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Subunit Structure of rDNA-Containing Chromatin†

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ABSTRACT: Recent studies indicate that chromatin has a repeating subunit structure. In an attempt to relate this organization to chromatin's role in selective gene transcription we have begun to examine the subunit structure of a specific gene. *Tetrahymena pyriformis* preferentially replicates the genes coding for rRNA (rDNA) during refeeding after prolonged starvation. By prelabeling cultures during exponential growth with [^{14}C]thymidine and pulse-labeling during refeeding with [^{3}H]thymidine, we have been able to differentially label bulk chromatin and rDNA-containing chromatin. Nuclei which contained at least 78% of their ^{3}H label in rDNA were digested with staphylococcal nuclease, and the DNA digestion products analyzed on agarose gels. Both the kinetics of digestion and the digestion products were similar for ^{14}C- and ^{3}H-labeled chromatin. In order to monitor protein exchange, digestions were also performed on partially purified rDNA-containing chromatin or free rDNA in the presence of nuclei. While the chromatin had a digestion pattern like nuclei, the rDNA was afforded no protection from digestion. Our conclusion is that the chromatin containing rDNA (a repeated, extrachromosomal gene in *Tetrahymena*) exhibits a particulate structure very similar to that of bulk chromatin. This organization does not exist in free rDNA and is not the result of protein exchange during the nuclease digestion.

Recent electron microscopic and nuclease digestion studies have provided evidence to support a particulate model of chromatin structure. Linear arrays of spherical particles have been observed in chromatin extruding from lysed nuclei (Olins and Olins, 1974) and in isolated chromatin depleted of histone F1 (Oudet et al., 1975). Arrays of nucleoprotein particles have also been detected after partial digestion of nuclei or chromatin with relatively nonspecific nucleases (Noll, 1974; Sahasrabuddhe and Van Holde, 1974; Sollner-Webb and Felsenfeld, 1975). To date, evidence of a repeating subunit structure has been found in chromatin from several vertebrates (Hewish and Burgoyne, 1973; Axel, 1975; Honda et al., 1975), a plant (McGhee and Engel, 1975), two lower eukaryotes (Lohr and Van Holde, 1975; Gorovsky and Keevert, 1975), and two animal viruses (Griffith, 1975; Louie, 1974).

Any model of chromatin structure must eventually be able to account for the transcription of selected DNA sequences. Thus, it is of interest to relate the apparent particulate structure of chromatin to proposed models of genome organization (Davidson and Britten, 1973). In this context, one can ask whether the subunit profile is similar for repeated vs. unique DNA sequences, expressed vs. repressed genes, or spacer vs. coding sequences. We have begun to examine these questions by comparing the subunit structure of bulk chromatin with that of chromatin containing a specific gene.

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The ciliate protozoan *Tetrahymena pyriformis* provides a system in which to study the chromatin containing the rRNA genes (rChromatin). Engberg and coworkers (1974) have shown that after a defined starvation–refeeding procedure, cultures of strain GL preferentially synthesize rDNA. Thus, by prelabeling cultures during exponential growth with [^{14}C]thymidine and pulse-labeling during refeeding with [^{3}H]thymidine we have been able to differentially label bulk and rDNA-containing chromatin. We present evidence based on staphylococcal nuclease digestion studies that rChromatin exhibits a subunit structure like that of bulk chromatin.

Experimental Procedures

Cell Culture and Labeling. Stock cultures of *Tetrahymena pyriformis*, strain GL (obtained from I. Cameron), were maintained in enriched proteose peptone as previously described (Gorovsky et al., 1975). The starvation–refeeding regime is a modification of the procedure of Engberg et al. (1974) as follows. Cells were prelabeled by growth for eight generations in enriched proteose peptone containing [^{14}C]thymidine at 0.05 or 0.1 μCi/ml. At densities of 200 000 to 300 000/ml the cells were spun at 250*g* for 5 min, washed by gentle refluxing in an equal volume of 0.010 M Tris (pH 7.5), spun again at 250*g* for 5 min, and finally resuspended at two-thirds to three-fourths the original culture volume in Tris supplemented with 1% Antibiotic-Antimycotic mixture (Grand Island Biological Co.). At this point, cell density was again 200 000 to 300 000/ml and increased 20–40% during 22–24 h starvation. An equal volume of refeeding medium (Table I) was added to the starved cells, and 2.5 or 5 μCi/ml of [^{3}H]thymidine pulsed for the indicated time interval. Throughout the procedure, cultures were maintained at 28 °C and were aerated by shaking at approximately 150 cycles/min.

