Isolation and Characterization of an RNA-Containing Nuclear Matrix from *Tetrahymena* Macronuclei†

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ABSTRACT: We have developed a procedure allowing the isolation of an RNA-containing nuclear matrix (RNM) from *Tetrahymena* macronuclei in high yield (~60%) by low- and high-salt extractions including DNase treatment. Electron microscopy reveals that the RNM still shows the nuclear sphere shape and is composed of condensed fibrillar–granular material in its interior, whereas the "residual nucleoli" located at the periphery and structurally linked to the "residual nuclear envelope" essentially consist of more loosened fibrillar material, including tiny fibrils (~4 nm in diameter). The protein pattern of the RNM, as revealed by electrophoresis on 10–15% $NaDodSO_4$–polyacrylamide gradient gels, is nearly identical with that of the RNA-free nuclear matrix showing bands in the molecular weight region between 12000 and 300000 with one prominent band of 18000 molecular weight. The majority of the RNA (~85%) included in the RNM consists of specific pre-rRNA fractions (A–D). Fraction A containing the primary stable transcription product of the rDNA seems to be almost completely retained in the RNM, whereas fractions B and D, the direct nuclear precursors to the cytoplasmic 26S and 17S rRNAs, are partly solubilized during RNM preparation. Moreover, some 5S rRNA and at least two specific low molecular weight (LMW) RNAs with low turnover (T_A and T_C) are part of the RNM. One LMW RNA component (T_D) which is contained in whole macronuclei is lost during RNM isolation, together with some material from the 5S region. [^{3}H]Uridine-labeling kinetics of the RNA recovered in the RNM compared with those of the solubilized RNA show that the rapidly labeled RNAs are at first tightly bound to the nuclear matrix and then successively become more susceptible to solubilization during the extraction procedure. Our results are compatible with the view that processing and intranuclear translocation, at least of rRNA, occur along the nuclear matrix elements in a highly ordered process.

A special nuclear structure, termed nuclear matrix, has recently been identified in various mammalian cells [Berezney & Coffey, 1974, 1976, 1977; Hildebrand et al., 1975; Comings & Okada, 1976; Hodge et al., 1977; cf. also Riley et al. (1975) and Riley & Keller (1978)] and in the ciliated protozoan *Tetrahymena pyriformis* (Herlan & Wunderlich, 1976; Wunderlich & Herlan, 1977). This nuclear matrix is essentially composed of protein—each nuclear matrix type exhibiting its own specific protein pattern—and obviously represents the fundamental framework determining the shape and integrity of cell nuclei [for reviews see Berezney & Coffey (1976), Wunderlich et al. (1976), and Wunderlich (1978)]. Recent findings indicate that the nuclear matrix may not be regarded as a "rigid skeleton" but rather as an elastic and dynamic structure (Wunderlich & Herlan, 1977; Herlan et al., 1978).

First indications suggest that the nuclear matrix, besides its structural role, may also be involved in specific metabolic functions of the nucleus, e.g., in the initiation and/or progression of DNA replication (Berezney & Coffey, 1975; Allen et al., 1977; Hemminki, 1977; Wanka et al., 1977), in the initiation of adenovirus assembly (Hodge et al., 1977), and in the mediation of hormonal signals (Barrack et al., 1977). In view of previous and recent investigations, the nuclear matrix may also be expected to play a significant role in the processing and ordered intranuclear translocation of RNA toward the nuclear pore complexes into the cytoplasm [for review see, e.g., Wunderlich et al. (1976)]. For instance, a "ribonucleoprotein (RNP) network" bound to the nuclear envelope was obtained after high-salt extraction of different mammalian nuclei [e.g., Smetana et al. (1963), Narayan et al. (1967), and Faiferman & Pogo (1975)], and a "nuclear skeleton" associated with RNA was isolated from rat liver nuclei (Herman et al., 1976; Zieve & Penman, 1976; Miller et al., 1978a,b). At the present stage of knowledge, it is reasonable to assume that these two nuclear residual fractions contain nuclear matrix elements.

A prerequisite step toward a more precise understanding of the role of the nuclear matrix in nRNA metabolism is the isolation of the nuclear matrix in high yield which still contains a large portion of nRNA. For this study we have chosen *T. pyriformis* since we have already isolated and characterized the RNA-free nuclear matrix from the macronucleus of this unicellular eukaryote (Herlan & Wunderlich, 1976; Wunderlich & Herlan, 1977; Herlan et al., 1978) and since the RNA metabolism is characterized by high rates of synthesis, processing, and nucleocytoplasmic translocation of RNA, especially rRNA (Kumar, 1970; Leick & Anderson, 1970; Prescott et al., 1971; Wunderlich, 1972; Eckert et al., 1975).

