A REVERSIBLY CONTRACTILE NUCLEAR MATRIX

Its Isolation, Structure, and Composition

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ABSTRACT

From *Tetrahymena* macronuclei we have isolated a reversibly contractile nucleoskeleton, i.e., an "expanded" nuclear matrix which reversibly contracts when the total concentration of the bivalent cations, Ca and Mg (3:2), is decreased to 5 mM or increased to 125 mM. During contraction the average diameter of the expanded matrix becomes reduced by about 24%; this corresponds to a volume contraction of about 55%. The reversible contraction of the nuclear matrix does not depend on ATP and cannot be inhibited by salyrgan. The expanded matrix is obtained by removing carefully from the macronuclei 89.7% of the phospholipid, 99.6% of the DNA, 98.5% of the RNA, and 74.8% of the protein by treatment with Triton X-100 and digestion with DNase and RNase followed by an extraction with 2 M NaCl. Electron microscopy reveals, within the expanded matrix, residual equivalents to the structures characteristic for macronuclei: (*a*) a residual nuclear envelope with nuclear pore complexes; (*b*) residual nucleoli at the periphery; (*c*) a fibrillar internal network. The expanded matrix is essentially composed of proteins (96.2%) and traces of DNA (0.8%), RNA (0.5%), phospholipid (1.6%), and carbohydrates (0.9%). The last, which have been determined by gas chromatography, contain glucose, mannose, and an unidentified sugar in the ratio 1:5.4:5.7. The ratio of acidic to basic amino acids of the expanded matrix is 1.55. Sodium dodecyl sulfate (SDS) gel electrophoresis reveals a predominant protein with a mol wt of 18,000 which is apparently involved in the reversible contractile process. The mechanism of this reversible contraction of the expanded matrix remains to be elucidated, but it differs both from actin-myosin contraction systems and from the contractile spasmoneme system in vorticellids.

Considerable evidence shows that cell nuclei vary in their size as for example during the cell cycle and cell differentiation, or with circadian periodicity (14). In particular, nuclear enlargement precedes as a prerequisite the onset of DNA synthesis in vivo (12, 15) and in vitro (3), while nuclear contraction appears to accompany cessation of DNA synthesis (13). LeStourgeon et al. (20) have previously suggested that nuclear contractile proteins of the actin-myosin type regulate nuclear contraction and swelling (cf. also reference 9).

Recent findings, however, suggest that not actin-myosin type proteins, but rather other acidic proteins determine the internal organization and overall form of cell nuclei. Thus, Berezney and Coffey (4) have isolated, from rat liver nuclei, a

residual fibrillar framework structure which still maintains the typical nuclear sphere geometry. This nucleoskeleton is composed essentially of three major proteins with molecular weights ranging between 50,000 and 70,000. This protein skeleton (termed nuclear matrix in the following) obviously represents the "equivalent structure" to the fibrillar framework which can be visualized by electron microscopy to extend throughout rat liver nuclei by the EDTA staining procedure (7). The nuclear matrix plays presumably a fundamental role in the initiation and replication of DNA (5) and in the synthesis, processing, and transport of RNA (for review see references 6 and 32). In the meantime, nuclear matrices have also been identified in Chinese hamster cell nuclei (17) and in the macronuclei of the ciliate protozoan *Tetrahymena* (16). Moreover, the rat liver nuclear matrix (4), the pore lamina fraction isolated from rat liver nuclei (1), and the nuclear ghosts of HeLa cell nuclei (23) reveal an identical peptide pattern, thus suggesting that these different structural entities probably contain a common component, i.e., the peripheral layer of the nuclear matrix.

Interestingly, in both *Tetrahymena* and rat liver the diameter of the nuclear matrix amounts to only about 70% of the nuclear diameter. This was ascribed to a contraction of the nuclear matrix during isolation, due to the removal of the nucleic acids which keep the nuclear matrix equivalent expanded within the nuclei (6). On the other hand, the question arises as to whether the nuclear matrix per se represents an actin-myosin independent contractile system and whether the nuclear matrix has been hitherto isolated under "contraction conditions." Indeed, we report here experimental conditions under which expanded matrices can be originally isolated from *Tetrahymena* macronuclei, which are capable of reversible contraction.

