

Organization of Nucleolar Chromatin

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I. Nucleolar Chromatin: Definition and Diversity of Contents and Arrangements of Genes

The definition of nucleolar chromatin as used in the context of this article includes all material that is associated with rDNA,* that is,

* The following terms and abbreviations are used in the context of this article: (1) *rDNA*: DNA that is enriched in, and may exclusively consist of, the genes for pre-rRNA and the interspersed apparent spacer regions. This definition of rDNA includes the possible occurrence of other genes in physical linkage with pre-rRNA genes, e.g., in rDNA spacer units (see below); (2) *pre-rRNA*: the largest size class of (stable) RNA molecules that contain 28, 18, and 5.8 S rRNA sequences (S values of ribosomal RNAs are nominal); (3) *intercept*: the region of a chromatin strand between any two given morphologically identified reference points, usually between two transcriptional complexes; (4) *transcriptional complex*; the particle containing the RNA polymerase, the associated chromatin region, and the attached nascent ribonucleoprotein (RNP) fibril; (5) *transcriptional unit*: the intercept that is transcribed by one RNA polymerase into one covalent ribopolynucleotide chain, i.e., an intercept, which is limited by a promotor

the DNA which contains the genes coding for the common precursor molecules of three of the constitutive ribosomal RNA's (25–28 S rRNA, 17–18 S rRNA, 5.8 S rRNA). The relative proportion of the nuclear DNA that is represented by rDNA is highly variable in different organisms and cell types. In most nuclei it constitutes only a quantitatively minor portion of the DNA but in some cell types the rDNA is greatly amplified and accumulated in extrachromosomal copies so that it represents a considerable proportion and can even exceed the total chromosomal DNA (for reviews, see Busch and Smetana, 1970; Birnstiel *et al.*, 1971; Tobler, 1975; Hadjiolov and Nikolaev, 1976; for plants, see also Ingle *et al.*, 1975). The numbers of copies of pre-rRNA genes present per nucleus also show great differences in different organisms and cell types, even in different nuclei of the same cell (in organisms with nuclear dimorphism): the extreme values are represented by the micronucleus of a ciliate, *Tetrahymena pyriformis*, which contains one copy (Yao and Gall, 1977), and the oocyte nuclei of various amphibia and insects which contain several millions of copies (for references, see the reviews quoted above and Trendelenburg *et al.*, 1977). Typically, rDNA also contains intercepts that are interspersed between the pre-rRNA gene regions and apparently are not transcribed from the same promotor as the pre-rRNA. Such rDNA spacer regions often appear as nontranscribed at times of full transcriptional activity of the adjacent pre-rRNA genes. It should be kept in mind, however, that arrays of transcriptional structures have some-

and a terminator site. This allows for the occurrence of processing in the nascent ribopolynucleotide; (6) *matrix unit*: an intercept covered with a series of lateral fibrils which increase in length from one point (the starting point) or, at least, all of which are longer than the fibril at the starting point (the latter to allow for potential processing events or differences of packing density in the nascent RNA). In discussions on nucleolar chromatin, matrix unit is often used in a restricted sense, i.e., matrix unit containing nascent pre-rRNA ("pre-rRNA matrix unit"); (7) *apparent spacer unit*: morphologically identified intercepts that lie between pre-rRNA matrix units and usually are not covered with lateral fibrils. In nucleolar chromatin, (apparent) spacer unit is usually meant to designate the intercepts which lie between pre-rRNA matrix units and contain sequences that are not transcribed from the same promotor as the pre-rRNA molecules. This definition does not exclude the possible occurrence of genes others than those coding for pre-rRNA in apparent spacer units; (8) *repeating unit*: intercept of chromatin strand which, in arrays of clustered genes, includes a matrix unit plus the adjacent (preceding or subsequent) apparent spacer unit. In nucleolar chromatin "repeating unit" is usually meant in the sense of the rDNA repeat unit, i.e., a given pre-rRNA matrix unit plus one of the two adjacent apparent spacer units (see also footnote 2); (9) *rDNA spacer unit*: regions in units of rDNA that do not contain sequences complementary to pre-rRNA; (10) *rDNA repeating unit*: the unit consisting of a sequence coding for pre-rRNA and the adjacent (subsequent or preceding) rDNA spacer unit.