The percentage of pulsed label attributed to rDNA under these starvation–refeeding conditions ranges from 40 to 90%; the variation seems to be related to culture age (unpublished observations).

Nucleus Isolation. Cells were pelleted at 2000*g* for 2 min. Detailed procedures for isolating nuclei from *Tetrahymena* have been published previously (Gorovsky et al., 1975). Modifications necessitated by the use of starved–refed cells are as follows: (a) medium A contained 0.5 M sucrose instead of 0.1 M; (b) medium B contained 0.5% instead of 0.63% *n*-octyl alcohol in medium A; (c) cells were lysed in medium B for two to three 45-s intervals at high speed in a Waring blender; (d) nuclei were pelleted by centrifuging at 16 000*g* for 5 min.

Subnuclear Fractionation. An rDNA-enriched fraction was isolated from *Tetrahymena* nuclei by the following procedure. Nuclei (4–8 × 10^{7}) from starved–refed cells were suspended in 5 ml of a buffer containing 0.5% Triton X-100, 0.25 M sucrose, 0.01 M $MgCl_2$, 0.01 M Tris (pH 8), and 0.05 M $NaHSO_3$. The nuclear suspension was homogenized 2 min at 28 V in the microcup of a Waring blender, and then centrifuged at 2000*g* for 5 min. The nuclei were resuspended in the Triton X-100 buffer and the homogenization and centrifugation steps repeated. The resulting pellet was dispersed by Dounce homogenization in 5 ml of 0.02 M EDTA, 0.01 M Tris (pH 8), and 0.05 M $NaHSO_3$. After pelleting at 16 000g for 10 min, the nuclei were suspended in 3 ml of deionized distilled water and sheared at setting 70 in the microcup of a Virtis Model 45. The shearing was monitored by phase contrast microscopy and care was taken to keep the preparation cold. When no nuclear fragments remained (1.5–5 min shearing), the material was diluted 1:1 with water and spun at 2000*g* for 5 min. The pellet was washed in 2 ml of water and respun at 2000*g*. The pooled supernatants were centrifuged at 4000*g* for 20 min and the resulting pellet was washed in 4 ml of water. The final 4000*g* pellet was suspended in the appropriate buffer and either frozen (rChromatin) or extracted for DNA (rDNA).

Table I: Composition of Refeeding Medium.[a]

Component	Concn (mg/ml)	Component	Concn (mg/ml)
L-Arg	0.30	Sodium acetate	2.00
L-His	0.22	Dextrose	2.00
DL-Ile	0.20	Adenylic acid	0.05
L-Leu	0.14	Cytidylic acid	0.05
L-Lys	0.07	Guanylic acid	0.05
L-Met	0.07	Dipotassium hydrogen phosphate	2.00
DL-Phe	0.20		
DL-Ser	0.36	Proteose peptone	0.40
DL-Thr	0.36		
L-Trp	0.04		
DL-Val	0.12		

[a] Adjusted to pH 7.2 and autoclaved 10–15 min.

The purity and yield of rDNA were monitored in selected fractions by counting aliquots on Whatman GF/A filters (Yao and Gorovsky, 1974). In order to determine these values it was necessary to calculate the initial ^{14}C/^{3}H ratio, to estimate the percentage ^{3}H label attributed to rDNA, and to know the percent total DNA comprised of rDNA.

Staphylococcal Nuclease Digestion. Nuclei were washed once in digestion buffer before suspension at about 10^{8}/ml. rDNA and rChromatin were digested in the presence of nuclei at DNA ratios approximating 1:50. Nuclei were assumed to have a DNA content of 10 μg/10^{6} nuclei (Mowat et al., 1974); the corresponding amount of rDNA was estimated from the preparation's absorbance at 260 nm given 1 A_{260} = 50 μg of DNA and given the calculated enrichment for rDNA in the preparation (Table I). Since rChromatin and rDNA derived from the same source and thus had the same specific activity, an equivalent amount of rChromatin was determined on the basis of Cl_3CCOOH precipitable ^{3}H counts.