Materials and Methods

Culture and Labeling Conditions of Cells. Static log-phase 5-L cultures of *T. pyriformis* (amicronucleate) were axenically maintained in proteose pepton yeast extract (PPY) medium as described previously (Ronai & Wunderlich, 1975). At a cell density of 30000–40000 cells/mL, the cells were concentrated into 400 mL of PPY medium by continuous flow centrifugation and then harvested as described elsewhere (Herlan et al., 1978). For labeling, the cells were incubated with 100 μCi of [^{3}H]uridine ([5,6-^{3}H]uridine; sp act. 40–50 Ci/mmol; NEN Chemicals), immediately after continuous flow centrifugation, for various times at 28 °C under aeration of the culture fluid. Labeling was stopped by pouring the cells onto 150 mL of frozen crushed PPY medium and immediate harvesting as described by Herlan et al. (1978).

Isolation of Macronuclei. Cells were lysed in PST buffer (0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.1 mM ATP, 1% poly(vinylpyrrolidone) K-90, and 20 mM Tris-HCl, pH 7.4) at 0–4 °C by addition of sodium deoxycholate to a final concentration of 0.08% (w/v) and immediate centrifugation at 2800*g* for 3 min (Herlan & Wunderlich, 1976). Macronuclei were isolated and purified according to our two-step

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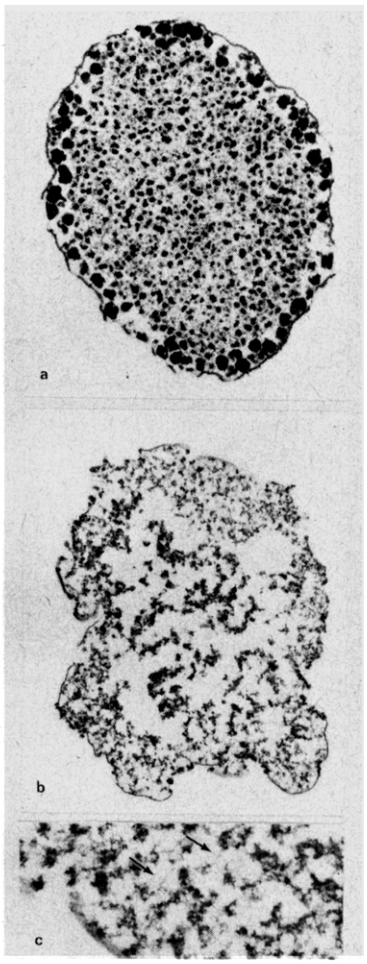

FIGURE 1: Thin-sectioning survey electron micrographs of (a) a representative macronucleus isolated from *Tetrahymena* and (b) an RNA-containing nuclear matrix (5000× magnification). (c) Residual nucleoli showing tiny 4-nm fibrils (arrows) (18 000× magnification).

sucrose gradient technique developed previously (Herlan & Wunderlich, 1976) with the modification of omitting the prepurification step over 72% sucrose and the inclusion of 0.1 mM ATP (Wunderlich & Herlan, 1977).

Electron Microscopy. The specimens were fixed with 1% glutaraldehyde and postfixed with 2% OsO_4, followed by an incubation in 1% uranyl acetate as described recently (Wunderlich & Herlan, 1977). Dehydration was carried out stepwise through graded solutions of ethanol and two final incubations in propylene oxide before embedding in Epon. Thin sections were cut on a OmU_2-Reichert ultramicrotome, double stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiscope I A.

Chemical Determinations. Samples were precipitated and washed with trichloroacetic acid as described recently (Wunderlich & Herlan, 1977). Total protein, DNA, and RNA were determined in the precipitates according to Lowry et al. (1951), Burton (1956), and Ogur & Rosen (1950), respectively.

$NaDodSO_4$ Disc Electrophoresis. The samples were solubilized in CMT (cf. legend to Figure 2), 4% $NaDodSO_4$, and 2.5% mercaptoethanol. After addition of 15% glycerol, the proteins were separated on 10–15% $NaDodSO_4$–polyacrylamide gradient slab gels according to Laemmli (1970), except that the electrode buffer contained 0.1% mercaptoethanol. Gels were stained with Coomassie blue.

RNA Analysis. RNA was isolated by proteinase K digestion (500 μg/mL in 0.1 M NaCl, 0.5% sodium dodecyl sulfate, and 10 mM Tris-HCl, pH 7.3; 20 min at room