times been observed in chromatin regions corresponding to apparent spacer units between pre-rRNA matrix units (e.g., Scheer *et al.*, 1973, 1977; see also below), and that, in some lower eukaryotes, genes coding for 5 S rRNA are located in such spacer regions where they represent independent transcriptional units (e.g., yeast: Rubin and Sulston, 1973; Bell *et al.*, 1977; Valenzuela *et al.*, 1977; *Dictyostelium*: Maizels, 1976). Consequently, it may be safe to include in the definition of both rDNA and nucleolar chromatin the possible existence of other, nonpre-rRNA genes.

Not only can the amount of total rDNA, usually determined as DNA complementary to pre-rRNA, be different in different organisms but there also can exist pronounced differences of the lengths of the pre-rRNA genes and their transcriptional units, the spacer regions, the resulting "units of repeat",* and the modes of arrangement of pre-rRNA genes among different organisms, cell types, and nuclei. Table I presents a compilation of data obtained with various methods which illustrate the perplexing diversity of the sizes of pre-rRNA genes, spacers, and repeating units and their patterns of arrangement. Moreover, it has been shown that DNA units containing pre-rRNA genes do not always occur in the typical tandem arrangements of repeating units but, in some organisms, can also exist in palindromic arrangements such as in the amplified rDNA units containing two genes that occur in the macronucleus of the ciliate, *Tetrahymena pyriformis* (Engberg *et al.*, 1976; Karrer and Gall, 1976), and in the plasmodial nuclei of the slime mold, *Physarum polycephalum* (Molgaard *et al.*, 1976; Vogt and Braun, 1976). A similar arrangement of matrix units with alternating polarity has also been suggested in the multigenic chromatin strands of the primary nucleus of a green alga, *Acetabularia exigua* (Berger *et al.*, 1978). The situation is even more complicated (1) by the existence of differences in the sizes and the pattern of the rDNA subunits among different individuals of the same species and (2) by the intra- and intermolecular heterogeneity described in some organisms

* The term "units of repeat" in the commonly used sense (cf. Footnote 1) is fully adequate only to the situation of series of tandemly arranged, similarly sized units of genes and/or matrix units, with or without similarly sized spacer regions. In strands containing heterogeneously sized spacer regions and/or pre-rRNA genes and matrix units (of identical nature), we extend the use of this term to designate any region in a strand of nucleolar chromatin and/or rDNA which contains a pre-rRNA gene (or matrix unit) plus the specific preceding or subsequent spacer unit. In amplified rDNA circles containing one pre-rRNA gene and in the arrays with alternating polarity, as, for example, the palindromic arrangements, we use the term "equivalent of repeat" (unit) in order to designate a unit containing one pre-rRNA gene (or matrix unit) and the corresponding portion (half) of associated spacer (cf. Table I).

TABLE I
Lengths (μm)^a of Repeat Units^b and Functional Subunits of rDNA of Various Eukaryotic Organisms

Species (type of cell and/or nucleus)	Method used ^a			References
	(1)	(2)	(3)	
Algae				
<i>Chlamydomonas reinhardi</i>	Repeat: ca. 2.9 Matrix: 1.9 Spacer: ca. 1.0			Woodcock <i>et al.</i> (1975)
<i>Acetabularia mediterranea</i> (primary nucleus)	Type Ia, mod. 1 Repeat: 2.6 Matrix: 2.1 Spacer: ca. 0.5 Type Ia, mod. 3 Repeat: 2.2 Matrix: 1.9 Spacer: ca. 0.3 Type IIa Repeat: 4.25 Matrix: 2.05 Spacer: 2.2 Selected examples of frequently observed size classes; intra- and intermolecular heterogeneity Repeat: 2.35 Matrix: 1.85 Spacer: ca. 0.5			Spring <i>et al.</i> (1976); cf. also Spring <i>et al.</i> (1974); Trendelenburg <i>et al.</i> (1974, 1975)
				Woodcock <i>et al.</i> (1975)

