Volume 13 Number 20 1985 Nucleic Acids Research

Low ionic strength extraction of nuclease-treated nuclei destroys the attachment of transcriptionally active DNA to the nuclear skeleton

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Received 9 May 1985; Revised and Accepted 9 September 1985

ABSTRACT

We have studied how the conditions in which the nuclear matrix is isolated influence the association of transcribing DNA with the nuclear matrix. Extraction of nuclease-treated nuclei with a low ionic strength solution before a high salt extraction completely abolishes this association. However, RNA removal by RNAase treatment does not affect the binding of transcribing DNA to the nuclear matrix. The nature of the association of active genes with the nuclear matrix is discussed.

INTRODUCTION

The association of active genes with nuclear skeleton elements has been extensively discussed in the literature during the recent years. In a number of cases, actively transcribed DNA sequences were found to be enriched in the nuclear matrix DNA /1-7/. Initiated RNA polymerase II complexes are also preferentially bound to this DNA fraction /8/. Yet some results obtained by other authors are contradictory of this conclusion /9-11/. Thus, the whole problem remains unsolved. In any case, the nature of the association of active genes with nuclear skeleton elements is unclear. It was suggested that the aggregation of nonhistone proteins and/or nascent RNA chains during high salt extraction of nuclei might be responsible for the observed phenomenon /12/ though other explanations are also possible.

To approach the problem, we made experiments to determinate what causes the aforementioned contradictions in the results obtained by different authors. With the aid of a simple experimental system, we studied how the association of active

genes with nuclear skeleton elements depends on several variations in the conventional nuclear matrix isolation procedures. It was found that the critical step in nuclei fractionation that leads to a random distribution of active genes versus nuclear skeleton elements is the extraction of nuclei with a very low ionic strength solution performed before a high salt extraction. On the other hand, it is demonstrated that RNA does not participate in maintaining the association of active genes with nuclear skeleton elements.

METHODS

Cell culture

Mouse hybridoma cells producing IgM were a generous gift of Dr.Roodin from the Moscow Cardiology Research Center. The cells were cultivated in the Dulbecco medium, supplemented with a 10% fetal calf serum. For DNA labelling, [^{3}H]thymidine was added to the culture medium 36 h before the experiment to a final concentration of 0.15 μCi/ml. To label RNA transcribed by RNA polymerase II, [^{14}C]uridine (10 μCi/ml) and actinomycin D (0.04 μg/ml) were added to the culture medium. The time of RNA labeling was 1 or 15 min in different experiments.

Isolation of nuclear matrices

All procedures except nuclease treatment were carried out at 0–4°C. All solutions contained 0.1mM phenylmethylsulphonyl fluoride. All centrifugations were run for 5 min at 1000 g.

Cells were suspended in the lysis buffer (50 mM Tris HCl, pH 7.5 - 25 mM NaCl - 25 mM KCl - 3 mM $MgCl_2$ - 2 mM $MnCl_2$ - 15% glycerol) at a concentration of 10^7 cells/ml. Nonionic detergents NP-40 and Triton X-100 were added to the suspension up to 0.5% and 0.1%, respectively. After a 10-min incubation in an ice bath, the cells were broken by 20 strokes in a Dounce tissue homogenizer. The nuclei were pelleted, washed twice with the lysis buffer, and suspended in the same buffer at a concentration of $4x10^7$ nuclei/ml. The necessary volume of a 4 M NaCl solution was added to the suspension in order to increase the concentration of monovalent cations up to 0.35 M. This step was supposed to eliminate the preferential sensitivity of active genes to nuclease treatment. After a 10-min in-

cubation in an ice bath, the nuclei were pelleted, washed twice with the cell lysis buffer, suspended in this buffer (10^8 nuclei/ml), and used for nuclease treatment. In some experiments, the above described preextraction of nuclei with a medium salt solution was omitted.