Staphylococcal nuclease was purchased from Worthington Biochemical Corp. Digestions were performed at 37 °C in 0.3 M sucrose, 2.5 × 10^{-5} M $CaCl_2$, and 5 mM phosphate buffer (pH 6.7). The reaction was stopped with 0.5 M ethylenediaminetetraacetate–18 mM Tris (pH 7.0).

Reaction progress was monitored by determining the percentage of DNA counts that were Cl_3CCOOH precipitable at progressive time points. Four aliquots of each digest were pipetted onto 2.4-cm Whatman GF/A filter disks. The filters were processed and counted by published methods (Gorovsky and Keevert, 1975).

DNA Isolation and Characterization. DNA was isolated from nuclei by a modification of the procedure of Kavenoff and Zimm (1973) as detailed by Gorovsky and Keevert (1975).

In order to estimate the contribution of rDNA to the ^{3}H profile, DNA from starved–refed nuclei was banded in cesium chloride gradients of density 1.685 gm/cm^{3}. The gradients were centrifuged 28–32 h at 42 000 rpm and the remainder of 72 h at 33 000 rpm in the 50 Ti head (Beckman)

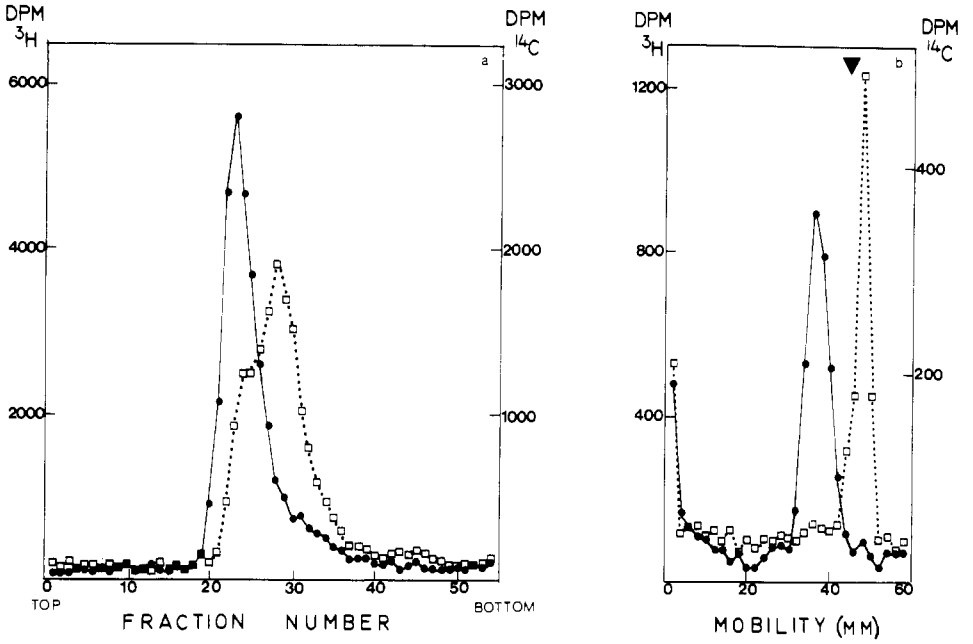

FIGURE 1: Equilibrium centrifugation and electrophoretic analysis of nuclear DNA from starved-refed cells. Cells were labeled with 0.1 μCi/ml of [^{14}C]thymidine during exponential growth and 2.5 μCi/ml of [^{3}H]thymidine during refeeding. DNA from isolated nuclei was examined by (a) equilibrium centrifugation in CsCl and (b) gel electrophoresis in 0.7% agarose. The arrow in b marks the position of the 13.6 × 10^6 mol wt Eco-RI fragment of λ DNA: (●—●) [^{14}C]DNA; (□···□) [^{3}H]DNA.