<i>Acetabularia major</i> (primary nucleus)	Repeat: 2.85 Matrix: 1.85 Spacer: 1.0 Most frequently observed type; intra- and intermolecular heterogeneity	Spring <i>et al.</i> (1976); cf. also Spring <i>et al.</i> (1974); Trendelenburg <i>et al.</i> (1974, 1975)
	Repeat: 3.4 Matrix: 1.9 Spacer: 1.5	Berger and Schweiger (1975a)
<i>Acetabularia cliftonii</i> (primary nucleus)	Repeat: 3.8 Matrix: 2.15 Spacer: 1.65 Most frequently observed type; intra- and intermolecular heterogeneity	Franke <i>et al.</i> (1976a); Spring (1977 and unpublished data)
<i>Acetabularia ryukyuensis</i> (primary nucleus)	Repeat: 2.55 Matrix: 1.85 Spacer: 0.7	Berger and Schweiger (1975b)
<i>Acetabularia dentata</i> (primary nucleus)	Repeat: 3.25 Matrix: 2.35 Spacer: 0.9	Berger and Schweiger (1975b)
<i>Acetabularia peniculus</i> (primary nucleus)	Repeat: 3.25 Matrix: 2.3 Spacer: 0.95	Berger and Schweiger (1975b)
<i>Acetabularia calyculus</i> (primary nucleus)	Repeat: 2.2 Matrix: 1.85 Spacer: 0.35 Intra- and intermolecular heterogeneity	Spring (unpublished data)

TABLE I (continued)

Species (type of cell and/or nucleus)	Method used ^a			References
	(1)	(2)	(3)	
<i>Acetabularia exigua</i> (primary nucleus)	<p>“Head-to-head” arrays of alternating polarity, indicative of palindromic arrangement</p> <p>Type 1 Equivalent of repeat: 1.6 Equivalent of matrix: 1.4 Equivalent of spacer: 0.2</p> <p>Type 2 Equivalent of repeat: 1.85 Equivalent of matrix: 1.4 Equivalent of spacer: 0.45</p>			Berger <i>et al.</i> (1978)
<i>Dasycladus clavaeformis</i> (primary nucleus)	<p>Repeat: 2.05 Matrix: 1.85 Spacer: ca. 0.2</p>			Berger and Schweiger (1975b)
<i>Cymopolia van bosseae</i> (primary nucleus)	<p>Repeat: 2.3 Matrix: 1.9 Spacer: 0.4</p>			Berger and Schweiger (1975b)
<i>Batophora oerstedii</i> (pri- mary nucleus)	<p>Type 1 Repeat: 1.95 Matrix: 1.95 Spacer: 0 (<0.1)</p> <p>Type 2 Repeat: 4.1 Matrix: 1.95 Spacer: 2.15</p>			Berger and Schweiger (1975c)

Higher plants				
<i>Glycine max</i>			Repeat: 2.8	Friedrich <i>et al.</i> (1978)
var. wayne			(5.9×10^6 MW)	
Slime molds				
<i>Physarum</i>	Amplified linear molecules containing two pre-rRNA genes in palindromic arrangement			
<i>polycephalum</i>				
(plasmodia)		Equivalent of repeat: 8.9	Equivalent of repeat: 8.9	Vogt and Braun (1976)
		(median)	(18.5×10^6 MW)	
			Equivalent of repeat: 9.4	Molgaard <i>et al.</i> (1976)
			(19.6×10^6 MW)	
	Equivalent of repeat: 7.5	Equivalent of repeat: 8.9		Grainger and Ogle
	Matrix: 4.2	(mode)		(1978)
	Equivalent of spacer:			
	3.0-3.3			
<i>Dictyostelium</i>			Repeat: 12.1	Maizels (1976)
<i>discoideum</i>			(38×10^3 NTP)	
			Repeat: 13.4	Cockburn <i>et al.</i> (1976)
			42×10^3 NTP)	
Fungi				
<i>Saccharomyces</i>			Repeat: 2.8	Bell <i>et al.</i> (1977)
<i>cerevisiae</i>			(5.8×10^6 MW)	
Protozoa				
<i>Paramecium</i>		Linear and circular amplified molecules		Findly and Gall (1978)
<i>tetraurelia</i>		repeat: 2.6 (5.5×10^6 MW)		
(macronuclei)		intra- and intermolecular heterogeneity		
<i>Tetrahymena</i>		Amplified linear molecules containing two pre-rRNA genes		Engberg <i>et al.</i> (1976);
<i>pyriformis</i> and		in palindromic arrangement		cf. Engberg <i>et al.</i>
<i>thermophila</i>		Equivalent of repeat: 3.0 (6.3×10^6 MW strain GL)		(1974)
(macronuclei)		Equivalent of repeat: 3.0 (6.3×10^6 MW; strain GL)		Karrer and Gall
		3.25 (6.75×10^6 MW; strain BVII, <i>T. thermophila</i>)		(1976); Gall
		range of mean values: $6.5-6.9 \times 10^6$ MW)		(1974)