MATERIAL AND METHODS

Cultures

Static 10 l-cultures of the ciliate protozoan *Tetrahymena pyriformis* (amicronucleate strain GL) were axenically grown at 28°C in the mid to late logarithmic growth phase (50,000–80,000 cells/ml) as described recently (24).

Isolation of Macronuclei and Nuclear Matrices

Macronuclei were isolated and purified according to our previous method (16), except that all isolation media were 0.1 mM ATP; the yield is thus raised to about 60%. For isolation of expanded matrices, these macronuclei were treated with a 0.3% Triton X-100 solution containing 20 mM Tris/HCl (pH 7.4), 0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.1 mM ATP, and 1% polyvinylpyrrolidone K-90 at 0°–4°C for 10 min; a 10-min centrifugation followed at 900 *g* in the cold. After the pelleted macronuclei were washed with 20 mM Tris/HCl (pH 7.4), 2 mM $MgCl_2$, 3 mM $CaCl_2$, and 0.1 mM ATP (CMT), they were incubated with ~100 μg/ml DNase (Worthington Biochemical Corp., Freehold, N. J.) and ~100 μg/ml RNase (Worthington Biochemical Corp.) in 5 ml of 20 mM Tris/HCl (pH 7.4), 50 mM $MgCl_2$, 75 mM $CaCl_2$, 0.1 mM ATP (HCMT) for 75 min at room temperature. The incubation was stopped by adding 45 ml 2.2 M NaCl buffered with CMT at ~4°C followed by a 20-min centrifugation at 1,300 *g*. Then a 30-min incubation with ~100 μg/ml DNase and RNase in HCMT at room temperature followed, which was stopped by twice washing with HCMT at 900 *g* in the cold. The yield of nuclear matrices amounts to ~30% of the total cells.

Electron Microscopy

Specimens were fixed with 1% glutaraldehyde buffered with 0.05 M Na-cacodylate (pH 7.4) at room temperature for 20 min. After washing out the glutaraldehyde, the specimens were postfixed in 2% OsO_4 buffered with cacodylate at ~4°C for 1 h. Then, the specimens were stained with 1% uranyl acetate, dehydrated stepwise through graded solutions of ethanol and propylene oxide, and embedded in Epon. Sectioning was performed on a OmU_2-Reichert ultramicrotome (C. Reichert, American Optical Corp., Buffalo, N. Y.). The sections were double stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop IA.

Chemical Determinations

GROSS COMPOSITION: The samples were precipitated with iced 10% TCA and washed three times with 5% TCA. The TCA precipitates were extracted with chloroform-methanol (2:1) according to Folch et al. (10). From the extract, we determined total phospholipid according to Gerlach and Deuticke (11). Total protein, DNA, and RNA were measured from the chloroform/methanol-extracted TCA precipitates according to Lowry et al. (21), Burton (8), and Ogur and Rosen (22), respectively.

SDS-DISCONTINUOUS ELECTROPHORESIS: The samples were solubilized in 50 mM Tris/HCl (pH 7.4), 30 mM EGTA, 4% sodium dodecyl sulfate (SDS), 2.5% mercaptoethanol, and 15% glycerol. Discontinuous SDS-polyacrylamide gel electrophoresis was carried out in 10 and 15% slab gels according to Laemmli (19), except that the electrode buffer contained 0.1% mercaptoethanol. Gels were stained with Coomassie blue.

