terations in base-pair structure (Sobell et al., 1976; Nuss et al., 1979). Nuss investigated the effect of ethidium and other dyes on the base-paired dinucleotides GpC and CpG and demonstrated that intercalation is accompanied by a change in the puckering of the ribose rings, a change in the dihedral angles of the sugar–phosphate backbone, and an increase in the distance between the bases of 3.4 Å. Although no work of this type has been done on $tRNA^{Phe}$, it is plausible to assume similar distortions in structure in the immediate neighborhood of the site.

Another possible contribution to the ΔV can come from release of a bound sodium ion simultaneously with ethidium binding, since hydration of ions is normally accompanied by a decrease in volume (Neuman et al., 1973). In this case replacement of sodium by lithium in the buffer should result in a change in ΔV due to the difference in hydration of the two ions. The ΔV's for the two buffer systems, however, are identical within the limits of experimental error, indicating that such ion release does not conspicuously contribute to ΔV.

The preceding observations can be related to the concept of hard and soft sites distinguished by Torgerson et al. (1979) on the basis of model studies. Hard sites are rigid and expel the ligand as pressure is raised, while soft sites are deformable and result in stabilized binding with increased pressure. The tRNA–ethidium system is therefore an example of a soft site, and we would conclude that when positively charged aromatics are intercalated into nucleic acid stacked structures the decrease in volume is considerably greater than that due to simple stacking of aromatic rings, and binding is readily stabilized by pressure. Although the present study refers to transfer RNA, the previous considerations indicate that qualitatively similar effects should be expected upon intercalation of ethidium in DNA and other nucleic acids.

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In Vitro Ribosomal Ribonucleoprotein Transport upon Nuclear Expansion†

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ABSTRACT: The interdependence of nuclear rRNA release and nuclear size is investigated in macronuclei isolated from *Tetrahymena*. Nuclei are induced to contract and to expand, without any structural disintegration of the nuclear envelope, by final Ca^{2+}/Mg^{2+} (3:2) concentrations of 5 and 1.5 mM, respectively. Upon expansion, the average volume of nuclei increases from 600 ± 42 to 811 ± 76 μm^3. Concomitantly, nuclei begin to release RNA following saturation kinetics. This RNA release stops immediately upon nuclear contraction. Similar to the in vivo situation, only advanced rRNA processing products are released in the form of ribosomal precursor particles, as identified in detail by polyacrylamide gel electrophoresis and rate zonal and isopycnic density gradient centrifugation. Three particle types are released having average buoyant densities of 1.495, 1.470, and 1.532 g/cm^3, exhibiting average sedimentation coefficients of 62, 62, and 35 S, and containing the immediate precursor to the 26S rRNA, 26S rRNA, and 17S rRNA, respectively. The rRNP release is ATP independent and noncoincident with the release of endogenous nuclear P_i, though it is Be^{2+} sensitive. Our data are compatible with the views that nuclear expansion is the prerequisite rather than the cause for the rRNP release and that nuclear pore complex associated ATPases play only, if at all, a minor role in nucleocytoplasmic exchange of rRNP.

The transport of RNA from the nucleus to cytoplasm presumably is an important multiregulated process in gene expression of eukaryotic cells. An intrinsic role herein is often ascribed to the nuclear envelope, especially to its pore complexes which represent the only direct contact sites and the major, if not exclusive, passageways for RNA between the nucleus and cytoplasm [for reviews see, e.g., Wunderlich et al. (1976), Fry (1977), and Harris (1978)]. Circumstantial evidence is often interpreted as to indicate an active, i.e., ATP

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consuming role of the pore complexes in RNA transport. For instance, ATPase activity has been localized in the pore complexes by means of electron microscopical cytochemistry [Klein & Afzelius, 1966; Yasuzumi & Tsubo, 1966; cf. also Agutter et al. (1976)]. Moreover, numerous in vitro studies have shown (1) that the release of RNA from isolated nuclei is stimulated by exogenous ATP [e.g., Schneider (1959), Ishikawa et al. (1970), Raskas (1971), Raskas & Rho (1973), Racevskis & Webb (1974), and Agutter et al. (1976, 1979a)] and (2) that the ATP-dependent RNA release is inhibited by ATPase blockers such as Be^{2+} (Schumm & Webb, 1975; Agutter et al., 1976). However, the actual role of pore complex associated ATPases in nucleocytoplasmic RNA transport remains to be elucidated [cf. Clawson & Smuckler (1979)].