(continued)

56 TABLE I (continued)

Species (type of cell and/or nucleus)	Method used ^a			References
	(1)	(2)	(3)	
	Equivalent of repeat: ca. 2.6 Equivalent of matrix: ca. 2.0 Equivalent of spacer: ca. 0.6, syngen I			Values estimated from data in Gall <i>et al.</i> (1977)
(micronucleus)			Single integrated gene in chromosome Equivalent of repeat: 3.2 (6.6×10^6 MW)	Yao and Gall (1977)
<i>Oxytricha</i> sp. (macronucleus)		In the developing macronucleus the DNA is fragmented into "gene-sized" pieces; transcribed pre-rRNA genes are contained in 14 S DNA size class Equivalent of repeat: ca. 2.0-2.2		Prescott <i>et al.</i> (1973)
<i>Oxytricha fallax</i>		Organization as in <i>Oxytricha</i> sp. Equivalent of repeat: 2.1 (6.7×10^3 NTP) Equivalent of matrix: 1.94 (minimum)		Rae and Spear (1978)
<i>Stylonychia</i> <i>mytilus</i> (macronucleus)		Organization as in <i>Oxytricha</i> Equivalent of repeat: 2.2 (4.5×10^6 MW)		Lipps and Steinbrück (1978)
<i>Amoeba proteus</i>	Matrix: 6.07			Murti and Prescott (1978)
<i>Leishmania</i> <i>donovani</i>		Repeat: 4.3 (13.51×10^3 NTP)		Leon <i>et al.</i> , 1978
Insects				
<i>Acheta domesticus</i> (oocytes)	amplified rDNA molecules; many of them in circles Repeat: 11.15 (mean value; range: 7.6-16.0)	Equivalent of repeat: broad range, 7.5-16.5		Trendelenburg <i>et al.</i> (1976); Trendelenburg

	Matrix: 5.4 (mean value; range: 3.8–7.5)	Heterogeneity	(1977); cf. Trendelenburg <i>et al.</i> (1973, 1975, 1977)
	Spacer: 5.85 (mean value; range 3.5–ca. 10.0)		
	Intra- and intermolecular heterogeneity		
<i>Oncopeltus fasciatus</i> (embryos)	Repeat: 2.9–3.4 (range)		Foe <i>et al.</i> (1976)
	Matrix: 2.4 (mean)		
	Spacer: 0.5–1.0 (range)		
<i>Dytiscus marginalis</i> (oocytes and/or whole ovaries)	Amplified rDNA molecules; most of them in circles		
	Repeat: 6.95 (peak mean; total range: 4.3–15.0)	Equivalent of repeat: ca. 7.0 (peak mean; total range: 6.0–ca. 12.0)	Trendelenburg <i>et al.</i> (1976); Trendelenburg (1977); cf. Trendelenburg (1974); Trendelenburg <i>et al.</i> (1977)
	Matrix: 3.6 (peak mean; total range: ca. 2.0–5.4)		
	Spacer: 3.4 (peak mean total range: ca. 1.5–12.2)		
	Intra- and intermolecular heterogeneity		
		Equivalent of repeat: 9.25 (19.2×10^6 MW)	Gall and Rochaix (1974)
<i>Colymbetes fuscus</i> (ovaries)		Equivalent of repeat: ca. 5.0 (10.04×10^6 MW)	Gall and Rochaix (1974)
<i>Trichosia pubescens</i> (spermatocytes)	Repeat: ca. 2.3		Amabis and Nair (1976)
	Matrix: 2.3		
	Spacer: 0–0.1 (range)		
<i>Chironomus thummi</i>	Repeat: 2.65		Derksen <i>et al.</i> (1973)
	Matrix: 2.2		
<i>Chironomus tentans</i> (embryo)		Repeat: 2.5 (7.8×10^3 NTP)	Degelmann <i>et al.</i> , 1978