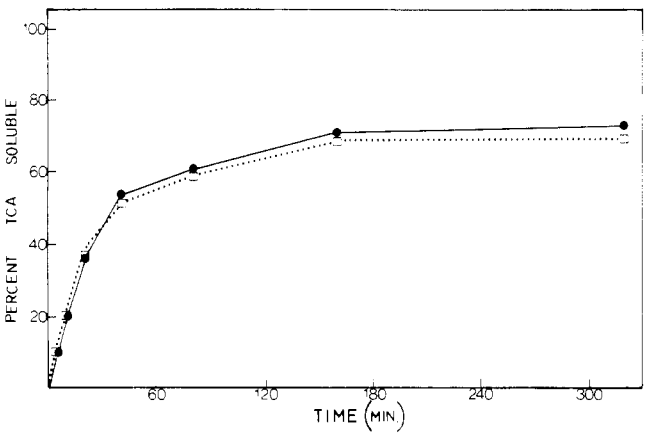

FIGURE 2: Kinetics of digestion of ^{3}H- and ^{14}C-labeled DNA in nuclei of starved-refed cells. Nuclei were digested 0–320 min with 48 units/ml staphylococcal nuclease. At selected time points, the percentage of DNA that was 5% Cl_3CCOOH soluble was determined by counting aliquots on filters: (●—●) [^{14}C]DNA; (□···□) [^{3}H]DNA.

at 22 °C. Fractions (0.1 ml) were collected from the top with a Buchler Auto-Densiflow (Buchler Instruments, Fort Lee, N.J.). Fractions were diluted with 0.5 ml of water and counted in 5 ml of an aqueous cocktail containing Triton X-100–toluene–2,5-diphenyloxazole–1,4-bis[2-(5-phenloxazolyl)]benzene in the ratio 1 l.:2 l.:18 g:0.225 g.

In order to visualize the subunit structure of chromatin, DNA from staphylococcal nuclease digested nuclei was electrophoresed on 1.5% agarose gels for 2.5–3 h at 22 °C by the method of Helling et al. (1974). After electrophoresis, the gels were stained overnight in 1 μg/ml of ethidium bromide in buffer and visualized with a short-wave ultraviolet lamp. Within 48 h after electrophoresis, the gels (either frozen or unfrozen) were sliced into 2-mm segments and counted as described (Gorovsky and Keevert, 1975).

DNA was isolated from the rDNA-enriched fraction by banding in cesium chloride. The material was banded in a gradient of starting density 1.685 g/cm^3 for 22 h at 22 °C in the 50 Ti. Fractions (0.2 ml) were collected and a small aliquot of each counted. Appropriate fractions were pooled, diluted 1:2 with water, and dialyzed 2 h against 10 vol of 1/10 × SSC. This material was pelleted 16 h in the 50 Ti at 42 000 rpm and 22 °C. The DNA pellet was suspended in digestion buffer and its absorbance at 230, 260, 280, and 320 read. For the DNA used in this study, the 260/320 ratio was 2.35 and the 260/280 was 1.86.

In order to characterize this DNA, it was examined on 0.7% agarose gels electrophoresed for 7 h at room temperature by the method of Helling et al. (1974). Eco-RI nuclease digested DNA from phage λ was included in each gel as a molecular weight marker. The λ DNA bands were marked with India ink and the gels sliced and counted as above.

Results

Preparation of Nuclei from Starved–Refed Cells. A culture of GL was prelabeled with [^{14}C]thymidine during exponential growth. The cells were starved, refed, and pulsed with [^{3}H]thymidine from 60 to 120 min after refeeding. DNA from isolated nuclei was examined on cesium chloride gradients and on agarose gels to assess the degree of enrichment for rDNA in pulse-labeled DNA. Figure 1 shows that the majority of ^{3}H label constitutes a discrete component that is G + C rich and has a molecular weight lower than bulk DNA. Both these properties are characteristic of rDNA in *Tetrahymena* (Engberg et al., 1972; Gall, 1975). The clear separation between bulk DNA and rDNA in Figure 1b allows us to say that at least 78% of the ^{3}H label can be attributed to rDNA. This estimate assumes that all the ^{3}H counts at the top of the gel are not contained in rDNA. Since the ^{3}H/^{14}C ratio of this material (2.59) is similar to that of the DNA which entered the gel (2.74), this assumption is probably not correct and the 78% value may be an underestimate.