On the other hand, evidence is recently accumulating that the pore complexes are an intimate part not only of the nuclear envelope but also of the nuclear matrix. This is the fundamental skeleton composed predominantly of acidic proteins, which extends throughout the whole nucleus as identified in a wide variety of cells [for reviews see, e.g., Berezney & Coffey (1976), Wunderlich (1978), Berezney (1979), and Shaper et al. (1979)]. The nuclear matrix may be regarded not as a "rigid skeleton" but rather as a dynamic framework structure (Wunderlich & Herlan, 1977; Herlan et al., 1978). In *Tetrahymena*, for example, the nuclear matrix per se exhibits elastic properties; i.e., it can be reversibly contracted by Ca^{2+}/Mg^{2+} ions (Wunderlich & Herlan, 1977). Moreover, first indications exist that the nuclear matrix is critically involved in the metabolism of different nuclear RNA species [e.g., Herman et al. (1976), Zieve & Penman (1976), Miller et al. (1978a,b), and Herlan et al. (1979a)]. For instance, our recent findings in *Tetrahymena* suggest that processing and intranuclear translocation of rRNA toward the pore complexes take place in a highly, possibly feedback coordinated mode along the elements of the nuclear matrix (Herlan et al., 1979a).

Accordingly, we wondered if the RNA transport capacity of the pore complexes changes upon expansion and contraction of nuclei, i.e., under conditions in which the nuclear matrix must be necessarily in a more expanded or contracted state. Here, we approach this problem in macronuclei isolated from the ciliate protozoan *Tetrahymena*.

Experimental Procedure

Cell Cultivation and Labeling Conditions. Axenic 5-L cultures of the ciliate protozoan *Tetrahymena pyriformis* (amicronucleate strain GL) were grown at 28 °C in proteose peptone–yeast extract medium up to the mid logarithmic growth phase (20 000–30 000 cells/mL) as described recently (Ronai & Wunderlich, 1975). The cells were harvested and labeled with [5,6-^{3}H]uridine (sp act. = 40–50 Ci/mmol; NEN Chemicals) as described elsewhere (Herlan et al., 1978).

Buffers Used. RM: 0.2 M sucrose, 0.5 mM $MgCl_2$, 0.75 mM $CaCl_2$, and 20 mM Tris-HCl at pH 7.4. SA: 0.5% sodium dodecyl sulfate, 5 μg/mL polyvinyl sulfate, and 10 mM sodium acetate at pH 5.0. SM: 0.2 M sucrose, 5.6 mM $MgCl_2$, 8.4 mM $CaCl_2$, and 20 mM Tris-HCl at pH 7.4. ST: 0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, and 20 mM Tris-HCl at pH 7.4. T: 2 mM $MgCl_2$, 3 mM $CaCl_2$, and 20 mM Tris-HCl at pH 7.4. TK: 0.6 mM $MgCl_2$, 0.9 mM $CaCl_2$, 50 mM KCl, and 20 mM triethanolamine hydrochloride at pH 7.4.

Isolation of Macronuclei. Cell disruption was carried out according to our glycerol technique (Wunderlich et al., 1978a; Giese et al., 1979a) and the nuclei were then isolated according to our two-step sucrose gradient method developed previously (Wunderlich & Herlan, 1977).

Determination of Nuclear Size. Nuclei were photographed in a Zeiss photomicroscope II using Nomarsky interference contrast optics. On calibrated positives, the area of single nuclei was determined automatically with a Leitz ASM computer. These areas were assumed to be circular for calculation of diameters and volumes of nuclei.

In Vitro Assay for Nuclear Release of [^{3}H]RNA. RNA release was induced by pipetting 0.2-mL aliquots of a given nuclear fraction suspended in ST into 2.8 mL of RM preequilibrated in 10-mL centrifuge tubes at 28 °C for 15 min. RNA release was stopped by adding 1 mL of ice-cold SM under gentle shaking and immediately putting the tubes in ice for 5 min before centrifugation at 900*g* for 7 min. Supernatants and pellets were mixed with 0.5 mL of bovine serum albumin (2 mg/mL) and then precipitated with 5% trichloroacetic acid at 0 °C. The precipitation was repeated twice. Then the precipitates were hydrolyzed with 0.5 mL of trichloroacetic acid at 90 °C for 30 min, mixed with 10 mL of Instagel (Packard, Frankfurt), and counted in a Packard liquid scintillation counter, Model 3380.

The effect of Be^{2+} on RNA release was tested by pipetting the nuclei into RM containing $Be(NO_3)_2$ at a concentration of 0.01 and 0.1 mM, respectively; the release was stopped after 2.5 min.

Determination of Inorganic Phosphate. RNA release from unlabeled nuclei was induced as described above and stopped, after 2.5 min, by adding trichloroacetic acid to a final concentration of 5%. After centrifugation (3000*g* for 10 min) the supernatants were assayed for inorganic phosphate according to either Fiske & Subbarow (1925) or Eibl & Lands (1969).