The nuclease treatment was performed at 30°C for 10 min while different amounts of either DNAase I or micrococcal nuclease were added. When micrococcal nuclease was used, $CaCl_2$ was added to a final concentration of 3 mM.

Further steps of nuclear matrix isolation varied in different experiments. According to the first protocol, the nuclei were pelleted and suspended in the extraction buffer (50 mM Tris-HCl, pH 7.5 - 25 mM NaCl - 25 mM KCl - 2 mM EDTA - 15% glycerol) up to a concentration of $4x10^7$ nuclei/ml. An equal volume of a cold 2 M NaCl solution was added to the suspension. After a 20-min incubation at 0°C, the matrices were pelleted and washed twice with a mixture of equal volumes of the extraction buffer and 2 M NaCl solution.

Alternatively, the pellet of nuclease treated nuclei was suspended in a low ionic strength solution (2 mM EDTA - 1 mM Tris-HCl, pH 7.5). After a 20-min incubation at 0°C, the nuclei were pelleted, washed twice with the same solution, suspended in the extraction buffer, and a high salt extraction was carried out as described above. DNA was isolated from the nuclear matrices using proteinase detergent extraction /13/.

Determination of RNA polymerase II activity

The RNA polymerase II activity of isolated matrices was assayed as described in our previous paper /8/. Briefly, the matrices were incubated for 15 min at 30°C in the following solution: 65 mM Tris-HCl, pH 7.9 - 250 mM $(NH_4)_2SO_4$ - 2 mM $MnCl_2$ - 25 mM KCl - 25 mM ß-mercaptoethanol - 5% glycerol - 0.5 mM ATP - 0.5 mM GTP - 0.5 mM CTP. 2 µCi of [^{3}H]UTP (25 Ci/mM) was added to each incubation tube containing 100 µl of the reaction mixture. Then the incorporation of [^{3}H]UTP into the acid-insoluble material was measured. To discriminate between the activities of RNA polymerase II and other RNA polymerases, control experiments were carried out with α-amanitin (1 µg/ml) included into the incubation mixture.

Hybridization experiments

A modification of the dot-blot hybridization technique was elaborated. DNA was immobilized on nylon filters by UV irradiation /14/ in order to prevent a subsequent loss of DNA from the filters during hybridization. Before the immobilization, DNA preparations were sheared and denatured by boiling for 20 min in a solution containing 0.3 M NaOH and 0.3 M NaCl. The denatured DNA was precipitated with ethanol and dissolved in an appropriate volume of bidistilled water to obtain a DNA solution (40 µg/ml). Round pieces (7 mm in diameter) were cut out from Gene Screen Filter (New England Nuclear, USA). These were washed in a 0.025 M sodium phosphate buffer solution (pH 7.0) and then dried on filter paper. The dry filters were placed on a sheet of Parafilm, and 8 µl of the denatured DNA solution (i.e. ~ 0.3 µg of DNA) was loaded on each filter. The filters were dried at room temperature and then for 40 min at 80°C. The completely dry filters were UV-irradiated at both sides (260 nm). The dose was 0.1 KJ/m^2 at each side.

Preliminary experiments have shown that over 95% of the loaded DNA is irreversibly bound to the filter under such conditions. The capacity of the filters was found to be rather low so that no more than 0.5 µg of DNA could be loaded on them without a considerable decrease in the effectivity of immobilization. When the dose of UV-irradiation was increased, higher amounts of DNA were immobilized but the hybridizability of such DNA fell down due to the damage produced by UV-irradiation.

Prehybridization was carried out for 24 h at 65°C in a 6xSSC - 10 X Denhardt solution - 300 µg/ml of denatured E.coli DNA - 100 µg/ml poly U - 0.15% SDS. The same solution was used for hybridization, but the concentration of SDS was increased to 1%. Hybridization was performed at 65°C for 24 h. Thereafter the filters were washed with a 2xSSC - 0.5% SDS solution twice for 15 min at room temperature and 6 times for 30 min at 65°C. The last washing was in 0.1xSSC - 0.5% SDS for 30 min at 42°C. Prehybridization, hybridization and all washings were carried with a continuous shaking.

