Alkali-Resistant "RNA" Oligomers: II. Association with Nuclear Matrix DNA in Mouse and Human Cells

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Condensed Title: Alkali-Resistant "RNA" Oligomers

Abstract

In part I in a series of two papers it was reported that alkali-resistant "RNA" (ARR)[1] oligomers had been isolated from mouse L-1210 cells and found to be tightly associated with high molecular weight DNA. In the present paper which is part II of this series it is reported that ARR was characterized to determine whether it was distributed uniformly through out the nuclear DNA or if it had a predilection for the nuclear matrix. Results suggested that the ARR/DNA ratio was as much as 17.5 times higher in matrix DNA than in non-matrix DNA. Similar results were found with human lymphocytes. It appeared that as much as 21% of the ARR was sequestered within as little as 1.5% of the total DNA. These results support earlier suggestions that ARR may be involved with the establishment of DNA domain attachment sites within the nuclear matrix. Non-matrix ARR appeared to be at least twice as methylated as matrix ARR. It is suggested here that non-matrix ARR may represent an inactivated form of ARR which has been released from the vicinity of the nuclear matrix and into the non-matrix DNA as a result of DNA loop fusions during cellular differentiation.

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[1]The abbreviations used are: DAR, DNA-associated "RNA", ARR alkali-resistant "RNA".

INTRODUCTION

In both prokaryotes and eukaryotes the DNA is held together in the form of independently supercoiled DNA domains. In prokaryotes RNA appears to be involved in this process (Hecht et al ., 1977; Kavenoff and Ryder, 1976; Pettijohn and Hecht, 1973: Stonington and Pettijohn, 1971, Worcel and Burgi, 1972; Worcel et al, 1973). The role of RNA in the establishment of independently supercoiled DNA domains in eukaryotes is less clear (Adolph et al, 1977; Adolph et al, 1977; Cook, 1984; Cook and Brazell, 1976; Cook and Brazell, 1978). Current evidence suggests that eukaryotic DNA is punctuated at replicon-sized intervals by alkali-sensitive linkers (Filippidis and Meneghini, 1977), regions recalcitrant to DNA cloning (Marx, 1985), and A+T-rich regions (Moreau et al, 1981; Moreau et al, 1982). The most likely area for such periodic perturbations to occur within the DNA are in the nuclear matrix at the level of DNA domain attachment sites. It was speculated that all three of these observations were the result of the same phenomenon, i.e., the presence of RNA linkers within the DNA. Therefore, our research has focused on the isolation and characterization of RNA which may be associated with the DNA in this region of the genome. In a recent report we described the isolation of DNA associated "RNA" (DAR) oligomers containing an ARR subcomponent from mouse L-1210 cells (Abernathy and Stephens, 1988). These RNA's are very tightly associated with high molecular weight nuclear DNA. It was suggested that they may be associated with DNA domain attachment sites within the confines of the nuclear matrix. If so, then, ARR should have a predilection for the nuclear matrix DNA. In this report we describe various experiments which support this hypothesis. It was suggested that the presence of ARR in the non-matrix DNA was due to inefficient separation of matrix and non-matrix components combined with the presence of an "inactive" ARR form which may be methylated in the 2' position of the RNA ribose. Double label experiments using L-[methyl-^{3}H]methionine as the methyl donor and [2-^{14}C]uridine as the RNA label supported this hypothesis.

EXPERIMENTAL PROCEDURES

Cell Culture, Harvesting, and Nuclei Purification

The mouse L-1210 cells used in these experiments were grown as a suspension culture and harvested in a manner similar to those discussed in a previous report (Abernathy and Stephens, 1988).

Human lymphocytes were isolated from 8 mls of freshly drawn blood. The blood was diluted in a 50 ml centrifuge tube in 25 mls of RPMI 1640 media (GIBCO) containing 0.5% fetal bovine serum (K.C. Biologicals). This was underlayered with 15 mls of Ficoll-Paque (Pharmacia) using a spinal tap needle. The samples were centrifuged at room temperature for 30 minutes at 2000 RPM (room temperature) in a Sorvall desktop centrifuge. The lymphocyte layer could be seen as a white turbid interface between the Ficoll-Paque and aqueous layers. This layer was gently collected with a 1 ml pipette and centrifuged for 5 minutes at 1000 RPM. The cell pellet was resuspended in 10 mls of media and centrifuged as before. The resulting pellet was resuspended and the cells were diluted in RPMI 1640 containing 10% fetal bovine serum, 50 units/ml of penicillin 50 ⫤ g/ml of streptomycin (Sigma), and 3% phytohemaglutinin-M (GIBCO) (to stimulate cell division). They were incubated for 24 hours at 37 ^{0}C, 5% CO_2, and maximum humidity.