Rate Zonal Density Gradient Centrifugation. Given fractions of unlabeled nuclei were assayed for RNA release as described above in a final volume of 8 mL at 28 °C for 10 min. After being cooled to 0–4 °C, the nuclei were spun down twice at 900*g* for 7 min. The supernatant was dialyzed against TK overnight, concentrated to 0.5–1.0 mL by poly(ethylene glycol), and then layered over a 27-mL continuous 15–30% (w/v) sucrose gradient buffered with TK and centrifuged at 21 000 rpm for 15 h (RPS25 rotor in a Hitachi ultracentrifuge). Fractions were collected from the bottom of the gradient. Aliquots were measured for optical density at 260 nm and for refractive index at 20 °C in a Zeiss refractometer. The fractions were corrected for volume, and sedimentation coefficients were calculated according to McEwen (1967). In some experiments, centrifugation was performed with 10-mL sucrose gradients at 40 000 rpm for 3 h (SW41 rotor in a Beckman ultracentrifuge). These gradients were automatically recorded in a flow-through cuvette at 260 nm.

Isolation of Ribosomes. Cytoplasmic monosomes and 50S and 30S ribosomal subunits, respectively, were isolated and separated on continuous 15–30% sucrose gradients as described previously (Nägel & Wunderlich, 1977).

Rapid Equilibrium Density Gradient Centrifugation. Samples were fixed with 6% freshly prepared formaldehyde at 0–4 °C for 24 h (Perry & Kelley, 1966) and then dialyzed overnight as described above. Step CsCl gradients were prepared according to Brunk & Leick (1969) and centrifuged at 30 000 rpm for 18 h (RP65 rotor in a Hitachi ultracentrifuge). The tubes were fractionated by underloading a heavy CsCl displacement solution. Continuous absorption at 260 nm and determination of refractive index were carried out as described above.

Extraction of RNA. (*a*) *Nuclei.* About (4–10) × 10^7 nuclei were suspended in 2 mL of ST containing 0.1 M NaCl and incubated with 100–150 μg of DNase (Sigma, München) at

Table I: Nuclear Size of Isolated *Tetrahymena* Macronuclei as a Function of Ca^{2+}/Mg^{2+}

	final Ca^{2+}/Mg^{2+} (3:2) concn of			
	5 mM		1.5 mM	
expt no.	diameter (μm)	vol (μm^3)	diameter (μm)	vol (μm^3)
1	10.2	556	11.3	756
2	10.5	606	12.0	905
3	10.7	641	11.8	860
4	10.2	557	11.1	716
5	10.7	641	11.6	817
av	10.5 ± 0.3	600 ± 42	11.6 ± 0.4	811 ± 76

28 °C for 5 min. Then, the suspension was made 1% with respect to sodium dodecyl sulfate and was incubated with 1–2 mg of proteinase K at 28 °C for 7–10 min. After cooling to 0–4 °C, we added an equal volume phenol saturated with distilled H_2O, containing hydroxychinoline (1 mg/g of phenol). This suspension was vigorously shaken on a whirl mixer and kept on ice for 5–10 min and then centrifuged at 2800*g* for 7 min. After rephenolization of the aqueous phase, the RNA was precipitated by addition of 2 volumes of ethanol at −20 °C overnight and collected by centrifugation at 6000*g* for 15 min. The pellets were resuspended in 2 mL of T containing 0.1 M NaCl and incubated with DNase as described above. Then, the suspension was made 1% with respect to sodium dodecyl sulfate, incubated with 0.5 mg/mL proteinase K for 5 min, phenolized, precipitated, ethanolized at −20 °C for at least 3 h, and centrifuged as described above. The pellet was resuspended in SA, from which the RNA was precipitated with ethanol overnight. Finally, the RNA was centrifuged as described above and dried under vacuum for ~2 min.

(*b*) *Released RNA.* Released nuclear material obtained as described above was mixed with sodium dodecyl sulfate and NaCl in final concentrations of 1% and 0.1 M, respectively. Then, 2 volumes of ethanol was added at −20 °C overnight before RNA was extracted with proteinase and phenol as described above.

(*c*) *RNP Particles.* Pooled fractions of sucrose gradients (~4 mL) were made 1% and 0.1 M with respect to sodium dodecyl sulfate and NaCl, respectively. This suspension was treated with a 1:1 (v/v) mixture of chloroform and phenol containing hydroxychinoline. The aqueous phase was reextracted with chloroform–methanol before ethanol was added at −20 °C.