The washed filters were exposed on a Fuji Film using

Dupont Intensifying screens. The exposition took from 7 to 10 days in different experiments.

RESULTS

The experimental system

Two independent experimental approaches were used in the present work to investigate the distribution of active genes versus nuclear skeleton elements.

First of all, we made hybridization experiments in order to check whether the nuclear matrix DNA from IgM-producing hybridoma cells is enriched in a DNA sequence coding for the constant region of IgM chains (C_μ sequences). The DNA fragment used as a hybridization probe was cleaved from the DNA of recombinant clone pμ107 /15/ by double digestion with SalI and BglII. The dot-blot hybridization technique was applied in all experiments. Total mouse DNA and different preparations of nuclear matrix DNA were immobilized on filters and hybridized with the nick-translated DNA fragment containing a C_μ sequence.

Such hybridization experiments involve two main technical problems that may highly affect the quality of results:

(i) a high background that can mask a weak signal obtained after hybridization with a unique DNA sequence,

(ii) a non-equal efficacy of the immobilization of DNA preparations which differ in the average fragment sizes and, as a result, an uncontrolled loss of immobilized DNA from the filters during prehybridization and hybridization.

The modification of the immobilization procedure and hybridization conditions described in Methods allowed us to solve both problems.

The results presented in Fig. 1b demonstrate that nuclear matrix DNA isolated from IgM-producing hybridoma cells by our standard procedure (with a high salt extraction only) is considerably enriched in C_μ sequences. At the same time, nuclear matrix DNA preparations obtained by the same method from SVT2 cells are not enriched in C_μ sequences (Fig. 1a). Thus, the apparent association of a C_μ sequence with nuclear skeleton elements strongly depends on its transcription, or, in other words, our system is adequate for investigating the so-called

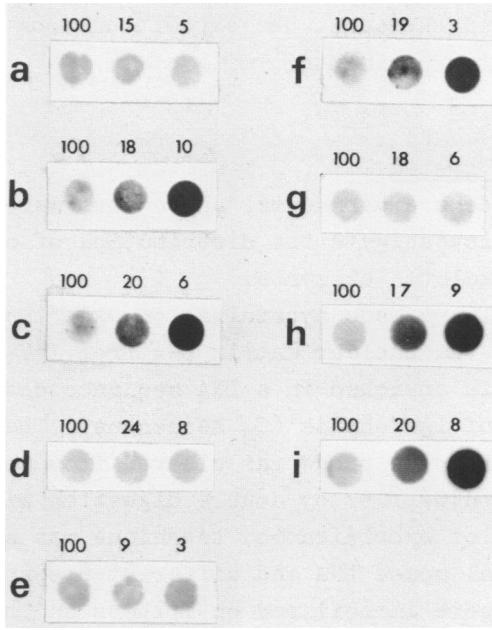

Fig. 1. Hybridization of the nick-translated DNA fragment containing a C_μ sequence with total mouse DNA and with different preparations of nuclear matrix DNA. (line a), nuclear matrix DNA from SVT2 cells isolated by a high salt extraction of nuclei pretreated with micrococcal nuclease; (line b and line h), nuclear matrix DNA from IgM-producing mouse hybridoma cells isolated by a high salt extraction of nuclei pretreated with micrococcal nuclease. In (h), the nuclei were not extracted with a medium salt solution before the nuclease treatment; (line c), nuclear matrix DNA from IgM-producing mouse hybridoma cells isolated by a high salt extraction of nuclei pretreated with DNAase I and RNAase A; (line d and line g), nuclear matrix DNA from IgM-producing mouse hybridoma cells isolated by a low salt extraction of nuclei pretreated with micrococcal nuclease. In (g), the nuclei were not extracted with a medium salt solution before the nuclease treatment; (line e), nuclear matrix DNA from IgM-producing mouse hybridoma cells isolated by successive low salt and high salt extraction of nuclei pretreated with micrococcal nuclease; (line f), nuclear matrix DNA from IgM-producing mouse hybridoma cells isolated by a high salt extraction of nuclei pretreated with micrococcal nuclease. The isolated matrices were extracted with a low salt solution. (line i), nuclear matrix DNA from IgM-producing mouse hybridoma cells isolated by successive low salt and high salt extractions of nuclei pretreated with micrococcal nuclease. The cells were lyzed in a buffer containing Cu^{2+} ions.