At the end of 24 hours of incubation both mouse and human cells were double labeled with 1 ⫤ Ci/ml of [methyl-^{3}H]thymidine (6.7 ci/mmol, New England Nuclear) and 0.1 ⫤ Ci/ml of [^{14}C]uridine (55.9 mCi/mM, New England Nuclear) in order to compare the ratio of ARR to total DNA isolated in the matrix and non-matrix fractions. In a separate experiment one set of mouse cells which had been grown in 50 ⫤ g/ml each of adenosine and guanosine were double labeled with 1 ⫤ Ci/ml of [^{3}H]methionine (200 mCi/mmol, Dupont) together with 0.1 Ci/ml of [^{14}C]uridine in order to study the ratio of alkali-resistant, methylated macromolecules with alkali-resistant RNA in matrix and non-matrix DNA. All nuclei were purified as described earlier (Abernathy and Stephens, 1988).

Ethanol Precipitation and Alkali Hydrolysis

Ethanol precipitation and alkali hydrolysis procedures were reported earlier (Abernathy and Stephens, 1988).

Matrix and Non-Matrix DNA Isolation

The production of matrix and non-matrix DNA by DNase I digestion was by a method modified from previously described procedures (Basler et al, 1981). Freshly purified nuclear pellets in polyallomer tubes were digested in 40 units of DNase I/mg of DNA sample in 10mM Tris, 5mM $MgCl_2$, (pH 7.4) for 5 minutes at 30 ^{0}C at a concentration of 200,000 nuclei/5 ≠l. It has been reported that these incubation parameters yield matrix DNA fragments with an average size range of 250-300 base pairs after phenol extraction (Basler et al, 1981). The reaction was terminated by the addition of one volume of cold 10mM Tris, 20mM EDTA, (pH 8.0). After mixing, 2 volumes of ice cold 10mM Tris, 4M NaCl, 10mM EDTA, (pH 8.0) was added, mixed, the contents transferred to a 1.5 ml microfuge tube, and allowed to sit on ice for 5 minutes to extract the non-matrix DNA from the matrix DNA. The microfuge tube was placed inside a 50 cc centrifuge carrier tube and spun down at 1000 X g for 10 minutes at 4 ^{0}C. The supernatant was decanted into a second microfuge tube and stored on ice. One volume of 10mM Tris, 2M NaCl, 10mM EDTA, (pH 8.0) was added to the pellet which was triturated to mix it with the buffer, incubated on ice for 5 minutes, and spun down as previously described. The first and second supernatants were pooled and the pellet was saved as the matrix DNA fraction. In all experiments except for the one described in Table I (Results) the supernatants were warmed to 37 ^{0}C and underlayered with 100 ≠l of 0.8% Sea Prep low melting temperature agarose (FMC Corp.) in 10mM Tris, 2M NaCl, 10mM EDTA, 20% sucrose, (pH 8.0). The tube was spun down at room temperature for 15 minutes at 1000 X g in a Sorvall desk top centrifuge to pull any remaining matrix DNA down into the Sea Prep layer and the tube was removed from the centrifuge and chilled on ice 15 minutes to gel the agarose. After gelling, the supernatant was decanted and the Sea Prep gel was rinsed and counted.