Polyacrylamide Gel Electrophoresis of RNA. RNA samples were dissolved in 2.5-fold concentrated electrophoresis buffer [for composition, see Loening (1969)] additionally containing 10% sucrose and 0.2% bromphenol blue. For partial denaturation, the samples were dissolved in a small volume of distilled H_2O before we added a 10-fold volume of formamide buffered with 20 mM barbituric acid at pH 9.0. The samples were heated to 65 °C for 5 min prior to electrophoresis on 2.2% aqueous polyacrylamide gels in 120 mm long plexiglass tubes with an internal diameter of 6 mm according to Loening (1969). Gels were washed in H_2O for 1–2 h and then continuously scanned at 260 nm.

Results

Nuclear Size and RNA Release. Upon isolation, *Tetrahymena* nuclei retain a structurally well-preserved nuclear envelope, as shown recently (Wunderlich et al., 1978a; Giese et al., 1979a). Without any disintegration of the nuclear envelope, these nuclei can be induced to change their size simply by varying the Ca^{2+}/Mg^{2+} concentration. The nuclei

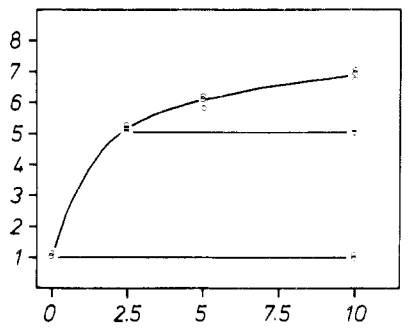

FIGURE 1: RNA release from [^{3}H]uridine prelabeled *Tetrahymena* macronuclei as a function of time (minutes). RNA release is induced by pipetting aliquots (3500 cpm) of a given nuclear fraction into release medium preequilibrated at 28 °C. RNA release is expressed in terms of percent radioactivity precipitated by trichloroacetic acid from nuclei-depleted suspensions from the total trichloroacetic acid precipitable radioactivity in a given sample. The latter remains constant during the incubation period. Inhibition of RNA release after 0 and 2.5 min and further incubation at 28 °C do not release any further radioactivity from the nuclei.

Table II: Effect of Be^{2+} on Nuclear RNA Release and Nuclear Size

	inhibn of RNA release	nuclear size	
$[Be^{2+}]$ (mM)	(%)	diameter (μm)	vol (μm^3)
0.00	0	12.0	905
0.01	59	11.9	882
0.10	99	11.3	755
0.00 + 5 mM Ca^{2+}/Mg^{2+}	100	10.5	606

are contracted or expanded at final Ca^{2+}/Mg^{2+} (3:2) concentrations of 5 or 1.5 mM, respectively (Table I). Upon expansion, the average nuclear diameter increases from 10.5 ± 0.3 to 11.6 ± 0.4 μm, corresponding to an increase of the average nuclear volume of ~30%.

Figure 1 shows that, upon expansion, nuclei prelabeled with [^{3}H]uridine begin to release RNA following saturation kinetics. At a first approximation, the RNA release is linear in the first 2–2.5 min and regularly reaches the maximum after ~10 min. The amount of maximal released RNA, in terms of the total trichloroacetic acid precipitable radioactivity, varies from experiment to experiment between 4 and 8%. This RNA release, however, is completely suppressed upon nuclear contraction (Figure 1). Once contracted, the nuclei do not release any significant radioactivity during a further incubation at the normal release temperature of 28 °C.

Surprisingly, the RNA release, though ATP independent, can be specifically blocked by Be^{2+} (Table II). Also, Be^{2+} causes a reduction in nuclear volume. However, we cannot find any proportionality between inhibition of RNA release and decrease in nuclear size. At a concentration of 0.01 mM, for example, Be^{2+} does not significantly affect nuclear size, though 59% of the RNA release is inhibited. At any rate, this Be^{2+} effect is the more surprising since the RNA release does not coincide with any detectable release of P_i from the nuclei.

Characterization of Nuclear and Released rRNA. Tetrahymena nuclei are known to contain numerous nucleoli located at the periphery and, hence, rRNAs as major RNA constituents [for reviews see, e.g., Hill (1972) and Conner & Koroly (1973)]. We therefore concentrate our analysis especially on the larger rRNA species in the following. Figure 2a shows that the RNA we extracted from *Tetrahymena* nuclei migrates as four monodisperse high molecular weight fractions in 2.2% polyacrylamide gels which are designated A, B, 26