AMINO ACIDS: Amino acid analysis was carried out as described by Kickhöfen et al. (18), using a Durrum 500 amino acid analyzer (Durrum Instrument

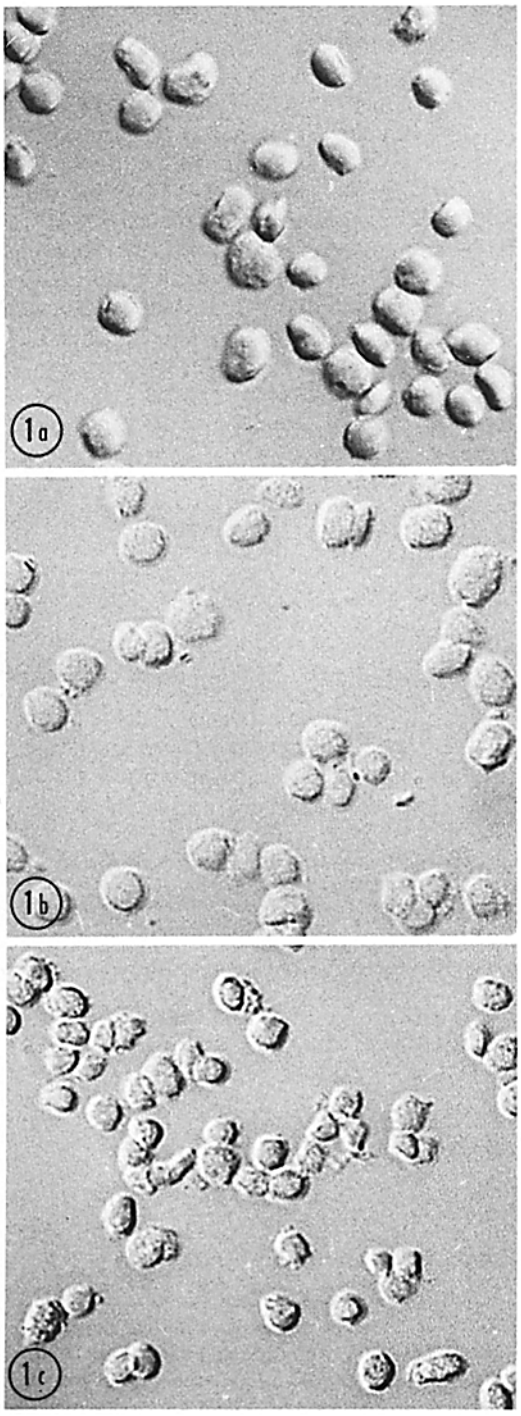

FIGURE 1 Survey micrographs (differential interference contrast) of macronuclei isolated from *Tetrahymena* (*a*), corresponding fractions of Ca/Mg-isolated expanded nuclear matrices (*b*), and contracted matrices (*c*). Macronuclei and matrices are suspended in Epon; this causes an aggregation which is only rarely observed in aqueous suspensions. All micrographs × 700.

Corp., Sunnyvale, Calif.). Cysteine was determined after oxidation with dimethylsulfoxide as described by Spencer and Wold (27).

CARBOHYDRATES: These were gas chromatographically determined according to Weckesser et al. (31).

RESULTS

Isolation procedure

Typical macronuclei isolated from *Tetrahymena* are shown in Fig. 1*a*. Their average diameters vary from experiment to experiment, ranging between 7.3 and 8.9 μm. They are composed of 66.0% protein, 24.0% DNA, 6.9% RNA, and 3.1% phospholipid (Table I). In preparing nuclear matrices from these macronuclei, we removed 89.7% of the phospholipid, 99.6% of the DNA, 98.5% of the RNA, and 74.8% of the protein by treatment with Triton X-100 and digestion with DNase and RNase followed by an extraction with 2 M NaCl (see details in Materials and Methods).

In a series of initial comparative experiments, we eventually found out that a contraction of nuclear matrices can be largely prevented when the enzymatic digestion is performed in the presence of the bivalent cations Ca and/or Mg in a total concentration of 125 mM, whereas monovalents such as Na in a total concentration of 250 mM were not so effective. In Table II, one can compare the diameters of the expanded matrices, which we have isolated from the same macronuclear fraction either with Ca alone or with Mg only, or with Ca and Mg in a 3:2 ratio. The Ca matrices contract by about 8%, whereas the Mg matrices and the Ca/Mg matrices are only contracted by about 2 and 3%, respectively. The Mg matrices, however, disintegrate after a prolonged time, whereas the Ca matrices and the Ca/Mg-matrices are stable (cf. also below for differences in the peptide pattern between these three expanded matrix types). Thus, we preferred the Ca/Mg method as final routine procedure for the isolation of expanded matrices. These expanded matrices can be still further slightly expanded for about 3 or 5% at incubation in 250 or 500 mM of a Ca/Mg (3:2) mixture, respectively. A progressive disintegration, however, accompanies this slight expansion.