Matrix and Non-Matrix Nucleic Acid Purification Procedures

The matrix and non-matrix samples were adjusted to 1% SDS and either dialyzed overnight or diluted in TE buffer to yield a final salt concentration of 0.1M NaCl. At this point the samples were made up to 10% in formamide and subjected to batch hydroxylapatite chromatography (Kanter and Schwartz, 1979). Hydroxylapatite was boiled briefly in 0.01M potassium phosphate buffer, pH 7.0 in 15 cc centrifuge tubes, then centrifuged 15 seconds at 600 X g (later changed to 1 minute at 1000 X g to insure that the hydroxylapatite fines were completely removed). The supernatant was discarded and the tubes were transferred to a 60 ^{0}C water bath. Five mls of sample were added to each tube of hydroxylapatite, vortexed briefly, and incubated at 60 ^{0}C for 15 minutes. Every five minutes the tubes were removed, vortexed briefly, and returned to the water bath. At the end of this time period the tubes were centrifuged as before and the supernatants removed. At this point, 5 mls of 0.01M potassium phosphate buffer containing 20% formamide was added to each tube, the tubes were vortexed, incubated for 10 minutes, and centrifuged. The supernatants were removed and the process was repeated. The process was repeated twice more using 0.5M potassium phosphate buffer with 20% formamide to remove all single and double stranded nucleic acids which at this point should be relatively free of all protein and other non-nucleic acid components. In the finalized protocol the two 0.5M potassium phosphate supernatants were pooled and dialyzed overnight against 50 volumes of distilled water. They were dialyzed at 37 ^{0}C to avoid precipitation. The dialysis was repeated twice at room temperature using 10mM Tris, 0.1M NaCl, (pH 7.4). The dialysates were removed, volumes measured, and 0.1 volumes of 10mM Tris, 0.1M NaCl, 0.1M $MgCl_2$, (pH 7.4) were added to bring the final magnesium concentration to 10 millimoles in preparation for ethanol precipitation.

Matrix and Non-Matrix Exhaustive Digestion by DNase I

The dried [^{3}H]thymidine and [^{14}C]uridine labeled matrix and non-matrix ethanol precipitates were dissolved in 0.9 volumes of 10mM Tris, 0.1M NaCl, (pH 7.4) by vortexing them and incubating them at 37 ^{0}C for 5 minutes followed by a second vortexing. Next, 0.1 volumes of TNM buffer (10mM Tris, 0.1M NaCl, 0.1M $MgCl_2$, pH 7.4) was added to bring the magnesium concentration up to 10 millimoles. The samples were divided into 0.5 ml aliquots, one of which was counted to determine total CPM. The other aliquots were placed into separate 4 ml polyallomer tubes along with 53 U/ml DNase I, incubated at 37 ^{0}C for about 24 hours, and then ethanol precipitated. Except for one set of samples described in Table I the precipitated pellets were dried, resolubilized, and exposed to a second treatment of DNase I. They were then ethanol precipitated in preparation for alkali hydrolysis .

RESULTS

Association of the ARR with the Nuclear Matrix

Table I contains [^{3}H]thymidine and [^{14}C]uridine counts for mouse nuclear matrix and non-matrix samples. It includes data on pre-DNase samples (those not yet exhaustively incubated in DNase I) and samples exhaustively incubated in DNase I once or twice. Those samples exposed to DNase I include those heated to 100 ^{0}C for 20 minutes in 0.1 M KCl (pre-ARR samples), and those heated to 100 ^{0}C for 20 minutes in 0.1M KOH (ARR samples). The samples exposed to DNase I twice include only ARR samples. The pre-DNase matrix and non-matrix samples taken together are considered here as total nuclear DNA. The pre-ARR samples include thermostabile alkali-sensitive RNA and ARR. Table II includes results similar to those in Table I except that additional purification of matrix from non-matrix fractions was achieved using Sea Prep underlayering and all pre-ARR and ARR samples were digested twice in DNase I. Table III contains data on human lymphocytes and is similar to Table II except for the absence of pre-ARR data. Low cell yields necessitated the exclusion of this data as well as a reduction in the total number of aliquots counted for other data.

Data from Tables I, II, and III were used in the computations shown in Table IV. These computations include the percentage of total nuclear DNA which is either matrix or non-matrix DNA, (data column 1), the percentage of total nuclear ARR which is either matrix or non-matrix ARR, (data column 2), and the percentage of pre-ARR (for a given sample) which is ARR, (data column 3). In data column 4 the ARR counts for a given sample have been divided by the pre-DNase thymidine counts for that same sample. When the matrix ARR/DNA ratio is divided by the non-matrix ARR/DNA ratios a " predilection index" is generated as shown in data column 5. A predilection index greater than 1 indicates that the ARR has a predilection for the nuclear matrix, i.e., it is more concentrated per unit of DNA in the matrix DNA than in the non-matrix DNA.