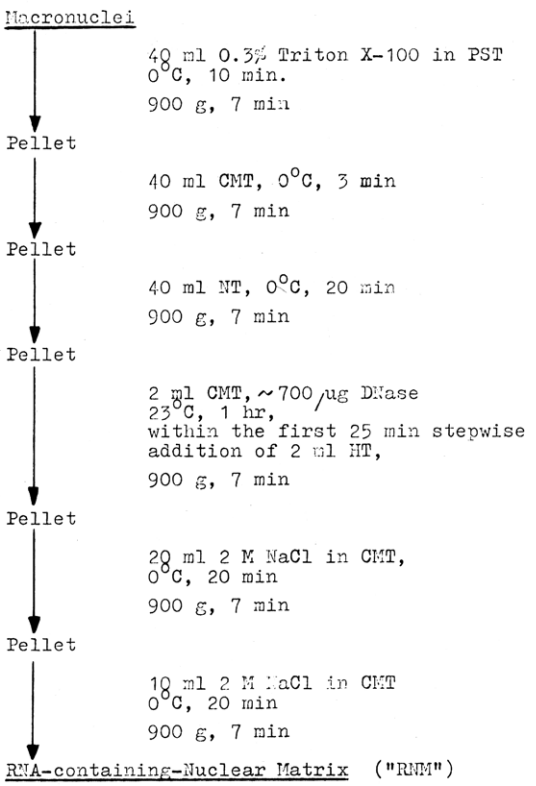

FIGURE 2: Scheme of the isolation method of the RNM from *Tetrahymena* macronuclei. Abbreviations for the buffers used are the following: PST (0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.1 mM ATP, 1% poly(vinylpyrrolidone) K-90, and 20 mM Tris-HCl, pH 7.4); CMT (2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.1 mM ATP, and 20 mM Tris-HCl, pH 7.4); NT (0.1 M NaCl, 0.4 mM $MgCl_2$, 0.6 mM $CaCl_2$, 0.1 mM ATP, and 20 mM Tris-HCl, pH 8.0); HT (100 mM $MgCl_2$, 150 mM $CaCl_2$, 0.1 mM ATP, 20 mM Tris-HCl, pH 7.4). DNase I was obtained from Worthington Biochemical Corp. (Freehold, NJ).

temperature), followed by DNase I treatment (50 μg/mL in 3 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 7.3; 30 min at 0 °C) and a modification of Kirby's cold "phenol–cresol method" (Kirby, 1968) essentially as described previously (Eckert et al., 1978). The separation of the RNAs on aqueous polyacrylamide gels has also been performed as described in detail recently (Eckert et al., 1978).

Results

Isolation, Structure, and Composition of the RNA-Containing Nuclear Matrix. The macronuclei which we have isolated from *Tetrahymena* have preserved their typical in situ appearance (Figure 1a). Most obvious are the numerous small condensed chromatin regions embedded in a fine fibrillar–granular network, the great number of peripherally located nucleoli, and the surrounding, largely intact nuclear envelope. From these nuclei we have isolated an RNA-containing nuclear matrix (RNM) by a series of extractions as outlined in Figure 2. In principle, the nuclei are treated with 0.3% Triton X-100 before being incubated at low ionic strength, i.e., at a final Ca/Mg (3:2) concentration of 1 mM, followed by digestion of DNA with DNase and two final extractions in 2 M NaCl. By this method the RNM can be isolated within 3 h with an average yield of approximately 60%; i.e., 6×10^5 RNMs are obtained from 10^6 nuclei.

The fine structure of a typical RNM is shown in Figure 1b. The interior is composed of condensed fibrillar–granular material, whereas the material at the periphery is more loose in appearance and regularly contains thin fibers of a diameter of about 4 nm (Figure 1c). This peripheral material obviously

Table I: Gross Composition of Macronuclei and RNA-Containing Nuclear Matrix of *Tetrahymena*[a]

	macronuclei (pg/nucleus)	RNA-containing nuclear matrix (pg/RNM)	recovery (%)
protein	74.9	10.8	14.2
DNA	27.6	0.6	2.1
RNA	12.4	7.4	59.7

[a] Averages of three experiments. The number of macronuclei and RNMs was determined in a Fuchs–Rosenthal counting chamber (Hecht, Sondheim, West Germany).

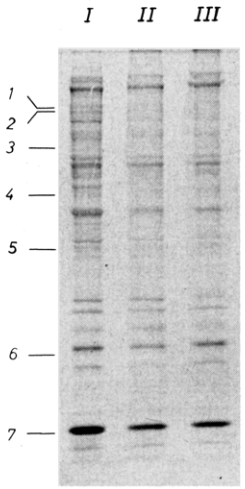

FIGURE 3: The 10–15% $NaDodSO_4$–polyacrylamide gradient gel of the RNA-containing nuclear matrix (I), the nuclear matrix (II) prepared as described previously (Wunderlich & Herlan, 1977), and the RNA-containing nuclear matrix after RNase treatment at 23 °C for 30 min (III). Standards: 1, 2, and 3, β', β, and σ subunits of RNA polymerase from *E. coli* (165 000, 155 000, 95 000); 4, bovine serum albumin (67 000); 5, ovalbumin (43 000); 6, chymotrypsin (23 000); 7, myoglobin (17 800).