Digestion of Nuclei from Starved–Refed Cells. Nuclei

Table II: Purity and Yield of rDNA during Subnuclear Fractionation.

Fraction	Total ^3H dpm	Total ^{14}C dpm	$^3H/^{14}C$	% ^3H dpm	% ^{14}C dpm	% rDNA/ Total DNA
Whole nuclei	5.44×10^6	1.89×10^6	2.88	100	100	2[a]
2000*g* pellet	1.25×10^5	2.98×10^4	4.19	2.29	1.58	
4000*g* supernatant	3.70×10^6	1.53×10^6	2.42	68.0	81.0	
4000*g* pellet	1.12×10^6	2.53×10^4	44.3	20.6	1.34	68[b]

[a] Yao and Gorovsky (1974). [b] Approximately 45% of the original ^3H counts were contained in rDNA. This value was determined by cesium chloride gradient analysis of DNA from whole nuclei.

from the preparation described above were digested with staphylococcal nuclease under low ionic strength conditions to inhibit protein exchange (Clark and Felsenfeld, 1971). Figure 2, a plot of percentage of Cl_3CCOOH soluble counts with time, demonstrates that ^3H- and ^{14}C-labeled chromatin are digested at approximately the same rate to approximately the same extent. Although the kinetics of digestion vary somewhat with each preparation, no repeatable difference in digestion of ^3H- vs. ^{14}C-labeled DNA was observed in three other experiments.

Figure 3 illustrates the progress by digestion by depicting the DNA products on agarose gels. Both ^3H and ^{14}C occur in large molecular weight DNA in undigested nuclei (Figure 3a). The slightly faster mobility of ^3H-labeled DNA reflects the fact that in *Tetrahymena,* rDNA occurs in discrete extrachromosomal pieces (Gall, 1975). After limited digestion (Figure 3b), both DNA products appear as a distinct series of bands. Gorovsky and Keevert (1975) have provided evidence that in *Tetrahymena* (strain B-1868-7) these bands constitute an oligomeric series containing multiples of a 150 base pair unit. ^3H- and ^{14}C-labeled products from starved-refed nuclei exhibit no obvious differences in pattern. Figures 3c and 3d demonstrate that ^3H- and ^{14}C-labeled chromatin also exhibit very similar products after extensive digestion. In both cases, the DNA appears in monomer-sized pieces which subsequently undergo further digestion. A comparison of Figures 3b, 3c, and 3d indicates that the smallest subunit undergoes a broadening while shifting to slightly faster mobility. The slight difference observed in the ^{14}C and ^3H profiles of Figure 3d may reflect true heterogeneity unresolvable in our gel system, or may simply reflect relatively low count levels.

Preparation of an rDNA-Enriched Nuclear Fraction. It is possible to isolate a fraction from *Tetrahymena* nuclei that is highly enriched for rDNA. The procedure, a modification of the chromatin isolation of Panyim et al. (1971), depends on the fact that rDNA-containing chromatin in *Tetrahymena* is not solubilized by shear forces that disperse bulk chromatin. Since the fractionation is performed on nuclei from starved-refed cells, the yield and purity of rDNA in the final pellet can be calculated by determining the $^3H/^{14}C$ ratio of selected fractions. Table II indicates that for this preparation the DNA in the final pellet is approximately 68% rDNA. Furthermore, it is clear from Figure 4 that essentially all the pellet's [^3H]thymidine counts occur in rDNA. Since our nuclease digestion studies will monitor only the ^3H counts in this pellet (its ^{14}C counts will be too low to detect), we will refer to the fraction as rChromatin and to the DNA isolated from it as rDNA.