The results derived from Table I indicate that 4.1% of the total nuclear DNA was isolated in the matrix fraction in the absence of Sea Prep underlayering. The percentage of ARR which was associated with the matrix fraction increased from 8.1% after one DNase I digestion to 13.5% when the samples were digested in DNase I twice. The percentage of pre-ARR that was ARR after one DNase I digestion was 17.5% and 14.0% for matrix and non-matrix fractions respectively. Similar information for samples incubated in DNase I twice was not obtained. The predilection index increased from 2.1 for samples incubated once in DNase I to 3.7 for samples incubated twice in DNase I. In both cases the indices indicate that there is a predilection of the ARR for the nuclear matrix DNA, but the index is increased after a subsequent DNase I incubation. This shift in the index is the result of a loss in non-matrix ARR during the second DNase I incubation. The ARR concentration in the matrix fraction was unaffected by this additional incubation. One explanation for this loss is that the non-matrix DNA was incompletely digested after only one DNase I digestion and served as a carrier for non-matrix ARR label. It is for

this reason that a second DNase I digestion was carried out in this particular experiment and why all subsequent experiments utilized two DNase I digestions.

The results derived from Table II indicate that only 1.5% of the total DNA was associated with the nuclear matrix in the presence of Sea Prep underlayering. Since Sea Prep underlayering should increase rather than decrease this number the reduction from 4.1% in Table I to 1.5% in Table II merely indicates that the DNase I digestion was more efficient in the latter experiment, resulting in a decrease in matrix DNA content. In Table II the percentage of ARR associated with the nuclear matrix after two DNase I digestions was 21.3%. In Table I in the absence of Sea Prep underlayering this percentage was only 13.5% in samples exposed to DNase I twice. How much of this increase in ARR content within the matrix fraction is the result of Sea Prep underlayering and/or experimental error is uncertain. The percentage of pre-ARR in Table II which was ARR was 40.8% and 84.2% for matrix and non-matrix fractions respectively. This is a dramatic upward shift from the numbers tabulated in Table I for samples exposed to DNase I one time. One explanation for this shift could be that more of the alkali-sensitive RNA was destroyed during the longer incubation period in DNase I thus increasing the relative amount of ARR within the pre-ARR sample. In samples exposed to DNase I twice there was a dramatic upward shift in the predilection index from 3.7 in Table I to 17.5 in Table II. This shift could be attributed to the more extensive digestion of the matrix DNA by DNase I (from 4.1% in Table I down to 1.5% in Table II) and/or to the use of the Sea Prep underlayering technique in the latter experiment which would remove more of the matrix DNA from the non-matrix DNA which would then be added back to the matrix fraction. In any case, the inverse relationship between matrix DNA content and the predilection index strongly indicates that the matrix ARR is located very near or at the nuclear DNA attachment sites.

The results from Table III indicate that only 1.2% of the total DNA was associated with human lymphocyte nuclear matrix. Again, this number only reflects the relative efficiency of the DNase I digestion during that particular experiment. The percentage of total ARR found within the matrix was 18.3% which is similar to the figure derived from Table II for mouse nuclear matrix ARR (21.3%). The percentage of pre-ARR which is ARR was not determined due to a paucity of sample material. The predilection index (18.6) was found to be remarkably similar to the one obtained for mouse cells (17.5) isolated under similar conditions, i.e., Sea Prep underlayering coupled with two DNase I digestions. Such a finding not only reinforces the idea that matrix ARR may be near or at the nuclear matrix DNA attachment sites but also that nuclear matrix ARR exists at these locations in at least two kinds of mammalian species. These results suggest that ARR sites within the nuclear matrix are strongly conserved. Therefore, ARR must be performing some very basic and important function(s) associated with the nuclear matrix.

Chemical Differences Between Matrix and Non-matrix ARR'

The purpose of this experiment was to test the hypothesis that methylation of the 2' hydroxyl position of ribose moieties may confer alkali resistance to the non-matrix ARR whereas both both methyl and phosphate groups may be conferring a similar resistance to the matrix ARR. It was suggested that the methylation of ARR may serve as a means for inactivating the 2' hydroxyl ribose sites so they would be incapable of forming diphosphate bridges in response to an internal Okazaki RNA primer signal sequence during the initiation of DNA synthesis (See Discussion). All of the non-matrix ARR would be methylated whereas only a portion of the matrix ARR would be methylated; the rest would be phosphorylated. Therefore, the ratio of methylated alkali-resistant oligomers to ARR should be higher in the non-matrix than in the matrix fraction.