TABLE I

Gross Composition of Macronuclei and Nuclear Matrices of Tetrahymena

	Macronuclei	Expanded matrices	Recovery
	pg/nucleus	*pg/matrix*	%
Protein	62.0	15.6	25.2
DNA	22.3	0.1	0.4
RNA	6.4	0.1	1.5
Phospholipid	2.9	0.3	10.3

Number of macronuclei and nuclear matrices were determined in a Fuchs-Rosenthal counting chamber (Hecht, Soudheim, West Germany).

TABLE II

Diameters of Macronuclei and Expanded Nuclear Matrices of Tetrahymena

	Nuclear matrices isolated with 125 mM:		
Macronuclei	Ca	Mg	Ca/Mg (3:2)
	diameter, μm		
8.15 ± 1.05	7.53 ± 0.92	7.98 ± 1.21	7.94 ± 0.95

A macronuclear fraction of *Tetrahymena* was divided into three aliquots from which the nuclear matrices were isolated in the presence of either Ca and Mg alone or Ca and Mg in a 3:2 ratio. For evaluating diameters, photographs were made from unfixed, freshly isolated fractions using differential interference contrast optics. On calibrated positives, at least 50 specimens were measured per fraction. Each specimen was measured twice (the long diameter and its orthogonal). Values are given with standard deviations.

Reversible Contraction

The expanded matrices are capable of contracting when we incubate them in 5 mM Ca/Mg (3:2) as one can see from Fig. 1*c* and Table III. The average diameter becomes reduced by about 24%. This would correspond to a volume contraction of about 55% (under the assumption that the expanded matrices are spheres). The contraction is reversed when we again raise the total Ca/Mg concentration to 125 mM. As Table III shows, the average diameter of the re-expanded matrices comes close to that of the original expanded matrices. A further raising of the bivalent concentration to 250 mM leads to an additional 3% increase in diameter of the re-expanded matrices, but also induces progressive disintegration. Re-expansion is only slightly possible with monovalents. When we incubate the contracted matrices with varying concentrations of NaCl, we observe no expansion with 125 mM NaCl whereas 250 mM and 500 mM NaCl induce ~8 and ~3% expansion of the diameter of the contracted matrices, respectively (cf. Table III).

In the contraction and re-expansion medium, we regularly included 0.1 mM ATP, which was found to have a stabilizing effect on the expanded matrices during isolation. However, ATP is not required for either contraction or re-expansion. In control experiments, we showed that these processes occur in the absence of ATP to the same extent as in the presence of 0.1 or 2.3 mM ATP. Moreover, we could not detect any significant phosphate release from ATP when it was present in a concentration of 0.1 mM. Furthermore, neither contraction nor re-expansion is inhibited by the sulfhydryl-blocker salyrgan (= mersalylnatrium; Serva, Heidelberg, W. Germany) even in such a high concentration as 5×10^{-2} M. Finally, it is still noteworthy that the expanded matrices isolated with either Ca or Mg alone are also capable of contracting in 5 mM Ca/Mg. These contracted Ca matrices and Mg matrices re-expand when the total Ca and Mg concentrations, respectively, are increased again to 125 mM.

Structure

In Fig. 2, one can compare the fine structure of a representative expanded matrix and a representative isolated macronucleus. The latter reveals: (*a*) a fine fibrillar granular network extending

TABLE III

Reversible Contraction of Tetrahymena Nuclear Matrices

	Experiment	Diameter	
		μm	%
Expanded matrix	1	8.52 ± 1.14	100
	2	7.94 ± 0.95	100
	3	7.47 ± 1.42	100
Contracted matrix	1	6.71 ± 0.93	79
	2	6.09 ± 0.69	77
	3	5.81 ± 0.95	78
Reexpanded matrix	1	8.13 ± 1.02	95
	2	7.59 ± 0.89	96
	3*	6.28 ± 0.83	84

Contraction and re-expansion of the nuclear matrices are induced as follows. The expanded matrices isolated in HCMT buffer are centrifugally concentrated at 900 *g* for 5 min and then incubated in CMT buffer, thus yielding contracted matrices. After centrifugal concentration, these matrices are re-expanded by incubating in HCMT buffer. Diameters are evaluated as described in Table II.
* In this experiment, contracted matrices have re-expanded in CMT containing 250 mM NaCl.