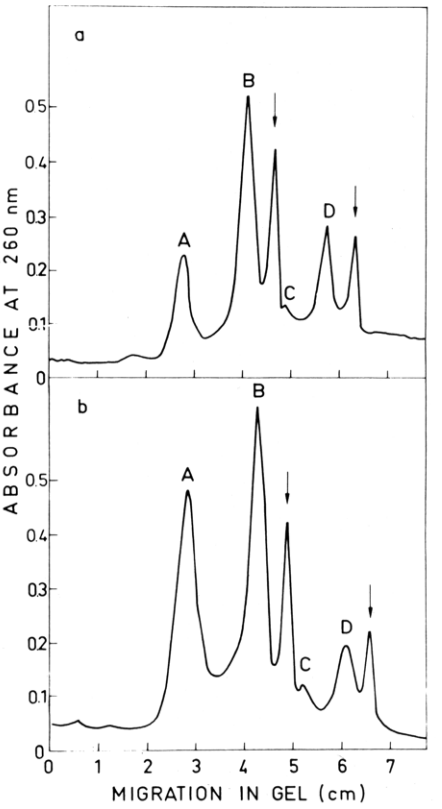

FIGURE 4: Gel electrophoretic separation of the high molecular weight RNAs contained in (a) nuclei and (b) the RNM. About 15 and 20 μg of the RNA extracted from isolated macronuclei and from the RNM, respectively, were dissolved in 20 μL of 2.5 × concentrated electrophoresis buffer (36 mM Tris-HCl, 30 mM $NaH_2PO_4 \cdot H_2O$, and 1 mM EDTA, pH 7.6) containing 0.1% $NaDodSO_4$ and 10% sucrose, layered onto cylindrical (6 mm in diameter and 80-mm long) 2.2% polyacrylamide gels prepared according to Loening (1969), and run for 3.5 h at 5 mA/tube. After electrophoresis the gels were scanned for absorbance at 260 nm. rRNA from *Escherichia coli* was added to all samples as an internal molecular weight standard (23 S: 1.05×10^6 and 16 S: 0.525×10^6).

represents the "residual nucleoli" which are somewhat separated from the "collapsed" internal material. The residual nucleoli are structurally linked to the surrounding peripheral layer revealing small rings and with an outer diameter of approximately 70 nm. This layer most probably represents the residual equivalent structure of the inner nuclear membrane still bearing residual nuclear pore complexes.

The *chemical composition* of the RNM is given in Table I. On the average an RNM consists of 57.4% protein, 3.2% DNA, and 39.4% RNA, which corresponds to 14.2, 2.1, and 59.7% of the total nuclear protein, DNA, and RNA, respectively.

The *protein pattern* of the RNM in 10–15% $NaDodSO_4$–polyacrylamide gradient gels is shown in Figure 3. It reveals many bands in the molecular weight region between about 12 000 and 300 000. The major peptide fraction has a molecular weight of approximately 18 000. A qualitatively similar protein pattern is also obtained after RNase treatment of the RNM. This pattern in turn is identical with that of the nuclear matrix (NM) prepared from *Tetrahymena* nuclei (Figure 3) as described previously (Wunderlich & Herlan, 1977). Only one RNM protein with a molecular weight in the region of 200 000 is not detected in the RNase-treated RNM and the NM (Figure 3). Moreover, some differences exist in the staining intensities of some peptides, in particular of those in the molecular weight range of 130 000, 55 000, and 43 000.

Characterization of the Nuclear Matrix-Bound RNA. (A) High Molecular Weight RNA. The RNA isolated from *Tetrahymena* nuclei reveals four fractions (designated A–D) in 2.2% polyacrylamide gels (Figure 4a), accounting for about 17 (A), 36 (B), 3 (C), and 14% (D) of the total. These fractions have recently been shown to contain rRNA precursor molecules (Eckert et al., 1978). Fraction A ($M_r = 2.3 \times 10^6$) contains the first stable transcription products of the rDNA; fraction B ($M_r = 1.34 \times 10^6$) and fraction D ($M_r = 0.66 \times 10^6$) represent the direct nuclear precursors to the cytoplasmic 26S rRNA ($M_r = 1.27 \times 10^6$) and 17S rRNA ($M_r = 0.66 \times 10^6$), respectively. The small fraction C contains very probably specific degradation products of fraction B as has been shown by hybridization to specific rDNA restriction fragments (Engberg, Din, Kaffenberger and Eckert, unpublished experiments). In the RNM the pre-rRNA fractions are also found as major components (Figure 4b), accounting for about 32 (A), 40 (B), 5 (C), and 13% (D) of total RNA. This shows that, compared with whole nuclei, fraction A is especially enriched and seems to be almost completely recovered in the RNM. Fractions B and D, however, have relatively decreased (compared to fraction A), indicating that molecules from these fractions have been preferentially solubilized during the preparation of the RNM.