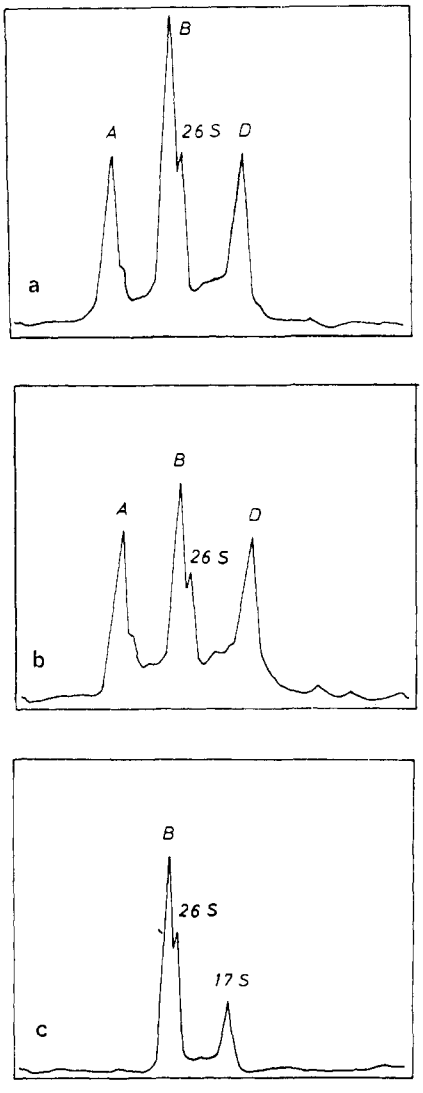

FIGURE 2: 2.2% polyacrylamide gel electrophoretograms of *Tetrahymena* macronuclei before (a) and after (b) RNA release and of the released rRNA (c). Migration is from left to right. Absorbance at 260 nm is plotted on the ordinate. This experiment was done with one given nuclear fraction.

S, and D according to the nomenclature of Eckert et al. (1978) [cf. also Herlan et al. (1979a)]. According to these authors, fraction A (M_r 2.3 × 10^6) contains the first stable transcription product of the rDNA. Fraction B (M_r 1.34 × 10^6) and fraction D (M_r 0.66 × 10^6) represent the direct nuclear precursors for the 26S rRNA (M_r 1.27 × 10^6) and the 17S rRNA (M_r 0.66 × 10^6), the major constituents of the large and small ribosomal subunits, respectively. Fraction B and 26S rRNA are considerably decreased relative to fraction A after a 10-min period of RNA release (Figure 2b). Also, fraction D reveals a slight reduction. The nuclei contain the same amount of fraction A before and after RNA release (cf. parts a and b of Figure 2), thus indicating that fraction A is not released and/or processed and/or degraded during RNA release.

Only fraction B, 26S rRNA, and 17S rRNA (=fraction D) are normally released from the nuclei (Figure 2c). Fraction B is always the predominant component while 17S rRNA regularly contributes only a small portion to the total released rRNA. Occasionally, however, the portion of 17S rRNA is about the same as that of 26S rRNA (Figure 3a). In this case, two further RNA fractions can be observed, designated F_1 (M_r 0.63 × 10^6) and F_2 (M_r 0.50 × 10^6) according to the nomenclature of Eckert et al. (1978). These F fractions still

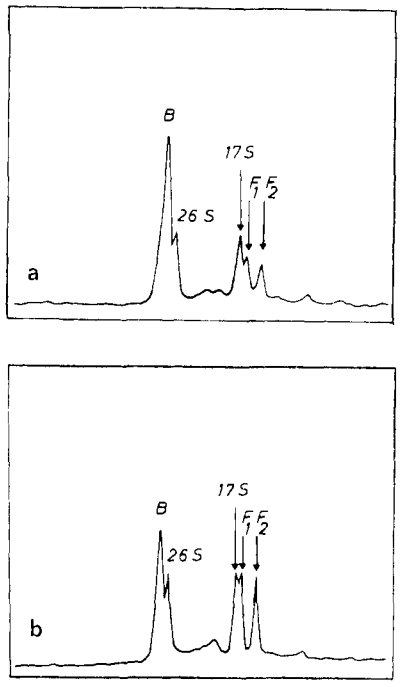

FIGURE 3: 2.2% polyacrylamide gel electrophoretograms of the rRNA released from *Tetrahymena* macronuclei for 10 min at 28 °C before (a) and after (b) partial denaturation in formamide. Migration is from left to right; the ordinate indicates absorbance at 260 nm. This experiment was done with one given nuclear fraction.

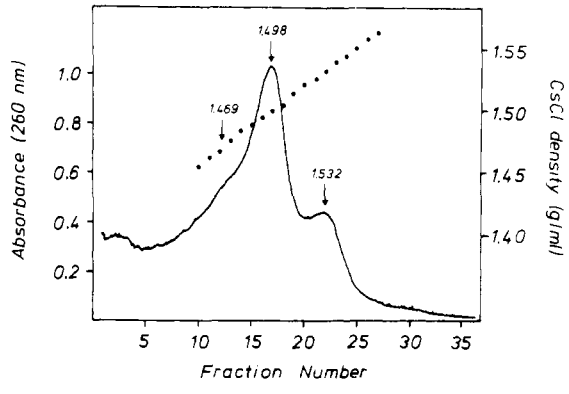

FIGURE 4: CsCl gradient profile of RNP released from *Tetrahymena* nuclei at 28 °C for 10 min.

increase upon partial denaturation in formamide, whereas fraction B, and to a lesser extent 26S rRNA, decreases in relation to 17S rRNA.