Figure 4 also indicates that the rDNA is of native molecular weight. This point is important in light of the nuclease digestion studies of Noll et al. (1975) on sheared chromatin

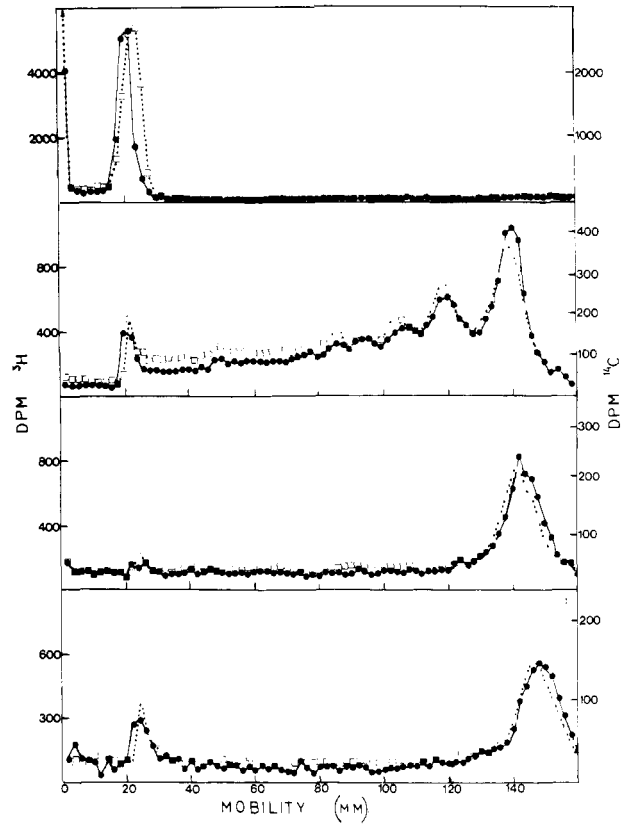

FIGURE 3: Electrophoretic analysis of ^3H- and ^{14}C-labeled DNA digestion products from nuclei of starved-refed cells. Nuclei were digested for 0 (a), 5 (b), 160 (c), and 320 (d) min with 48 units/ml staphylococcal nuclease. The DNA digestion products were electrophoresed on 1.5% agarose gels for 3 h. The bands which appear at approximately 22 mm are probably due to undigested DNA. This material varies in amount with different preparations: (●—●) [^{14}C]DNA; (□ · · · □) [^3H]DNA.

preparations. Their data imply that shear forces strong enough to break covalent DNA bonds may also disrupt native chromatin subunit structure. rDNA from our enriched pellet and rDNA from isolated nuclei have approximately the same electrophoretic mobility with respect to λ DNA molecular weight markers (cf. Figures 1 and 4). Therefore, our fractionation procedure is probably not subject to this artifact.

We would like to emphasize that the exact composition of the rDNA-enriched fraction is not known. We have not yet determined whether the final pellet contains nucleoli or a chromatin aggregate. In addition, we do not know whether contaminants such as membrane fragments or ribonucleoprotein particles are present. This fractionation is merely the first step in a procedure we are developing to isolate

Table III: Staphylococcal Nuclease Digestion of rDNA and rChromatin.

DNA Source[a]	Digestion Time (min)	% Digestion[b]	DNA Source[a]	Digestion Time (min)	% Digestion[b]
^{14}C-Labeled nuclei	10	30.1	^{14}C-Labeled nuclei	10	36.2
^{3}H-Labeled rDNA	10	48.3	^{3}H-Labeled rChromatin	10	35.6
^{14}C-Labeled nuclei	320	53.8	^{14}C-Labeled nuclei	320	65.6
^{3}H-Labeled rDNA	320	84.7	^{3}H-Labeled rChromatin	320	66.1

[a] Each pair was digested in the same test tube. [b] Percentage of thymidine counts that were 5% Cl_3CCOOH soluble.