A presumptive test was performed to test this hypothesis by double labeling mouse cells with [^{3}H]methionine and [^{14}C]uridine in the presence of 50 ug/ml each of adenosine and guanosine to prevent the non-specific uptake of methyl label into purine biosynthetic pathways. Table V contains [^{3}H]methionine and [^{14}C]uridine counts for nuclear matrix and non-matrix samples purified with Sea Prep underlayering. It includes data on pre-DNase samples (those not yet exhaustively incubated in DNase I) and samples exhaustively incubated in DNase I twice. Those samples exposed to DNase I include those heated to 100 ^{0}C for 20 minutes in 0.1M KCl

(pre-ARR samples), and those heated to 100 ^{0}C for 20 minutes in 0.1M KOH (ARR samples). The pre-ARR samples include thermostabile alkali-sensitive RNA and ARR. Data from Table V were used in the computations shown in Table VI. These computations include the percentage of total nuclear ARR which is either matrix or non-matrix ARR, (data column 1), and the percentage of pre-ARR (for a given sample) which is ARR, (data column 2). In data column 3 the alkali-resistant tritiated methylated counts (ARCH3) for a given sample have been divided by the ARR counts for that same sample. When the non-matrix ARCH3/ARR ratio is divided by the matrix ARCH3/ARR ratio a "methylation index" is generated as shown in data column 4. A methylation index greater than 1 indicates that the non-matrix ARR is more "methylated" than the matrix ARR.

The percentage of ARR associated with the matrix was 13.8% and the percentage of pre-ARR that was ARR was 13.4% and 27.8% for the matrix and non-matrix samples respectively. These numbers are considerably lower than their thymidine/uridine labeled counterparts described for Table II in Table IV, i.e., 21.3%, 40.8%, and 84.2%. Interestingly, the percentages of non-matrix ARR's for both groups is twice that for their matrix counterparts. Both sets of cells were incubated and processed at the same time and under similar conditions except for the addition of adenosine and guanosine to the methionine/uridine group. This suggests that the exogenous adenosine and guanosine may have influenced these percentages. The methylation index was found to be 2.19 which indicates that the non-matrix ARR may be more methylated than the matrix ARR. This supports the hypothesis that chemical differences exist between matrix and non-matrix ARR. The ability of adenosine and guanosine alone to completely inhibit non-specific methylation of RNA nucleotides has been met with variable results (Weinberg and Penman, 1968; Maden and Salim, 1974). It is possible that some non-specific methylation occurred in the ARR's thereby quenching the signal provided by the specific methylation which could be occurring in the 2' hydroxyl ribose position. If such is the case, then, the methylation index may be considerably larger than 2.18.

DISCUSSION

The relationship between the pre-ARR isolated from matrix and non-matrix DNA and the DAR isolated from gel insert studies (Abernathy and Stephens, 1988) is not yet clear. In both cases there exists a combination of what appears to be both alkali-sensitive and alkali-resistant RNA's. However, the combination of the two has varied widely from experiment to experiment. The DAR contains a percentage of ARR which ranges from 40% to 82% (Abernathy and Stephens, 1988). Matrix and non-matrix pre-ARR's contain percentages of ARR's ranging from 13% to 41% and from 14% to 84% respectively. The percentage of whole cell RNA which has been isolated as DAR by electroelution is less than 1% (Abernathy and Stephens, 1988) whereas matrix and non-matrix pre-ARR's can be isolated in quantities which are roughly 10 times this amount. This 10 fold difference in DAR and pre-ARR quantity may be attributable to radical differences in how DAR and pre-ARR are isolated. It seems reasonable to conclude that at least a portion of the matrix and non-matrix ARR's are equivalent to ARR isolated during gel electroelution studies and, therefore, are forms of DAR's. If these relationships can be established, then, the possibility that matrix ARR is somehow involved with permanent DNA attachment sites will be strengthened.