Identification of rRNP Particles. Figure 4 shows the sedimentation profile of the total material released from nuclei upon expansion in rapid equilibrium CsCl gradients. The major component has a buoyant density of 1.498 g/cm^3. This major peak always reveals a distinct shoulder with a buoyant density of 1.469 g/cm^3. Moreover, a third minor fraction always bands at a density of 1.532 g/cm^3. The amount of this heavy fraction, however, varies from experiment to experiment, while the relative proportion of the major peak to the light fraction is almost always the same. The densities of all three fractions are much lower than those of the cycloplasmic monosomes and the 50S and 30S ribosomal subunits, respectively, which obviously corresponds to a higher protein content (Table III).

In velocity 15–30% sucrose gradients, the material released from nuclei always sediments as one major fraction with an average sedimentation coefficient of ~62 S (Figure 5 and Table IV). Besides this major fraction, a minor fraction

Table III: Buoyant Density and Protein Content of Ribosomes, Ribosomal Subunits, and Nuclear Released rRNP Particles in *Tetrahymena*[a]

particles	buoyant density (g/cm³)	protein content (wt %)[b]
70 S	1.563 ± 0.002	46.7
50 S	1.580	43.9
30 S	1.556	47.9
62 S	1.470 ± 0.003	63.4
	1.495 ± 0.003	58.4
35 S	1.532 ± 0.003	52.0

[a] All particles, except the 35S particles, have been prepurified on continuous 27-mL sucrose gradients before CsCl gradient centrifugation was performed. The 35S particles found in sucrose gradients are assumed to be identical with the heavy fraction in CsCl gradients. [b] Weight percent protein = $100 \times [\rho_P(\rho_N - \rho)/[\rho(\rho_N - \rho_P)]]$; ρ = density of particles in CsCl and ρ_P and ρ_N = densities of protein and RNA, assumed to be 1.3 and 1.9 g/cm³, respectively.

Table IV: Sedimentation Coefficients of rRNP Particles Released from *Tetrahymena* Macronuclei

no. of sucrose gradients	$s_{20,w}$ values (S) major fraction	slow sedimenting fraction	fast sedimenting fraction
1	62.6	30.7	81.4
2	60.8	39.2	78.4
3	64.7	39.2	87.2
4	63.8	32.2	
5	58.2		
6	64.3		
av	62.4 ± 2.5	35.3 ± 4.5	82.3 ± 4.5

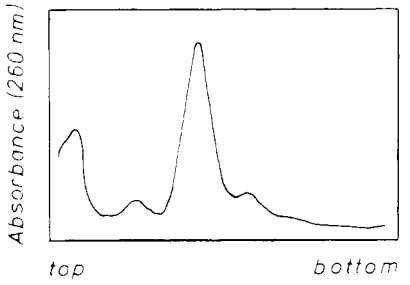

FIGURE 5: Sedimentation profile (10-mL 15–30% sucrose gradient) of RNP released from *Tetrahymena* nuclei at 28 °C for 10 min.

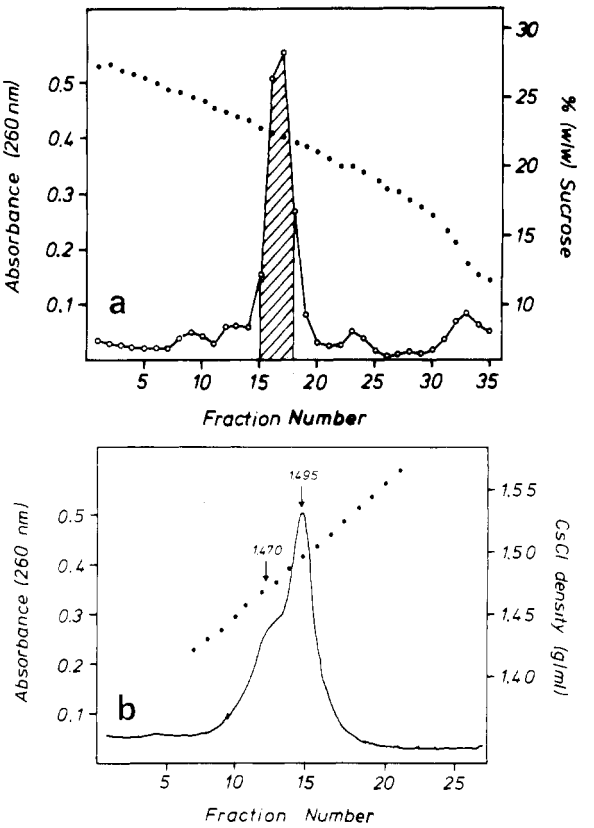

FIGURE 6: Sedimentation profile (27-mL 15–30% sucrose gradient) of RNP released from *Tetrahymena* nuclei at 28 °C for 10 min (a) and recentrifugation of the hatched fractions in a CsCl gradient (b).