(B) Low Molecular Weight RNA. Tetrahymena nuclei also include specific low molecular weight (LMW) RNAs as can be detected in 7.5% gels (Figure 5a). Besides a prominent peak in the 5S region (containing, inter alia, the 5S rRNA; see below), three additional significant fractions are found,

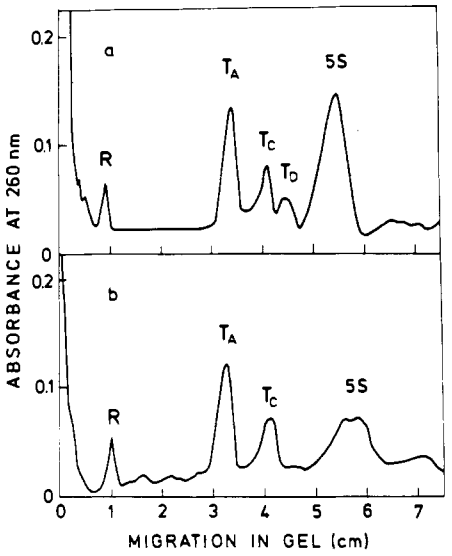

FIGURE 5: Gel electrophoretic separation of the low molecular weight RNA components in (a) nuclei and (b) the RNM. About 45 μg of the RNA extracted from (a) macronuclei and 40 μg extracted from (b) the RNM were separated in 7.5% polyacrylamide gels at 5 mA/tube for 3 h.

designated T_A, T_C, and T_D, which account for 1.8, 0.6, and 0.4% of the total nRNA, respectively. They are probably similar to the T components (T_1–T_3) described for *Tetrahymena* nuclei by Hellung-Larsen et al. (1971). From their electrophoretic mobilities relative to tRNA and 5S rRNA, we have determined molecular weights of approximately 73 000 (T_A), 66 000 (T_C), and 52 000 (T_D) for these components. With these molecular weights they are comparable in size to the major snRNA fractions found in mammalian cell nuclei [A, C, and D according to the nomenclature of Weinberg & Penman (1969) or U_1, U_2, and U_3 as designated by Ro-Choi & Busch (1974)]. In addition to the 5S fraction and the T components, we found a further LMW RNA fraction, designated R, which just entered the 7.5% gels. Possibly, this fraction might correspond to the T_4 component detected in macronuclear as well as in cytoplasmic RNA of *Tetrahymena* by Hellung-Larsen et al. (1971).

Compared with whole nuclei, T_A and T_C are relatively enriched in the RNM (Figure 5b) accounting for about 3.3% and 1.1% of total RNA, while T_D is absent. The material in the 5S region has relatively decreased and shows some microheterogeneity. The latter finding suggests that this region, in addition to 5S rRNA, contains specific RNA molecules that are confined to the nucleus, comparable with the situation in mammalian cells (cf. Weinberg & Penman, 1969; Ro-Choi et al., 1971; Ro-Choi & Busch, 1974; Zieve & Penman, 1976), and that these molecules show some specific retention in the RNM.

Labeling Kinetics of the Nuclear Matrix-Bound RNA. Under our labeling conditions, *Tetrahymena* cells incorporate [^{3}H]uridine into total cellular RNA at a linear rate for at least 60 min (Figure 6, inset). Figure 6 shows the kinetics of [^{3}H]uridine incorporation into the RNA which is associated with the RNM and that portion of macronuclear RNA which is solubilized during the isolation procedure of the RNM. The latter portion accounts for approximately 40% of total nRNA. The specific radioactivity of the matrix-bound RNA rapidly increases during the first 10 min and then continues to rise at a much lower rate for up to 40 min of labeling, whereas the "solubilized" nRNA initially has a much lower specific radioactivity which then progressively increases and nearly

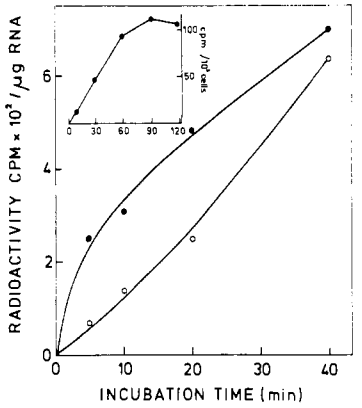

FIGURE 6: [^{3}H]Uridine incorporation into whole *Tetrahymena* cells (inset), the RNM (●), and the solubilized nRNA (○). Solubilized RNA and corresponding cpm values are the difference between RNA and cpm per nucleus and RNA and cpm per RNM, respectively.

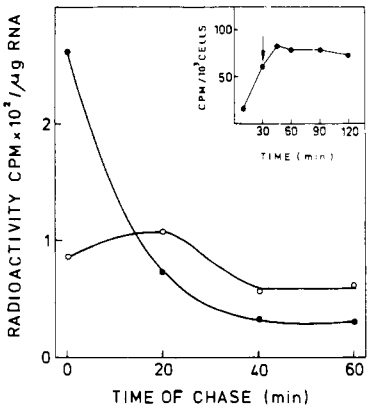

FIGURE 7: Pulse–chase labeling kinetics of nuclear matrix bound RNA (●) and solubilized RNA (○). *Tetrahymena* cells were pulse labeled with [^{3}H]uridine for 5 min and then chased by addition of a 10^6-fold excess of nonradioactive uridine to the medium. Inset shows incorporation of [^{3}H]uridine into whole *Tetrahymena* cells; arrow indicates the time of addition of the unlabeled uridine.

reaches the level of the specific radioactivity of the nuclear matrix-bound RNA after 40 min of labeling (Figure 6).