In the previous paper a model was proposed involving DAR's which suggested that they may be involved in the establishment of DNA domain attachment sites at the origins of replication within the nuclear matrix. Origins of replication in Drosophila appear to be clustered together in chromomere sized (30 kb) arrays that initiate DNA synthesis at approximately the same time (Blumenthal et al, 1973). Similar clustering has been observed in Physarum (Funderud et al, 1978). These observations suggest that the cluster is initiated into DNA replication by a master "switch". It is postulated here that each replicon in the cluster is activated into replication by a master origin of replication. The DNA domain attachment sites may represent the connection of the replicon cluster to the nuclear matrix by the master replicon. The DAR regions were postulated to be within each origin of replication. A signal sequence within each DAR could be responsible for achieving three things following the initiation of DNA replication: 1) preventing the loss of the Okazaki RNA primer region which constituted the DAR, 2) allowing the tetrameric base pairing of the newly replicated replicons within the DAR region, and 3)

establishing the diphosphate ARR bridges which may be flanking alkali-labile tetrameric regions. Therefore, DAR would be responsible for establishing both DNA domain attachment sites to the nuclear matrix and the structure of the replicon cluster itself.

During the course of cellular differentiation in lymphocytes there may be a decrease in the total number of DNA domains. Such a decrease could be the result of two different phenomena: 1) The DNA domains may be fusing together to make larger domains. 2) Some of the DNA domains may be removed entirely from the cellular genome and lost as intact circles of DNA. The former case is suggested by the fact that the DNA domains in leukemic lymphoblasts are about one-half the size of their counterparts in normal lymphocytes (Hartwig, 1982) and during the development of frog embryos the average nucleoid loop size apparently increases in later stages of development (Flickinger et al, 1986). The latter case is suggested by the releasing of large numbers of small circular DNA's which are heterogenous in size during the course of development of embryonic chicken bursa lymphocytes (Delap and Rush, 1978) and during the aging of fibroblasts in culture (Icard-Liepkalns et al, 1986). In the latter case, new gene sequences were found associated with the circular DNA's of older fibroblasts that were not present in the circles of the younger fibroblasts. It is suggested here that such loop fusions and deletions are the result of changes in the DAR attachment sites which establish replicon cluster formation. Loop fusions could be generated by hydrolysing the central tetrameric regions binding two origins together (Figure 1). This would inactivate the origins of replication for both replicons. Therefore, as loop fusions progressed during the course of cellular differentation the number of active replicons would diminish correspondently. Evidence that less differentiated cells contain more replicons than more highly differentiated cells has been shown in Drosophilia studies (Blumenthal et al, 1973). The flanking diphosphate ARR bridges could be inactivated during the next round of replication by substituting methylation for phosphorylation at the 2' hydroxyl position of the ribose. The diphosphate bridge which is immediately adjacent to the hydrolyzed section would not be methylated and would maintain strand continuity. As loop fusions continued to progress, more and more of the ARR sites would be inactivated by methylation and released into the non-matrix region of the DNA. This could account for the difference in methylation content which has been observed for matrix and non-matrix ARR's. Such an inactivation process would be analagous to what is suspected to be occuring during the methylation of cytosine in DNA, i.e., gene inactivation. During loop deletions the domains would be released as intact circles of DNA leaving behind a "scar" of DAR within the replicon cluster. The regions responsible for ARR formation in this "scar" could be inactivated by methylation. Once loop fusions and deletions were completed, the replicon cluster would be reduced to one or more DNA domains with an average size of about 100-300 kb depending on cell type resulting in a 1:1 correlation between DNA domain numbers and alkali-sensitive sites.

In this series of papers, DAR's and their ARR subcomponents have been implicated in establishing DNA domain attachment sites within the nuclear matrix, establishing complex non-linear DNA superstructures (replicon clusters), and providing the plasticity necessary for DNA loop fusions and deletions during cellular differentiation. These phemonena and others have been discussed elsewhere relative to the structure, function, and evolution of the eukaryotic chromosome (Abernathy, 1988).