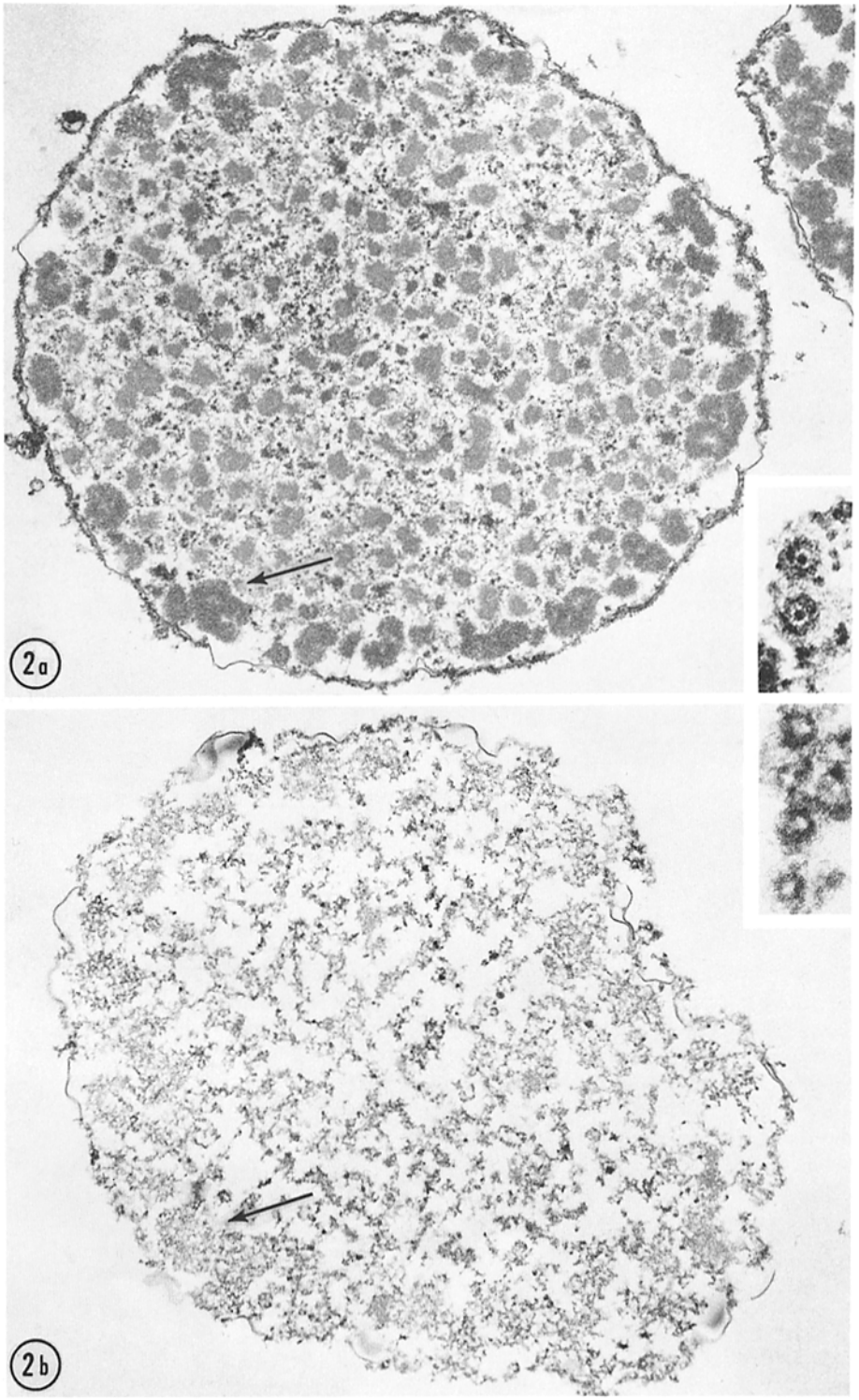

FIGURE 2 Electron micrographs of a representative macronucleus (*a*) isolated from *Tetrahymena* and a representative expanded matrix (*b*). The latter is composed of ~96% protein. Macronuclei and nuclear matrices are surrounded by a nuclear envelope and residual nuclear envelope, respectively. Arrows indicate nucleoli and residual nucleoli. Insets show nuclear pore complexes and the corresponding residual equivalents. × 20,000. Insets, × 80,000.

throughout the whole nucleus and, in between, a great many chromatin bodies uniformly distributed; (*b*) numerous peripherally located nucleoli; (*c*) a largely well-preserved nuclear envelope with characteristic nuclear pore complexes filled with central material. Residual equivalents to these structures, apart from the chromatin bodies, can also be detected in the expanded matrices: (*a*) a fibrillar granular network extending throughout the whole matrix; (*b*) residual nucleoli at the matrix periphery; (*c*) a residual nuclear envelope; this is composed of one peripheral layer, which can be resolved at some sites as a typical triple-layered "unit membrane" and which still bears residual nuclear pore complexes revealing only annular ring material. Occasionally, a second layer is closely attached to the outside of the residual nuclear envelope, which thus appears to correspond largely to the inner nuclear membrane.

Composition