The numbers above the spots show DNA percentage in the particular preparations of nuclear matrices. The total DNA spots are marked with number 100.

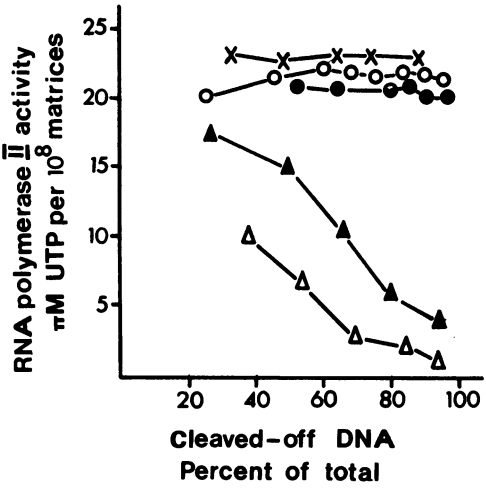

Fig. 2. RNA polymerase II activity of nuclear matrix preparations obtained by different procedures and containing different portions of the initial amount of DNA.

O—O ... a high salt extraction of nuclei predigested with micrococcal nuclease,

X—X ... a high salt extraction of nuclei predigested with DNAase I,

●—● ... a high salt extraction of nuclei predigested with DNAase I and RNAase A,

▲—▲ ... a low salt extraction of nuclei predigested with micrococcal nuclease,

Δ—Δ ... successive low salt and high salt extractions of nuclei predigested with micrococcal nuclease

"temporal" interactions of transcriptionally active DNA with the nuclear skeleton.

Another approach used to follow the distribution of active genes among skeleton-associated and cleaved-off DNA fractions was based on the determination of endogenous RNA polymerase II activity. We have demonstrated recently that practically all initiated RNA polymerase II complexes are concentrated in the DNA associated with the nuclear matrix /8/. This follows from the comparison of RNA polymerase II activities recovered in nuclear matrices containing different portions of total DNA. RNA polymerase II activity detected in the matrices isolated after a high salt extraction of nuclei hardly depends on the percentage of nuclear skeleton DNA within a wide range from ~80% to ~3% (Fig. 2). It should be mentioned

that the reinitiation of RNA synthesis is unlikely in our mixture for the determination of RNA polymerase II activity /16/ and, indeed, it has not been observed in the isolated matrices /8/. Thus, all the measured RNA polymerase II activity results from an elongation of the previously initiated RNA chains.

These data independently prove the association of transcriptionally active DNA with the nuclear skeleton. Otherwise one should observe a decrease in the activity of matrix-bound RNA polymerase II along with a decrease in the amount of DNA remaining bound to the nuclear skeleton.