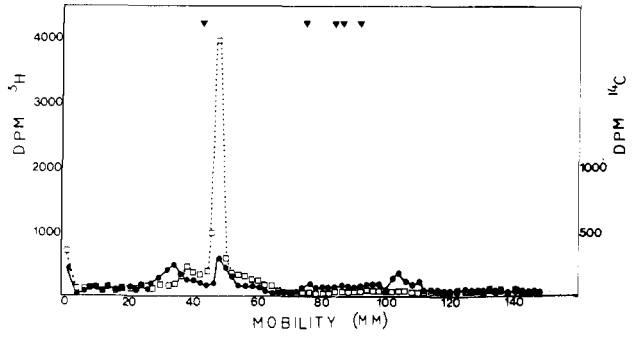

FIGURE 4: Electrophoretic analysis of DNA extracted from 4000*g* pellet. Cells were labeled with 0.05 μCi/ml [^{14}C]thymidine during exponential growth and 5 μCi/ml [^{3}H]thymidine during refeeding. Isolated nuclei were fractionated as described, and DNA from the 4000*g* pellet was examined on 0.7% agarose gels. The arrows mark the positions of Eco·RI λ molecular weight markers: (●—●) [^{14}C]DNA; (□ · · · □) [^{3}H]DNA.

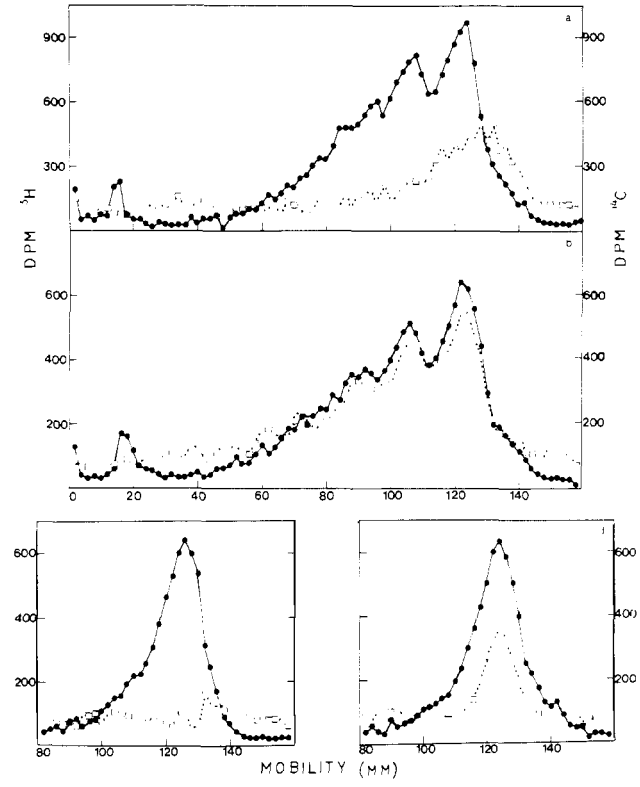

FIGURE 5: Electrophoretic analysis of DNA digestion products from [^{14}C]-labeled starved-refed nuclei, ^{3}H-labeled rDNA, and ^{3}H-labeled rChromatin. The following digestions were performed at 48 units of staphylococcal nuclease/ml: (a) ^{14}C-labeled nuclei, [^{3}H]rDNA for 10 min; (b) ^{14}C-labeled nuclei, [^{3}H]rChromatin for 10 min; (c) ^{14}C-labeled nuclei, [^{3}H]rDNA for 320 min; (d) ^{14}C-labeled nuclei, [^{3}H]rChromatin for 320 min. The DNA digestion products were electrophoresed on 1.5% agarose gels for 2.5 h: (●—●) [^{14}C]DNA; (□ · · · □) [^{3}H]DNA.

rChromatin in pure form. The important considerations for this set of experiments are that the fraction's ^{3}H counts are essentially all contained in rDNA and that this rDNA is not detectably sheared.

Digestion of rChromatin and rDNA This experiment was designed to test the premise that rDNA-containing chromatin exhibits a typical subunit profile because protein exchange with bulk chromatin occurs during the nuclease digestion. Half the final pellet described in Table II was frozen (rChromatin), while DNA was isolated from the other half (rDNA). Staphylococcal nuclease digestions were performed for 10 and 320 min on starved-refed nuclei (^{14}C prelabeled only) plus ^{3}H-labeled rDNA or rChromatin in a ratio approximating 50:1.