The expanded matrix is composed essentially of proteins (96.2%) and traces of DNA (0.8%), RNA (0.5%), phospholipid (1.6%), and carbohydrates (0.9%). The last are composed of glucose, mannose, and an unidentified sugar in the ratio of 1:5.4:5.7. Table IV shows that the ratio of acidic to basic amino acids of the expanded matrix is 1.55, which is similar to that recently described for rat liver nuclear matrices (6). Figs. 3 and 4

TABLE IV

Amino Acid Composition of the Tetrahymena Nuclear Matrix

	mol/100 mol
Lysine	9.79
Arginine	1.85
Histidine	4.58
Aspartic acid	11.76
Threonine	5.15
Serine	7.35
Glutamic acid	13.45
Proline	4.13
Glycine	5.98
Alanine	6.33
½ Cystine	1.40
Valine	4.47
Methionine	2.13
Isoleucine	5.15
Leucine	7.85
Tyrosine	3.64
Phenylalanine	4.69
Ratio $\frac{\text{acidic}}{\text{basic}}$	1.55

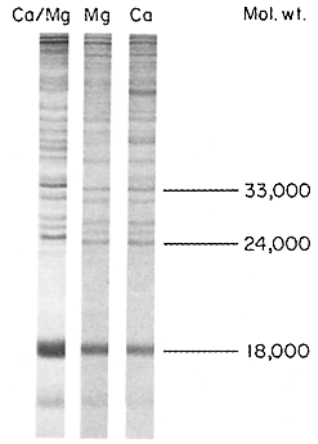

FIGURE 3 Stained 15% SDS-polyacrylamide gel electrophoretograms of *Tetrahymena* nuclear matrices isolated with 125 mM Ca and Mg in the ratio 3:2, or with Ca or Mg alone.

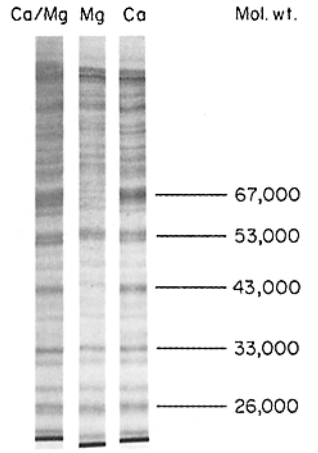

FIGURE 4 Stained 10% SDS-polyacrylamide gel electrophoretograms of *Tetrahymena* nuclear matrices isolated with Ca/Mg (3:2), or only Ca or only Mg.

reveal the peptide pattern of the expanded matrix in 15 and 10% SDS electrophoretograms. The most prominent peptide fraction exhibits a mol wt of ~18,000. The other outstanding peptides have mol wt of ~24,000, ~33,000, ~43,000, ~53,000, and ~67,000. It is noteworthy that the same peptide pattern is also found in that expanded matrix type which was isolated in the presence of Ca only. The expanded Mg matrix, however, which disintegrates after prolonged time intervals, contains only traces of the 43,000 and 67,000 mol wt proteins. These might be responsible for the stability of the nuclear matrix.