The influence of RNAase treatment on the distribution of active genes among the nuclear matrix and cleaved-off DNA fractions

It seemed reasonable to suggest that RNA and, in particular, nascent RNA chains might play some role in maintaining the association of transcribed DNA sequences with the nuclear skeleton. Indeed, it is well known that RNA molecules can precipitate in concentrated salt solutions. Several authors demonstrated that hnRNA remained associated with the nuclear skeleton upon a high salt extraction of DNAase-treated nuclei /17-19/. Being independently bound to the nuclear skeleton, nascent RNA chains can ensure the indirect attachment of transcribed DNA sequences. To check such a possibility, we compared matrices isolated with and without RNAase pretreatment. In this series of experiments, the RNA polymerase II transcripts were labeled by incubation of cells for 1 or 15 min in the medium containing [^{14}C]uridine and actinomycin D at a concentration selectively inhibiting the transcription of ribosomal genes. Thus, the distribution of both DNA (labeled with [^{3}H]thymidine) and RNA (labeled with [^{14}C]uridine) could be easily analyzed. Isolated nuclei were treated with different amounts of DNAase I. The latter was inhibited by adding a two-fold excess of EDTA, and each preparation was divided into two samples. One was treated with RNAase A (15 μg/ml, 20 min, 37°C) while the other was incubated in the same conditions but without the enzyme. Then the both were extracted with a high salt solution, and the nuclear matrix DNA was isolated accor-

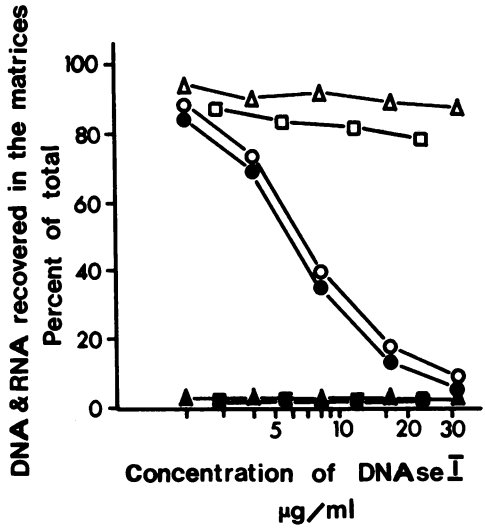

Fig. 3. Kinetics of DNA (circles) and RNA (squares - 1-min pulse; triangles - 15-min pulse) solubilization upon a high salt extraction of nuclei treated with increasing amounts of DNAase I and treated (closed) or non-treated (open) with RNAase A.

ding to the standard procedure (see Methods).

The curves presented in Fig. 3 demonstrate the kinetics of matrix DNA solubilization upon the treatment of nuclei with increasing amounts of DNAase I. One can see that RNAase treatment did not solubilize any additional amount of nuclear matrix DNA though more than 95% of nuclear skeleton-bound RNA labeled either for 1 or 15 min was solubilized as a result of such a treatment (Fig. 3).

At the same time, the hybridization experiments showed that RNAase treatment of nuclei did not destroy the associations of transcribed DNA sequences with the nuclear skeleton (Fig. 1c). RNAase A pretreatment also did not influence the association of endogenous RNA polymerase II activity with nuclear matrix DNA (Fig. 2).

Low ionic strength extraction of nuclease-treated nuclei

Chromatin may be solubilized from nuclease-treated nuclei either by the extraction with a high ionic strength solution

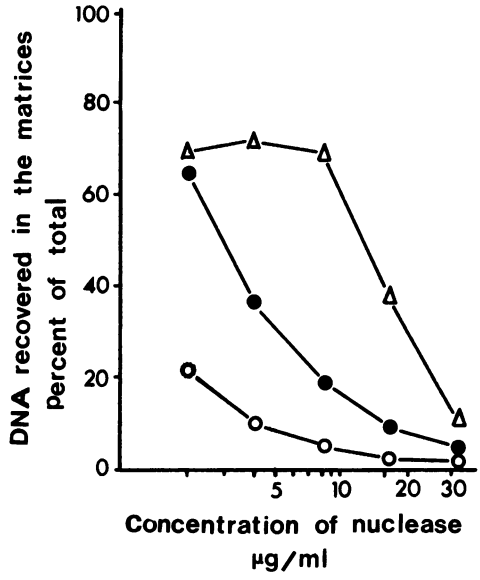

Fig. 4. Kinetics of DNA solubilization upon different extractions of nuclei predigested with increasing amounts of micrococcal nuclease.
●—●... a high salt extraction,
Δ—Δ... a low salt extraction,
O—O... successive low salt and high salt extractions.