TABLE I (continued)

Species		Method used ^a		
(type of cell and/or nucleus)	(1)	(2)	(3)	References
	Repeat: — Matrix: 2.55 Spacer: —			McKnight and Miller (1976)
	Type 1 Repeat: 3.3 Matrix: 2.5 Spacer: 0.8			Laird and Chooi (1976); Laird <i>et al.</i> (1976)
	Type 2 Repeat: 3.9 Matrix: 2.5 Spacer: 1.4			
	Repeat: 3.45 (range 2.95–3.75) Matrix: 2.65 Spacer: 0.8 (range 0.3–1.1)			Chooi (1976)
(ovarian nurse cells)	Repeat: 3.95 (range 3.85–4.55) Matrix: 2.65 Spacer: 1.3 (range 1.2–1.9)			Chooi (1976)
(whole pupae)			Repeat: 4.4 (13.74×10^3 NTP; basic unit; total range of repeat units containing insertions: 4.7–6.9) Heterogeneity	Pellegrini <i>et al.</i> (1977)
(whole embryos)		Repeat: 3.5 (11×10^3 NTP; basic unit; total range of repeat units with insertions: 3.65–5.4) Heterogeneity		Wellauer and Dawid (1977); cf. Tartof and Dawid (1976)

(rDNA fragment Dm 103 inte- grated in plasmid, and whole embryos)		Repeat: 3.6 (11.4×10^3 NTP; basic unit; another size class of 5.4, i.e., 17×10^3 NTP, contains an insertion)	White and Hogness (1977); Glover and Hogness (1977)
<i>Drosophila</i> <i>virilis</i> (whole DNA)		Repeat: 6.7 (21×10^3 NTP; most frequent size class containing a ca. 2.2 μm insertion; basic unit without insertion ca. 4.5)	Barnett and Rae (1977)
<i>Drosophila hydei</i> (spermatocytes)	Repeat: 3.4 (total range: 2.0–4.2) Matrix: 2.5 (total range: 1.5–2.7) Spacer: 0.9 (total range: 0.5–1.7) Repeat: 3.5–5.1 (total range) Matrix: 2.6–3.2 (total range) Spacer: 0.9–1.9 (total range)		Meyer and Hennig (1974)
<i>Sciara coprophila</i> (spermatocytes)	Repeat: 2.75 (median) Matrix: 2.15 (median) Spacer: 0.60 (median)	Similar values as under (1)	Glatzer (1975) Renkawitz <i>et al.</i> (1978)
Echinoderms <i>Lytechinus</i> <i>variegatus</i> (sperm)		Repeat: 3.85 (8×10^6 MW) Repeat: 3.6 (11.4×10^3 NTP)	Wilson <i>et al.</i> (1976) Wilson <i>et al.</i> (1978)

(continued)

TABLE I (continued)

Species (type of cell and/or nucleus)	Method used ^a			References
	(1)	(2)	(3)	
Fish				
<i>Brachydanio rerio</i> (oocytes)	Repeat: 2.70 Matrix: 1.85 Spacer: 0.85			Trendelenburg (1977)
Amphibia				
<i>Andrias davidianus</i> (oocytes)	Repeat: 3.40 (median) Matrix: 2.15 (median) Spacer: 1.25 (median) Most frequently observed size class; hetero- geneity			Trendelenburg (1977)
Pleurodeles <i>waltli</i> (oocytes)	Repeat: 3.25 (median) Matrix: 2.40 (median) Spacer: 0.85 (median) Most frequently observed size class; intra- and intermolecular hetero- geneity			Trendelenburg (1977); cf. Angelier and Lacroix (1975)
<i>Notophthalmus viridescens</i> (oocytes)	Repeat: 2.9–3.3 (range) Matrix: 2.2–2.5 (range) Spacer: 0.7–0.8 (range) Heterogeneity observed			Miller and Beatty (1969a,b,c); Miller and Bakken (1972); Miller and Hamkalo (1972)
<i>Triturus helveticus</i> (oocytes)	Repeat: 4.8 Matrix: 2.6 Spacer: 2.2 Heterogeneity observed			Scheer <i>et al.</i> (1973)