The data in Table III indicate that while rChromatin is protected from digestion to a degree comparable with bulk chromatin, rDNA is more readily degraded to acid-soluble material. Since rDNA is not totally degraded at 320 min, these data do not rule out the possibility that some protein exchange occurred leading to limited protection. However, it is clear from the gels illustrated in Figure 5 that the Cl_3CCOOH insoluble products of the rDNA digestion do not exhibit a typical subunit structure. There is no evidence of an oligomeric series after limited digestion, and there are no detectable 150 base pair monomers after extensive digestion. Since the ^{14}C-labeled DNA products appear normal, the lack of typical ^{3}H products cannot be attributed to a faulty digestion. In addition, since ^{3}H-labeled rChromatin exhibits both an oligomeric series after 10 min digestion and monomers after 320 min, the subnuclear fractionation did not disrupt native subunit structure.

Discussion

By differentially labeling bulk DNA and rDNA we have been able to study the subunit structure of chromatin containing a specific gene. Our data indicate that rChromatin exhibits a particulate structure indistinguishable from that of bulk chromatin. There are no obvious differences in either rate or extent of digestion. Likewise, the DNA digestion products are similar after both limited and extensive digestion.

Any study which proposes to compare bulk chromatin with a specific chromatin fraction must consider the possibility of protein exchange. In our experiments, exchange is a potential artifact during the nucleus isolation and during the nuclease digestion. We have not tested for protein rearrangement during the nucleus isolation since with present technical skills it is not possible to detect intranuclear protein exchange. It is probably worth noting that rDNA is encased within nucleoli and that in *Tetrahymena,* nucleoli are not intimately associated with chromatin (Nilsson and Leick, 1970).

However, we have provided evidence that protein exchange during the nuclease digestion cannot account for the particulate structure displayed by rDNA-containing chromatin. Free DNA, digested in the presence of nuclei, was degraded in a random fashion by staphylococcal nuclease. This DNA was predominantly rDNA (about 68%) and was present in a ratio to total DNA similar to that of rDNA in situ (1:50) (Yao and Gorovsky, 1974). Since *Tetrahymena* nuclei lyse during digestion in our low Ca^{2+} buffer (2.5×10^{-5} M), exchange between chromatin and exogenous DNA should have been possible.

Our conclusion from this set of experiments is that rDNA-containing chromatin exhibits a repeating subunit structure. This organization does not exist in free rDNA and is not the result of protein exchange during the nuclease digestion. Thus, we have shown that particulate chromatin structure is also characteristic of an extrachromosomal gene (Gall, 1975) that is repeated approximately 200 times/haploid genome (Engberg and Pearlman, 1972). This conclusion is supported by the report of Higashinakagawa and Reeder (1975) that the chromatin containing amplified rDNA in *Xenopus* also has a repeating subunit structure.

We cannot state with certainty that the rDNA-containing chromatin we examine contains active genes. Nilsson and Leick (1970) have shown that cultures of GL exhibit a rapid rate of RNA synthesis and a net accumulation of total cellular RNA from approximately 60 min after refeeding. Since rRNA accounts for about 85% of cellular RNA, it is assumed that rDNA is transcribed at this time. These studies were performed on cultures starved 3 h in an inorganic phosphate medium and refed with 2% proteose peptone-0.1% liver extract. Similarly, Hallberg (personal communication) has been able to detect newly synthesized RNA in isolated ribosomes within 15 min after refeeding. This RNA is not released with either EDTA or puromycin. Hallberg's studies employed cultures of B7 starved for 12–15 h in 50 mM Tris and refed in 1% proteose peptone. Although these studies imply that rRNA is being synthesized during the time of refeeding we examined, both use different conditions of starvation and refeeding and one deals with a different strain. Furthermore, it is not possible to say whether newly synthesized rDNA (i.e., that labeled from 60 to 120 min after refeeding) is being transcribed.

Because rDNA-containing chromatin consists of a complete functioning unit (transcribed and nontranscribed spacer regions as well as the sequences coding for 25S + 17S rRNA) with a well-characterized product, it provides a good system in which to begin a detailed dissection of chromatin structure. It should be possible to determine whether spacer and coding sequences are equally represented in nuclease sensitive and resistant regions of rChromatin. It may also be possible to study the subunit structure of rChromatin in different states of genetic activity.

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