or by extraction with a very low ionic strength solution. Recently, Long and Ochs /20/ have described a new procedure for nuclear matrix isolation. They used 1 mM Tris-HCl, pH 7.5 - 2 mM EDTA to solubilize cleaved-off DNA from the nuclei exhaustively treated with DNAase I. We have compared the efficiency of solubilization of the cleaved DNA by this procedure (low salt extraction), by extraction with a solution containing 1 M NaCl (high salt extraction) and by successive low salt and high salt extractions. Kinetic curves were constructed by repeating the experiment several times with the treatment of the nuclei by increasing amounts of micrococcal nuclease. The results are presented in Fig. 4. One can see that the low salt extraction makes the cleaved-off DNA be effectively solubilized only after a rather extensive nuclease treatment of the nuclei though even here the low salt extraction is less effective than the high salt extraction. On the other hand, succes-

sive extractions of the nuclei with low salt and high salt solutions ensure the solubilization of additional DNA amounts comparing to a high salt extraction alone. The amount of DNA recovered in the matrices obtained after the high salt extraction was from four to five times as high as that after successive low salt and high salt extractions at any point of digestion with micrococcal nuclease (Fig. 4). Naturally, the average size of nuclear matrix DNA fragments decreased when the nuclei were treated with increasing nuclease amounts (not shown).

The distribution of RNA polymerase II activity among DNA fractions also changed drastically after either low salt or low salt plus high salt extractions (Fig. 2). In contrast to the above mentioned experiments, RNA polymerase II activity recovered in the nuclear matrix decreased almost proportionally to the decrease of DNA remaining bound to the nuclear skeleton. One may conclude that the low salt extraction of the nuclei destroys the attachment of transcribed DNA sequences to the nuclear skeleton.

Interestingly, the low salt extraction of nuclease-treated nuclei not only solubilizes transcriptionally active DNA sequences, but also stimulates their further solubilization by the subsequent high salt extraction. In fact, at the early stage of nuclease digestion a considerable amount of cleaved-off DNA is solubilized only after a high salt extraction.

The hybridization experiments showed that the nuclear matrix DNA isolated either after the low salt extraction alone or after successive low salt and high salt extractions was not enriched in actively transcribed sequences (Fig. 1d, e). Thus, they confirm the above conclusion.

According to the standard procedure used for isolation of matrices in all the above discussed experiments, the nuclei were preextracted with a medium salt solution before digestion of DNA. We believe that it is a useful step as it eliminates the preferential sensitivity of active genes to nuclease treatment. However, the preextraction of nuclei with a medium salt solution was not commonly used by other authors in the course of the nuclear matrix isolation /9, 10, 23/. To make our data

more comparable with those of other authors, we studied whether the omission of a medium salt preextraction influence the distribution of active genes in the course of subsequent low salt and high salt extractions. It was found that in this case again the active genes were separated from the nuclear skeleton by the low salt extraction (Fig. 1g). The matrices isolated without the low salt extraction were still enriched in active genes even if preextraction of nuclei with a medium salt solution was omitted (Fig. 1h).

Low ionic strength extraction of isolated nuclear matrices

It was demonstrated in the previous section that the low salt extraction of nuclease-treated nuclei destroyed the attachments of transcribed DNA sequences to the nuclear skeleton. The question arises whether the same effect can be achieved with an isolated nuclear matrix. To answer the question, we isolated matrices, then washed the material with 2 mM EDTA - 1 mM Tris-HCl, pH 7.5, and incubated it in the same solution at $0^{o}C$ for 1 h. Such an incubation solubilized less than 5% of nuclear matrix DNA probably due to a non-specific degradation.