<i>Triturus alpestris</i> (oocytes)	Repeat: 4.8 (median) Matrix: 2.7 (median) Spacer: 2.1 (median) Median values of most frequently observed size class; heterogeneity observed	Trendelenburg (1977); cf. Scheer <i>et al.</i> (1973)
<i>Triturus cristatus</i> (oocytes)	Repeat: 4.25 (median) Matrix: 2.50 (median) Spacer: 1.70 (median) Most frequently observed size class; heterogeneity observed	Trendelenburg (1977); cf. Scheer <i>et al.</i> (1973); Franke <i>et al.</i> (1976a)
<i>Rana pipiens</i> (oocytes)	Repeat: 3.2 (median) Matrix: 2.2 (median) Spacer: 0.8 (median)	Trendelenburg and McKinnell (1978), pers. communication.
<i>Xenopus laevis</i> (oocytes)	Repeat: 2.7–3.3 (range) Matrix: 2.0–2.5 (range) Spacer: 0.7–0.8 (range) Heterogeneity observed Repeat: 3.35 (median; total range ca. 2.4–7.0) Matrix: 2.40 (median; total range up to 4.2) Spacer: 0.95 (median; total range ca. 0.5–2.7) Median values are for a frequently observed type of pattern; intra- and intermolecular heterogeneity; for detailed quantitative analyses see Scheer <i>et al.</i> , 1977; Reeder <i>et al.</i> (1978b)	Miller and Beatty (1969c) Scheer <i>et al.</i> (1977); cf. Scheer <i>et al.</i> (1973); Franke <i>et al.</i> (1976a)

TABLE I (continued)

Species	Method used ^a			References
(type of cell and/or nucleus)	(1)	(2)	(3)	
(erythrocytes)		Repeat: 4.0 (8.3×10^6 MW)		Henikoff <i>et al.</i> (1974)
(ovaries and somatic cells)		Repeat: 5.4 (8.7×10^6 MW)		Wensink and Brown (1971)
		Repeat: 3.25–5.05 ($6.8–10.5 \times 10^6$ MW; total range observed; discrete size classes characteristic for the specific individual or nucleolar state are found; pronounced heterogeneity in regions not coding for pre-rRNA)		Forsheit <i>et al.</i> (1974); Morrow <i>et al.</i> (1974); Wellauer <i>et al.</i> (1974, 1976a,b); Wellauer and Dawid (1974); Southern (1975); Reeder <i>et al.</i> (1976a,b); Buongiorno-Nardelli <i>et al.</i> (1977)
		Circular amplified rDNA molecules Equivalent of repeat: 3.5–4.0 (total range of reported mean values)		Hourcade <i>et al.</i> (1973); Rochaix <i>et al.</i> (1974); Rochaix and Bird (1975); Bakken (1975); Buongiorno-Nardelli <i>et al.</i> (1976)

Xenopus borealis
(erythrocytes)
(ovaries and
somatic cells)

Repeat: 4.25 (8.8×10^6
MW)

Repeat: 5.0 (8.5×10^6
MW)

Repeat: 3.45–3.8 (7.2 – 7.9×10^6 MW; heterogeneity
observed in regions not coding for pre-rRNA;
discrete size classes occur)

Henikoff *et al.* (1974)

Brown *et al.* (1972)

Wellauer and Reeder
(1975); cf.
Forsheit *et al.*
(1974)

Mammals

Mus musculus
(liver tissue)

Repeat: ca. 14
(44×10^3 NTP;
heterogeneity
observed)

Cory and Adams
(1977); cf.
Southern (1975)

(various tissues
and cultured
cells)
(cultured
kidney cells)

Repeat: 14–15
Spacer: 9–10

Repeat: 11.5
(24×10^6 MW;
heterogeneity
indicated)

Arnheim and
Southern (1977);
cf. Southern
(1975)

Mus poschiavinus
(cultured cells)