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Table I

Double Radiolabel Counts Isolated From the Nuclear Matrix and Non-matrix of Mouse L-1210 Cells After One or Two DNase I Treatments Followed by Heating in the Presence or Absence of Alkali

Treatment	Nuclear Fraction	[^{3}H]Thymidine†	[^{14}C]Uridine†	N††
Pre-DNase	Matrix	259	591	1
0.1M KCl, 100 ^{0}C	Matrix	37 ±4	97 ±11	2
0.1M KOH, 100 ^{0}C	Matrix	7 ±3	17 ±1	2
0.1M KOH, 100 ^{0}C	Matrix*	6	17	1
Pre-DNase	Non-matrix	6130	7785	1
0.1M KCl, 100 ^{0}C	Non-matrix	774 ± 48	1371 ±47	2
0.1M KOH, 100 ^{0}C	Non-matrix	217 ±16	192 ±7	2
0.1M KOH, 100 ^{0}C	Non-matrix*	60	109	1

Mouse L-1210 cells were double labeled in log phase with [^{3}H]thymidine and [^{14}C]uridine. They were incubated for 24 hours in low serum media to remove extraneous label and to inhibit further DNA replication. Additional separation of the matrix and non-matrix fractions by Sea Prep underlayering was not used here. Purified matrix and non-matrix aliquots were counted or exhaustively digested in DNase I once or twice. The DNase I digests were heated to 100 ^{0}C for 20 minutes in either 0.1M KCl or 0.1M KOH, neutralized where appropriate, ethanol precipitated, rinsed twice, resolubilized, and counted. *Aliquots which were digested twice in DNase I, †average and standard error, ††number of aliquots counted.

Table II

Double Radiolabel Counts Isolated From the Nuclear Matrix and Non-matrix of Mouse L-1210 Cells After Two Successive DNase I Treatments Followed by Heating in the Presence or Absence of Alkali

Treatment	Nuclear Fraction	[^{3}H]Thymidine*	[^{14}C]Uridine*	N†
Pre-DNase I	Matrix	1291±25	823±8	3
0.1M KCl, 100 ^{0}C	Matrix	617 ±8	255 ±6	3
0.1M KOH, 100 ^{0}C	Matrix	278±3	104±4	3
Pre-DNase I	Non-matrix	84513±1188	6316±102	3
0.1M KCl, 100 ^{0}C	Non-matrix	3211 ± 69	457 ±7	2
0.1M KOH, 100 ^{0}C	Non-matrix	1393±18	385±10	3

Mouse L-1210 cells were double labeled in log phase with [^{3}H]thymidine and [^{14}C]uridine in the absence of exogenous deoxynucleosides. They were incubated for 24 hours in low serum media to remove extraneous label and to inhibit further DNA replication. Additional separation of the matrix and non-matrix fractions was achieved by Sea Prep underlayering. Purified matrix and non-matrix aliquots were counted or exhaustively digested in DNase I for two successive treatments. The DNase I digests were heated to 100 ^{0}C for 20 minutes in either 0.1M KCl or 0.1M KOH, neutralized where appropriate, ethanol precipitated, rinsed twice, resolubilized, and counted. *Aliquots which were digested twice in DNase I, *average and standard error, †number of aliquots counted.

Table III

Double Radiolabel Counts Isolated From the Nuclear Matrix and Non-matrix of Human Lymphocytes

Treatment	Nuclear Fraction	[^{3}H]Thymidine*	[^{14}C]Uridine*	N†
Pre-DNase I	Matrix	539	250	1
0.1M KOH, 100 ^{0}C	Matrix	36±2	22±0	2
Pre-DNase I	Non-matrix	45166	3093	1
0.1M KOH, 100 ^{0}C	Non-matrix	636±0	98±4	2

Human lymphocytes were stimulated to replicate using phytohemagglutin and double labeled with [^{3}H]thymidine and [^{14}C]uridine in the absence of exogenous deoxynucleosides. They were incubated for 24 hours in low serum media to remove extraneous label and to inhibit further DNA replication. Additional separation of the matrix and non-matrix fractions was achieved by Sea Prep underlayering. Purified matrix and non-matrix aliquots were counted or exhaustively digested in DNase I for two successive treatments. The DNase I digests were heated to 100 ^{0}C for 20 minutes in 0.1M KOH, neutralized, ethanol precipitated, rinsed twice, resolubilized, and counted. *Aliquots which were digested twice in DNase I, *average and standard error, †number of aliquots counted.