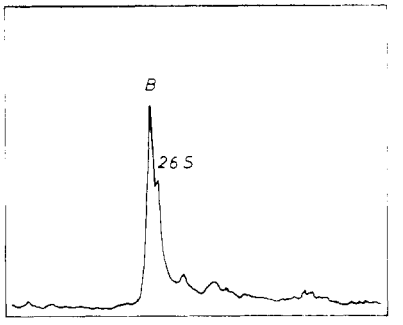

FIGURE 7: 2.2% polyacrylamide gel electrophoretogram of the rRNA extracted from the 62S particles released from *Tetrahymena* nuclei. Migration is from left to right; absorbance at 260 nm is on the ordinate.

sedimenting at 35 S can be regularly detected in most experiments. Occasionally, some gradients reveal an additional minor fraction sedimenting at 82 S. Figure 6 shows that a recentrifugation of the 62S fraction in isopycnic CsCl gradients yields two components. The main component has an average buoyant density of 1.495 g/cm³ while the average density of the minor component is 1.470 g/cm³ (Table III). The 62S fraction also contains two rRNA components, namely, fraction B and 26S rRNA (Figure 7).

Taken together, our data indicate that fraction B, 26S rRNA, and 17S rRNA are released in the form of ribosomal precursor particles having average *s* values of 62, 62, and 35 S as well as average buoyant densities of 1.495, 1.470, and 1.532 g/cm³, respectively.

Discussion

Our data demonstrate that macronuclei isolated from *Tetrahymena* can be induced to release RNA simply by lowering the final Ca^{2+}/Mg^{2+} (3:2) concentration from 5 to 1.5 mM. This RNA release exhibits saturation kinetics and is obviously not due to a simple nuclear leakage and/or elution since only rather specific RNAs are released. Indeed, the first stable rDNA transcription product is never released; only 17S rRNA and 26S rRNA, including the immediate precursor to the latter, i.e., fraction B, are released within ~5 min. Some molecules of fraction B and 26S rRNA contain a central hidden break. Moreover, we find that these rRNAs are released not in the form of mature ribosomal subunits but rather of ribosomal precursor particles. Thus, the in vitro system we developed here resembles the in vivo situation in so far as rRNA is also predominantly transported from the nucleus to cytoplasm in *Tetrahymena* cells. Through reliable data on the transport of fraction B are not yet available to date, the 26S and 17S rRNA are synthesized, processed, and finally transported to the cytoplasm within less than 5 min under optimal growth conditions [Leick & Andersen, 1970; cf. also Kumar (1970) and Wunderlich (1972)]. Also, the cytoplasmic 26S rRNA contains a central hidden break (Eckert et al.,

1978). Moreover, the 26S and 17S rRNAs appear in the cytoplasm as 60S and 40S precursor particles, where they mature to the 50S and 30S ribosomal subunits (Leick, 1969; Leick et al., 1970). It is totally unknown, however, how far Ca^{2+} and/or Mg^{2+} ions are also in vivo critical for the rRNP transport.

A further in vivo equivalence of our in vitro system also exists with respect to the nuclear envelope. In fact, not only are the nuclei we used surrounded by a structurally well-preserved nuclear envelope including pore complexes (Wunderlich et al., 1978a; Giese et al., 1979a) but also this nuclear envelope exhibits a typical in vivo property, namely, temperature sensitivity. In *Tetrahymena* cells, nuclear membranes can undergo reversible thermotropic lipid–protein segregations (Wunderlich et al., 1974; Nägel & Wunderlich, 1977), obviously due to a lateral lipid clustering (Wunderlich et al., 1975, 1978b). This lipid clustering coincides with a decrease in nucleocytoplasmic RNA transport. Also, thermotropic lipid clustering obviously occurs in nuclear membranes of isolated nuclei (Giese et al., 1979a) which also coincides with a reduction of the RNA release (Herlan et al., 1979a,b). These coincidences found in vivo and in vitro support the view that RNA transport in vitro proceeds in a similar way as in vivo. In vivo, RNA is predominantly transported through nuclear pore complexes, the transport capacity of which is probably modulated by the lipid fluidity of nuclear membranes (Wunderlich et al., 1974; Nägel & Wunderlich, 1977). Recently, Agutter et al. (1979b) also suggested that the RNA transport capacity of nuclear pore complexes is controlled by nuclear membrane lipids in SV40-3T3 cells.