Pattern slightly different
from that of *Mus*
musculus; hetero-
geneity indicated

Puvion-Dutilleul and
May (1978)
Arnheim and
Southern (1977)

Rattus norvegicus
(hepatocyte
nuclei from rat
liver)

Matrix unit: 4.0–4.5
(total range observed)

Puvion-Dutilleul
et al. (1977a)

Cricetulus griseus
(cultured
Chinese hamster
ovary cells)

Repeat: 10.2
Matrix: 3.8
Spacer: 6.4
Heterogeneity especially
in spacer lengths

Puvion-Dutilleul
et al. (1977b)

TABLE I (continued)

Species (type of cell and/or nucleus)	Method used ^a			References
	(1)	(2)	(3)	
(cultured Chinese hamster lung cells)			Repeat: 7.8 (16.2×10^6 MW; heterogeneity observed)	Stambrook (1978)
<i>Bos taurus</i> (calf thymus tissue)			Repeat: 10.25 (21.3×10^6 MW)	Blin <i>et al.</i> (1976)
<i>Homo sapiens</i> (human placenta and several cell culture lines) (HeLa cells)	Repeat: ca. 7.0 Matrix: 3.5 Spacer: ca. 3.5		Repeat: 9.6 (20×10^6 MW; heterogeneity indicated)	Arnheim and Southern (1977) Miller and Bakken (1972)

^a The following factors for the interconversion of values for lengths, molecular weights (MW) and numbers of nucleotide pairs (NTP) in double-stranded DNA have been used: $1 \mu\text{m} = 2.08 \times 10^6 \text{ MW} = 3143 \text{ NTP}$ (Stüber and Bujard, 1977).

^b For the extended definition of the term "repeat unit" see footnote 2 of the text.

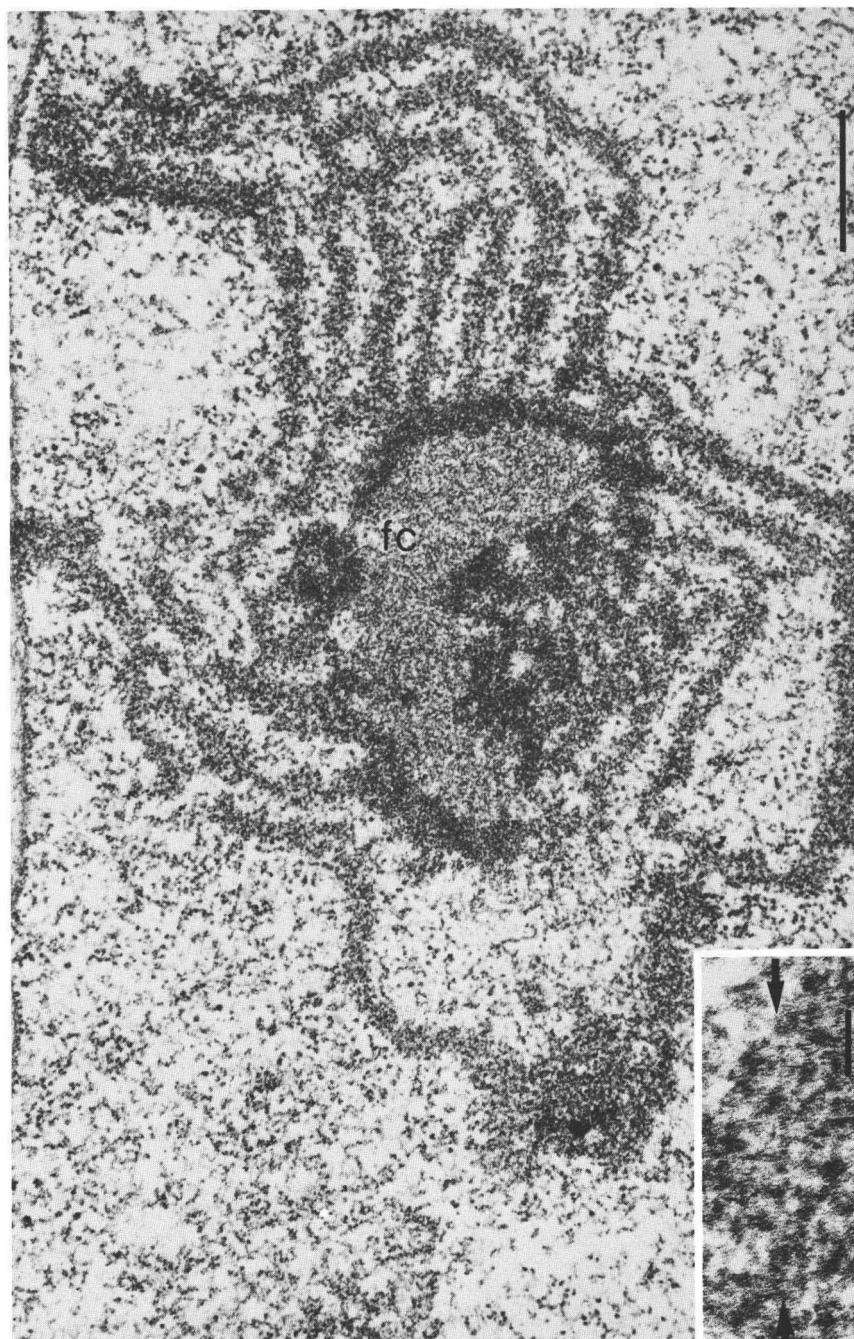
^c Key to Methods: (1) electron microscopy of spread preparations of transcribed chromatin (mean values are given, if not indicated otherwise); (2) biochemical and/or electron microscopic determination of sizes of rDNA molecules and mapping after partial denaturation or heteroduplex formation; (3) biochemical determination of sizes of fragments obtained after digestion with restriction endonucleases.