In the pulse–chase experiments cells were labeled with [^{3}H]uridine for 5 min and then "chased" with a 10^6-fold excess of unlabeled uridine. Under these conditions the net increase of radioactivity in RNA of whole cells was stopped after about 10 min (Figure 7, inset). The specific radioactivity of the matrix-bound RNA rapidly decreases during the chase to a relatively low constant level after 40 min. In contrast, the specific radioactivity of the "solubilized" RNA (which was only about one-third of that of the matrix-bound RNA after the 5-min pulse; cf. also Figure 6) slightly increases at first, then decreases and remains constant after 40 min.

The radioactivity patterns of the matrix-bound RNA on 2.2% gels reveal that pre-rRNA fraction A has at least a threefold higher specific radioactivity than fraction B after 5 min of labeling with [^{3}H]uridine (Figure 8a). Some labeled heterogeneously migrating material was found in the high molecular weight region with an electrophoretic mobility lower than that of fraction A, as well as between fraction C and D. The radioactivity in fraction A after 30 min of labeling (Figure 8b) was still somewhat higher than in B, and significant amounts of label were also found associated with the peaks C and D.

The labeling pattern of the LMW RNAs associated with the RNM shows that T_A and T_C are only slightly labeled after 30 min incubation of the cells with [^{3}H]uridine (Figure 9).

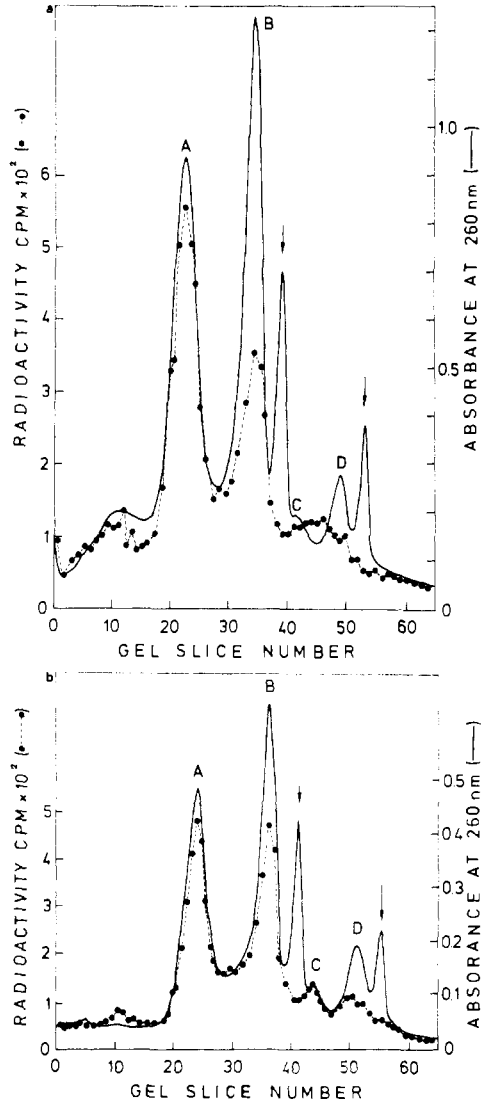

FIGURE 8: Radioactivity pattern of the high molecular weight RNAs contained in the RNM. Cells were labeled with [^{3}H]uridine for (a) 5 min and (b) 30 min, respectively, and the RNM was isolated as outlined in Figure 2. Aliquots of the RNA [about 30 μg in (a) and 20 μg in (b)] were separated on 2.2% polyacrylamide gels as described in the legend to Figure 4. After electrophoresis the gels were cut into 1-mm slices and assayed for radioactivity. *E. coli* rRNAs are marked by arrows.

Higher label is found in the 5S region and in the monodisperse fraction R.

Discussion

Characteristics of the RNA-Containing Nuclear Matrix. From purified *Tetrahymena* macronuclei we have isolated an RNA-containing nuclear matrix (RNM) in high yield (~60%) by a series of low- and high-salt extractions including DNase treatment. This fractionation procedure preserves the typical spherical shape and the basic structure of the original nuclei. The RNM reveals condensed internal material which is clearly separated from the peripheral residual structures of the numerous extrachromosomal nucleoli. This peripheral material contains tiny fibrils (~4 nm in diameter), probably representing RNP strands. In this regard, the fine structural appearance of the RNM differs from that of the RNA-free nuclear matrix isolated previously from the same *Tetrahymena* cells (cf. Wunderlich & Herlan, 1977).