The in vitro rRNP transport in *Tetrahymena* differs from other cell-free systems of RNA transport in at least two important aspects. Firstly, the rRNP release from *Tetrahymena* nuclei does not need any supplementation of the release medium with cytosol as it has been reported, for example, in rat liver nuclei [Sato et al., 1977; cf. also Schumm et al. (1979)]. Secondly, the rRNP transport from *Tetrahymena* nuclei does not depend on exogenous ATP which is needed as an energy source in other RNA release systems (Sato et al., 1977; Schumm et al., 1979; for further references, see the beginning of this paper). The ATP-dependent release is normally blocked by the general phosphatase inhibitor Be^{2+} (Schumm & Webb, 1975; Agutter et al., 1976). Surprisingly, however, Be^{2+} also inhibits the rRNP release from *Tetrahymena* nuclei. This could possibly indicate an intranuclear energy store, e.g., energy-rich phosphate bound to protein of the nuclear matrix [cf. Allen et al. (1977)] and/or even ATP remaining trapped in nuclei during nuclear isolation. Inconsistent with this view is our finding that inorganic phosphate is not released during rRNP release. However, we cannot exclude that nuclear phosphate possibly liberated during rRNP release is immediately transferred to and/or unspecifically "precipitated" onto other nuclear components. At present, it must therefore remain open whether these differences between the different rRNP release systems are of phylogenetic origin or reflect different regulatory mechanisms existing in even one cell type. At any rate, the fact that rRNP particles can be released from *Tetrahymena* nuclei similarly as in vivo would consequently mean that ATPases possibly located in nuclear pore complexes play only, if at all, a secondary role at least in the rRNP release from *Tetrahymena* nuclei.

Conspicuously, we find that nuclear RNA release critically relates to nuclear size. Lowering the final Ca^{2+}/Mg^{2+} concentration from 5 to 1.5 mM induces not only the onset of RNA release but also an expansion of nuclear volume of ~30%. Conversely, nuclear contraction coincides with the immediate inhibition of RNA release. Incidentally, the size of expanded nuclei closely resembles that of in situ nuclei [cf. for discussion Wunderlich et al. (1978a)]. Moreover, we have preliminary evidence that expansion of *Tetrahymena* nuclei can be induced by increasing concentrations of Na^{+}, Ca^{2+}, and Mg^{2+}, which also coincides with RNA release (W. C. Nägel and F. Wunderlich, unpublished results). Similarly, ATP stimulates RNA release accompanied by nuclear expansion, which is presumably due to chelation of the bivalent ions by ATP. (G. Herlan and F. Wunderlich, unpublished results). Such an interdependence of nuclear size and RNA release appears to exist in other cell-free systems too. For instance, polyribosomes are released from HeLa cell nuclei in the presence of polyanions such as polyuridine (Goidl et al., 1975). The latter in turn are known for their capacity to swell nuclei (Coffey et al., 1974). Furthermore, even the ATP-driven release of mRNA from rat liver nuclei is inhibited by factors causing nuclear shrinkage (Clawson et al., 1978).

Our data, however, cannot provide any evidence for the mechanisms underlying both nuclear expansion and nuclear RNA release. It is only known that the expansion of *Tetrahymena* nuclei coincides with a dramatic dispersion of chromatin and nucleoli (Wunderlich et al., 1978a). Such a dispersion can most straightforwardly be explained in terms of a repulsion of negative charges of nuclear components, including those of the nuclear matrix (Wunderlich & Herlan, 1977; Leake et al., 1972). Though such electrostatic rearrangements could conceivably facilitate nuclear RNA release, e.g., by impairment of steric hindrances, they cannot be per se the primary reason for RNA release. Firstly, we find that Be^{2+} causes a dramatic deterioration of RNA release at concentrations which do not significantly affect nuclear size. Secondly, the RNA release from *Tetrahymena* nuclei can be blocked by proteinase inhibitors (Giese et al., 1979b), thus suggesting an involvement of proteinase(s). Interestingly, Stevenin et al. (1977, 1979) recently reported that hnRNP particles extracted from rat liver nuclei are associated with endogenous proteinase(s). Thirdly, *Tetrahymena* nuclei lose their potentiality for RNA release upon increasing incubations under nonrelease conditions. Hereby, nuclei retain undegraded rRNA and their capability for expansion (Giese et al., 1979b). In particular, the latter finding indicates that nuclear expansion is only a "structural" prerequisite for nuclear RNA release rather than the primary reason, as is also the case in DNA and RNA synthesis [for reviews see, e.g., Coffey et al. (1974), Berezney & Coffey (1976), and Berezney (1979)].

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