ($\chi_{CN} \approx 120°$) prefer a left handed base stacking with a right handed helical organization

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