Approximately, 60% of the total nRNA can be recovered in the RNM. This RNA content, at first sight, appears to be

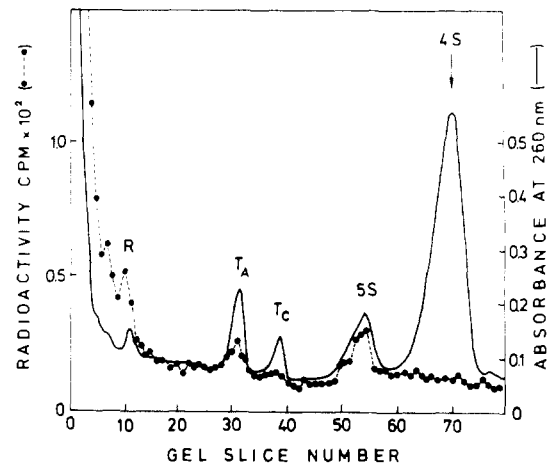

FIGURE 9: Radioactivity pattern of the low molecular weight RNAs associated with the RNM. About 50 μg of RNA extracted from the RNM isolated from cells prelabeled with [^{3}H]uridine for 30 min was separated on a 7.5% gel, together with about 10 μg of tRNA from yeast. After UV scanning, the gel was sliced and assayed for radioactivity. Arrow marks tRNA.

twice as high as reported for comparable subfractions of rat liver cell nuclei: namely, the nuclear matrix fraction before RNase treatment (about 30% RNA; Berezney & Coffey, 1976) and the "nuclear skeleton" fraction (about 31% RNA; Miller et al., 1978a). The recovery of RNA in these fractions, however, was determined relative to total RNA in the starting nuclei. Such calculations neglect the fact that a more or less considerable portion of the nuclei is lost during these fractionation procedures. Indeed, when Miller et al. (1978a) recalculated their data on the basis of recovery, the nuclear skeleton was found to include about 50% of total nRNA. In rat liver cells, the nuclear matrix reveals an almost identical protein pattern before and after RNase treatment (Berezney & Coffey, 1976). This corresponds to the situation in *Tetrahymena*: the protein pattern of the RNM is very similar before and after RNase treatment. This pattern in turn is very similar to that of the nuclear matrix isolated, though with a somewhat different method, from *Tetrahymena* nuclei. This demonstrates that the RNM indeed includes the nuclear matrix as major protein components. At least some of these protein components and not the RNA components are responsible for the structural integrity of the RNM, since RNase treatment, in contrast to Pronase treatment (Wunderlich & Herlan, unpublished experiments), does not disintegrate the structure of the RNM.

Characterization and Dynamics of the Nuclear Matrix-Bound RNA. The majority of the RNA (about 85%) recovered in the RNM represents the specific rRNA precursor fractions which are also found in whole *Tetrahymena* nuclei. The presence in the RNM of a high proportion of the first stable transcription product of the rDNA (in fraction A; cf. Eckert et al., 1978) as well as the [^{3}H]uridine labeling kinetics shows that the pre-rRNA molecules become rapidly bound to the nuclear matrix elements after their synthesis, specifically with those of the numerous extrachromosomal nucleoli of the macronucleus. Moreover, the presence of the direct nuclear precursor of the cytoplasmic 26S and 17S rRNAs (fractions B and D) indicates that the pre-rRNAs are at least partly associated with these structures throughout their lifetime in the macronucleus. This confirms the notion, mainly suggested from fine structural observations, that the rRNA precursor molecules, at least until their passage through the nuclear pore complexes, are integrated into continuous structures (Monneron & Bernhard, 1969; for review see Busch & Smetana,

1970; Wunderlich et al., 1976). Our findings, however, also indicate that the association of the rRNA precursors with the nuclear matrix decreases as the processing and/or the intranuclear translocation of these molecules proceeds [cf. also Augenlicht & Lipkin (1976)]. This is in accord with the general finding, in various cells, that the RNP particles containing the immediate precursors to the cytoplasmic rRNAs can be liberated more easily from nuclei or nucleoli than the "preribosomes" (80S) containing the stable transcription product of the rDNA [e.g., Liau & Perry, (1969); for review see Warner (1974)].

Besides the specific pre-rRNA fractions we have observed only a very small amount of rapidly labeled heterogeneously migrating RNA of high molecular weight associated with the nuclear matrix. This could be due to several reasons. First, our RNA-extraction procedure, though optimal for rRNA, may be not suited for extracting hnRNA. Secondly, though hnRNA has not yet been characterized in *Tetrahymena* it may be that, in contrast to mammals, the proportion of hnRNA to pre-RNA is lower in *Tetrahymena* as has been found in other lower eukaryotes (cf. Shulman et al., 1977). Thirdly, the hnRNA could have been extracted during the isolation of the RNM. Consistently, most of the hnRNA in mammalian cells can normally be extracted from "intact" nuclei in the form of RNP particles by low-salt buffers (0.1–0.14 M NaCl at pH 8.0; cf. Samarina et al., 1968; Martin & McCarthy, 1972; Beyer et al., 1977). Recently, however, Pogo and collaborators (Faiferman & Pogo, 1975; Miller et al., 1978a) and Herman et al. (1976) have found hnRNA as the major labeled component in the nuclear skeleton isolated from mammalian cell nuclei by DNase and high-salt treatment (0.5–0.9 M NaCl).