Table IV

Data Derivatized From Tables I, II, and II

Table	Nuclear Fraction	Sea Prep†† Underlayer	% DNA (M+N)	% ARR (M+N)	% ARR in pre-ARR	ARR+DNA	P. I.
I	Matrix*†	No	4.1	8.1	17.5	0.0656	2.1
I	Non-matrix*†	No	95.9	91.9	14.0	0.0313	---
I	Matrix†	No	4.1	13.5	---	0.0656	3.7
I	Non-matrix†	No	95.9	86.5	---	0.0178	---
II	Matrix	Yes	1.5	21.3	40.8	0.0806	17.5
II	Non-matrix	Yes	98.5	79.7	84.2	0.0046	---
III	Matrix	Yes	1.2	18.3	---	0.0408	18.6
III	Non-matrix	Yes	98.8	82.7	---	0.0022	---

Data column one represents percentages of total matrix and non-matrix (M + N) DNA prior to exhaustive DNase I digestion. Data column two represents percentages of total matrix and non-matrix ARR isolated. Data column three represents the percentage of pre-ARR (within a given sample) which is ARR. Pre-ARR is a combination of alkali-sensitive RNA and ARR and its value is determined by heating RNA label at 100 ^{o}C for 20 minutes in 0.1M KCl. Data column four is the ratio of matrix or non-matrix ARR counts to their respective total DNA counts. Data column five is called the "predilection index" (P.I) and it is determined by dividing the matrix ARR/DNA ratio by the non-matrix ARR/DNA ratio. An index greater than 1 indicates a predilection of the ARR for the nuclear matrix DNA. *These aliquots were digested only once in DNase I. †These samples were isolated from cells which has been grown in the presence of 50 ug/ml deoxynucleosides. ††Indicates which samples were or were not further purified with Sea Prep underlayering.

Table V

Radiolabel Counts Isolated From from Mouse Nuclear Matrix and Non-matrix Double Labeled With [^{3}H] Methionine and [^{14}C] Uridine

Treatment	Nuclear Fraction	[^{3}H]Thymidine†	[^{14}C]Uridine†	N††
Pre-DNase I	Matrix	676±62	624±24	3
0.1M KCl, 100 ^{0}C	Matrix	116 ±4	112 ±3	2
0.1M KOH, 100 ^{0}C	Matrix*	7±0	15±0.6	3
Pre-DNase I	Non-matrix	4989±57	3972±49	3
0.1M KCl, 100 ^{0}C	Non-matrix	463 ± 3	338 ±8	3
0.1M KOH, 100 ^{0}C	Non-matrix*	96±1	94±4	4

Samples are from mouse cells in log phase labeled with with [^{3}H] Methionine and [^{14}C]uridine. Cells were grown in the presence of 50 ug/ml of adenosine and guanosine to inhibit the uptake of labeled methyl groups into the purine biosynthetic pathway. They were incubated for 24 hours in low serum medium with adenosine and guanosine (but no exogenous deoxynucleosides) to remove extraneous label and to inhibit further DNA replication. Additional separation of the matrix and non-matrix fractions was achieved by Sea Prep underlayering. Purified matrix and non-matrix aliquots were counted or exhaustively digested in DNase I for two successive treatments. The DNase I digests were heated to 100 ^{0}C for 20 minutes in either 0.1M KCl or 0.1M KOH, neutralized where appropriate, ethanol precipitated, rinsed twice, resolubilized, and counted. *Aliquots which were digested twice in DNase I. *Fifty minute counts, †average and standard error, ††number of aliquots counted.

Table VI

Ratios of Alkali-Resistant [^{3}H] Methionine Labeled and [^{14}C] Uridine Labeled Oligomers

Nuclear Fraction	% ARR (M+N)	% ARR in Pre-ARR	$ARCH_3$+ ARR	M. I.
Matrix	13.8	13.4	0.467	2.19
Non-matrix	86.2	27.8	1.021	---

Data column one represents percentages of total matrix and non-matrix (M+N) ARR isolated. Data column two represents the percentage of pre-ARR (within a given sample) which is ARR. Pre-ARR is a combination of alkali-sensitive RNA and ARR and its value is determined by heating RNA label at 100 ^{0}C for 20 minutes in 0.1M KCl. Data column three is the ratio of matrix or non-matrix alkali-resistant methylated ($ARCH_3$) counts to their respective ARR counts. Data column four is called the "methylation" index (M. I) and it is determined by dividing the non-matrix $ARCH_3$/ARR ratio by the matrix $ARCH_3$/ARR ratio. An index greater than one indicates that the non=matrix ARR is more "methylated" than the matrix ARR.

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