DISCUSSION

We have isolated from *Tetrahymena* macronuclei an expanded nuclear matrix which is capable of reversible contraction. This nuclear matrix is composed essentially of "acidic" proteins, which contribute ~25% to the total nuclear proteins. The predominant protein has a mol wt of ~18,000. This protein has not been detected in the "contracted" nuclear matrix of rat liver nuclei (4, 6) and has been found only in minimal traces in that nuclear matrix type that we have recently isolated from *Tetrahymena* macronuclei under contraction conditions (16). Interestingly, this contracted nuclear matrix type isolated from *Tetrahymena* can be only slightly expanded under our present reexpansion conditions (G. Herlan and F. Wunderlich, unpublished results). However, this expansion never reaches the extent observed with our present expanded matrix. Thus, we conclude that the 18,000 mol wt protein is somehow involved in the reversible contractile process of the expanded nuclear matrix.

Remarkably, a protein of similar molecular weight is also found in actin-myosin controlled contraction systems, namely the troponin C (for review see reference 28), and in the contractile spasmonemes of Vorticellida (for review see reference 2). Our data, however, indicate that the reversible contraction differs from both the Vorticellid and the actin-myosin systems, though we find matrix proteins similar in molecular weights to actin and troponins. The actin-myosin system depends on ATP and is inhibited by sulfhydryl-blockers such as salyrgan, in contrast to the Vorticellid-system and the *Tetrahymena* matrix system. The Vorticellid system is specifically regulated by Ca, i.e., $<10^{-5}$ M Ca induce contraction whereas higher Ca concentrations induce expansion. The nuclear matrix, however, contracts and expands at 5 and 125 mM Ca/Mg concentrations, respectively. Though the mechanism of the reversible contraction of the nuclear matrix remains to be elucidated, a plausible explanation would at present be that this mechanism operates on a simple bivalent electrostatic basis. For instance, negatively and positively charged groups of the nuclear matrix proteins mutually attract each other in the contracted state. In the expanded state, the Ca and Mg cations are concentrated around the negatively charged groups which thus become neutralized or even could gain a surplus of positive charge, which then leads to electrostatic repulsion.

At the moment, we can not determine whether the reversible contractility of the nuclear matrix has any biological significance at all. Indeed, we can induce swelling and contraction of the isolated macronuclei under the same conditions that we use with the isolated nuclear matrix. However, we are unable to determine whether this is due to reversible contraction of the matrix equivalent in these macronuclei or to possible swelling and contraction of the nuclear chromatin. On the other hand, Ca and Mg are accumulated more in the cell nucleus than in the cytoplasm in vivo (26, 29). In rat liver cells, for example, the nuclear Ca/Mg concentration can be approximated to be ~25 mM (for review see reference 25). Interestingly, Ca and Mg can be either uniformly distributed or sequestered at particular sites, depending on the RNA synthetic activity as revealed, for example, in Sertoli cell nuclei (30). Thus, it would be attractive to speculate that, in vivo, the matrix equivalent is also capable of only local swelling and contraction. Finally, however, one must await the isolation of further reversible contractile nuclear matrix types from other cell nuclei and see whether they follow the same contraction-expansion conditions as the nuclear matrix isolated from *Tetrahymena* macronuclei.

We are indebted to Miss C. Bucherer for technical assistance, to Dr. I. Fromme for determining the carbohydrates, to Dr. R. Warth for performing the amino acid analysis, and to Dr. J. Thompson for critical reading of the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft (Wu 73/5).

Received for publication 2 September 1976, and in revised form 14 December 1976.

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