In addition to the high molecular weight RNAs, at least two specific LMW RNA fractions are part of the *Tetrahymena* RNM. From their relative enrichment, the components T_A and T_C seem to be completely retained in the RNM, whereas the material in the 5S region has relatively decreased compared with whole macronuclei. This loss possibly includes, besides the free nuclear 5S rRNA (cf. Auger & Tiollais, 1974), those 5S rRNA molecules which are part of the RNP particles containing pre-rRNA fraction B which were solubilized during RNM preparation. The fact that specific LMW RNAs are part of the RNM in *Tetrahymena* corresponds to the situation in mammalian cell nuclei where a more or less high proportion of snRNAs has been found to be associated with the nuclear RNP network (Busch & Smetana, 1970) or with the nuclear skeleton (Zieve & Penman, 1976; Miller et al., 1978b). Whether some of these RNAs with low turnover are permanent structural components of the nuclear matrix or whether some are specifically associated with the RNP particles carrying hnRNA (cf. Deimel et al., 1977; Miller et al., 1978b) will have to be clarified by future investigations.

There are some indications that component T_A in the *Tetrahymena* RNM may be related to a specific small nuclear (sn) RNA component (sn A or U_3 according to Zieve & Penman (1976) and Ro-Choi & Busch (1974)) in mammalian cells, which has been found to be exclusively located in the nucleoli (Weinberg & Penman, 1969) and transiently associated with 28–30S pre-rRNA [Prestayko et al., 1970; for reviews see Herman et al. (1976) and Zieve & Penman (1976)]. (a) T_A has a similar size (molecular weight about 75 000 corresponding to about 230 nucleotides). (b) T_A is tightly bound to the nuclear matrix which includes a substantial amount of residual nucleolar structures. (c) A component with identical electrophoretic mobility with that of T_A was found hydrogen-bonded to nuclear rRNA precursor fraction B (Eckert et al., 1978). The high level of T_A compared to other snRNAs in *Tetrahymena*, which is different from the proportion of component A in mammalian cells [especially compared with components C and D; cf. Weinberg & Penman (1969) and Hellung-Larsen & Frederiksen (1972, 1977)], could be explained by the large number of extrachromosomal nucleoli in the macronucleus, including a high level of pre-rRNA fraction B.

Although from the present results final conclusions about the precise role of the nuclear matrix in RNA metabolism are premature, our data are compatible with the view that the known coupling of transcription, processing, and translocation of rRNA in *Tetrahymena* [cf. Wunderlich (1972), Eckert et al. (1975), and Eckert (1977)] occurs along the nuclear matrix elements in a highly ordered, possibly feedback controlled fashion (Wunderlich et al., 1976).

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Nitrogen-15 and Carbon-13 Dynamic Nuclear Magnetic Resonance Study of Chain Segmental Motion of the Peptidoglycan Pentaglycine Chain of ^{15}N-Gly- and $^{13}C_2$-Gly-Labeled *Staphylococcus aureus* Cells and Isolated Cell Walls†

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ABSTRACT: The 9.12-MHz ^{15}N and 67.89-MHz ^{13}C NMR spectra were obtained for ^{15}N uniformly labeled *Staphylococcus aureus* and ^{15}N-Gly and $^{13}C_2$-Gly specifically labeled *S. aureus*. ^{15}N NMR measurements were made on normal intact cells, chloramphenicol-treated cells with thickened walls, partially autolyzed cells, and cell walls isolated from normal cells. ^{13}C NMR measurements were made on normal cells and cell walls and on partially autolyzed cell walls. The ^{15}N and ^{13}C intact-cell and cell wall spectra were dominated by resonances originating from N-terminal and nonterminal positions of the pentaglycine segment of the cell wall peptidoglycan. ^{13}C and ^{15}N NMR spectral parameters (T_1, line width, and NOE) could be accounted for by a log-χ^2 distribution of correlation times. The rapid, but highly correlated, chain motion of the pentaglycine bridge differed from that found in synthetic polymers and was characterized by shorter average correlation times and broader distributions of correlation times. The effect of temperature on the distribution of the correlation times was anomalous, with increasing temperature producing a wider distribution of correlation times. Chain motion of the pentaglycine bridge appeared to be dependent on the degree of packing of glycan strands rather than on noncovalent bonding interactions between adjacent peptide chains. Guanidine hydrochloride (6 M) had no effect on the spectral parameters of ^{15}N-Gly-labeled *S. aureus* cell walls. The anomalous temperature dependence of the distribution of correlation times could be accounted for by the transition from a flexible coil to a fully extended chain that accompanies the separation of the glycan strands and swelling of the cell wall. ^{15}N spectral parameters indicated that the degree of packing of the glycan strands is decreased by cell wall elongation and cell turgor.

The Gram-positive bacterial cell wall consists of an amphoteric polyelectrolyte gel of peptidoglycan and teichoic acid, whose main functions are to preserve the morphogenetically determined shape of the cell and to provide the fragile cytoplasmic membrane with the mechanical support needed to withstand the high osmotic pressures associated with hypotonic environmental conditions (Rogers, 1974; Shockman et al., 1974). The primary structures of peptidoglycan and teichoic

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