The hybridization experiments showed that the nuclear matrix DNA was as much enriched in transcribed sequences as that before a low salt treatment (Fig. 1f). Thus, the association of transcriptionally active DNA with the nuclear skeleton is resistant to the low salt treatment if it is performed after the nuclear matrix isolation.

Stabilization of the attachment of transcriptionally active DNA to the nuclear skeleton by Cu^{2+} ions

Several authors used Cu^{2+} ions to stabilize the internal structures of the nuclear matrix /21, 22/. We checked whether such a stabilization interfered somehow with the solubilization of transcriptionally active DNA sequences by the low salt extraction of nuclei. 1 mM $CuSO_4$ was included in the cell lysis mixture only at the stage of detergent treatment. Then the nuclear matrices were isolated either by a high salt extraction or by successive low salt and high salt extractions. Roughly, the same DNA amount was found in both preparations of the matrices. The hybridization experiments demonstrated that both preparations of nuclear matrices were equally en-

riched in transcribed DNA sequences (Fig. 1i). Thus, the treatment of nuclei with Cu^{2+} ions stabilizes the attachments of transcriptionally active DNA to the nuclear skeleton in such a way that they become resistant to the low salt extraction of nuclei.

The treatment of nuclei with Cu^{2+} ions also resulted in the retention of initiated RNA polymerase II molecules in the matrices obtained with the low salt extraction or with subsequent low salt and high salt extractions (not shown).

Solubilization of RNA during different extractions in the course of nuclear matrix isolation

The last series of experiments was carried out in order to answer the question whether hnRNA and transcriptionally active DNA sequences were solubilized under the same conditions. The cells were labeled for 15 min with ^{14}C uridine either in the presence or in the absence of actinomycin D at a concentration selectively inhibiting the transcription of ribosomal genes (0.04 µg/ml). The isolated nuclei were treated with DNAase I and extracted either with a high salt solution or with low salt solution and the high salt solution.

Even after successive low salt and high salt extractions (i.e. when transcribed DNA sequences were solubilized), the major portion of hnRNA remained associated with the nuclear skeleton (Table 1).

It was found that hnRNA solubilization by successive low salt and high salt extractions required an addition of 0.5% ß-mercaptoethanol to the extraction medium (Table 1). Thus, it is clear that the removal of hnRNA is not a prerequisite for the solubilization of transcribed DNA.

DISCUSSION

In the present paper, we have demonstrated that a low salt extraction preceding a high salt extraction of nuclease-treated nuclei destroys the attachment of transcriptionally active DNA to nuclear skeleton elements. This observation accounts for the reported contradictions in the results of different authors who analyzed the distribution of active genes versus the points of DNA attachment to the nuclear skeleton.

Table 1. Solubilization of RNA upon different extractions of nuclei predigested with DNAase I

Type of extraction	Percentage of nucleic acids recovered in the residual structures		
	total RNA	RNA synthesized in the presence of actinomycin D (0.04 µg/ml)	DNA
high salt extraction	91	94	9
low salt extraction	92	87	32
successive low salt and high salt extractions	86	83	2.5
successive low salt and high salt extractions in the presence of 0.5% ß-mercaptoethanol	14	9	1.2

Indeed, one can see that a low salt treatment was used at the early stages of nuclear matrix isolation in all the papers in which the random distribution of active genes was demonstrated /9-11/.

However, the results of our investigations contradict to the data of Rose and Garrard /23/ who have found that actively transcribed genes are preferentially retained in matrices isolated by a low salt extraction of nuclease treated nuclei (without a high salt extraction at all). Some peculiarities of the isolation method used by Rose and Garrard might account for the above mentioned contradiction. We have found that incubation of nuclei in the media containing Cu^{2+} ions interfere with the subsequent solubilization of active genes by the low salt extraction. One could speculate that some other factors may cause the same effect.

Our results may be interpreted in two quite different ways.

(i) The association of transcribing DNA with the nuclear matrix is an artefact depending on non-histone protein aggregation.

(ii) This association does exist <u>in vivo</u>, but a low salt

extraction destroys the interaction.

Several authors have suggested that the association of active genes with the nuclear skeleton is a result of protein and/or nascent RNA aggregation /12, 25/. The data presented in this paper, as well as some data of other authors /24, 25/ rule out the involvement of nascent RNA.

As for a possible role of non-histone protein aggregation, the question remains open for further investigations. Kirov et al. /25/ found active genes in the nuclear matrices only when a nuclease treatment was done after a high salt extraction of nuclei. In the present work, we were not able to reproduce this result with our cells and our conditions used for isolation of nuclear matrices. However, there is some other evidence that the rearrangement and aggregation of non-histone proteins may take place in the high salt extraction of nuclei /26/. Obviously, some DNA sequences may be artificially entrapped in the residual nuclear structures during such an aggregation.

Yet, a non-histone protein responsible for the aggregation should be firmly associated with transcriptionally active DNA in medium and high salt solutions and dissociate from it in a low salt buffer. So far, no such protein has been found in cell nuclei.

It has been suggested that the side-to-side aggregation of transcriptional complexes plays an important role in maintaining the structure of residual nucleoli /27/. In the case of genes transcribed by RNA polymerase II, a similar explanation is hardly feasible due to a rather low concentration of transcriptional complexes. Besides, potentially active genes were also found to be associated with the nuclear skeleton /28/.

Clearly, our results as well as the results of other authors do not exclude the possibility that specific interactions of transcriptionally active DNA with nuclear skeleton elements do exist in the living cell. If this is the case, one may suggest that a low salt extraction destroys some structures of the nuclear matrix to which transcribed DNA sequences are bound. It is known, for example, that a low ionic

strength induces the depolymerization of intermediate filaments of the vimentin type. F actin which was shown to be a structural component of the nuclear matrix /29/ is also not stable in low ionic strength solutions. The incubation of nuclei in media containing Cu^{2+} ions can stabilize the structures of the internal matrix by S-S bonding /21/ which makes them insensitive to the low salt extraction of nuclei. It is more difficult to account for the failure to solubilize transcriptionally active DNA by a low salt extraction of the isolated nuclear matrix. Here, aggregation hypothesis seems to be more applicable. However, this argument is far from being conclusive.

It is proposed to discriminate between stable (structural) and temporal (functional) attachments of DNA to the nuclear skeleton, the latter being associated with transcription and replication.

Still the quantitation of these fractions of attachment sites has not been possible. Assuming that the attachment sites associated with transcription correspond to those which are labile to a low salt treatment, one may conclude that they comprise about 3/4 of all the attachment sites. However, it is not unlikely that some other nuclear matrix DNA sequences are also solubilized by a low salt preextraction.

While this paper was in preparation, the work of Mirkovitch et al. /30/ was published. They demonstrated that some specific non-transcribed DNA sequences remained associated with the nuclear skeleton after histones removal by lithium diiodosalicylate treatment in a low ionic strength solution. However, it was not clear whether a low ionic strength treatment itself or the specific detergent used for the histones removal were responsible for the detachment of transcriptionally active DNA from the nuclear skeleton. Our results reinforce the first possibility. We have mapped recently the positions of DNA attachment to the nuclear skeleton inside the chicken α-globin gene domain. The transcription-associated attachment sites and the permanent ones were identified, the latter being positioned at the 5'-end of the domain /7/. Traditional high salt extraction method was used for matrix isolation in

these experiments. The discrimination of the permanent and transcription-associated attachment sites was based on the comparison of the positions of DNA attachments in functionally active and inactive nuclei (erythroblasts and erythrocytes were used, respectively). Now, it seems interesting to reinvestigate the specificity of DNA sequences which remain bound to the nuclear skeleton of erythroblasts even after successive low salt and high salt treatments. These experiments are in progress now.

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