and cell types, respectively, which often is especially prominent in the spacer regions (see Table I and below; references are given in Table I).

The location and accumulation of rDNA in specific chromosomal regions, the "nucleolar organizers" (for review, see Busch and Smetana, 1970), its occurrence, in various cells, in separate extra-chromosomal units and, in most cell types, the high proportion of pre-rRNA genes that are transcriptionally active, makes the nucleolar chromatin a suitable, well-defined object for studies of chromatin organization. At present, however, one has to state that isolation and purification of nucleolar chromatin, without considerable contamination by associated nonnucleolar material, is still a problem that has so far been solved only with limited success (for references, see Busch and Smetana, 1970; Matsui and Busch, 1977; for procedures of isolating chromatin of amplified nucleoli, see the work done with *Tetrahymena* macronuclei by Leer *et al.*, 1976; Piper *et al.*, 1976; Mathis and Gorovsky, 1976; see the work done with *Xenopus* oocytes by Higashinakagawa *et al.*, 1977; Reeder *et al.*, 1978a).

Depending on the specific state of transcriptional activity, the morphology and the composition of nucleolar chromatin can vary greatly. It is obvious that during states of high transcriptional activity, as they are characteristic for many cells, the nucleolar chromatin contains large amounts of RNA polymerase A, nascent pre-rRNA, and RNA-associated proteins, which are absent from inactive nucleolar chromatin. Consequently, in such states of high transcriptional activity the DNA is present in a highly dispersed and "diluted" form and represents only a minor component of the whole nucleolar body. This situation is in contrast to the appearance of transcriptionally inactive nucleolar chromatin which is characterized by high DNA package per volume unit and a strong reaction with dyes that stain DNA, including the Feulgen reaction. The fact that in some processes of cell differentiation relatively large amounts of rDNA are present which, naturally or after certain experimental treatment, change from a transcriptionally inactive state to periods of high transcriptional activity and/or vice versa (see below) also makes nucleolar chromatin an object of high potential value in studying general aspects of the regulation of transcription.

Ribosomes are basic structures of all cells, and the composition of ribosomes is rather similar in eukaryotic cells. Ribosomal RNA's show a relatively high degree of conserved sequences (for references, see Busch and Smetana, 1970; Birnstiel *et al.*, 1971; Gerbi, 1976). This conservatism of function and composition of rDNA throughout the eukaryotes is in contrast to the great differences in the size and pat-



terns of arrangement of the pre-rRNA genes and their transcriptional units which apparently do not follow an obvious evolutionary order (Table I; cf. Hadjiolov and Nikolaev, 1976). From the data presented in Table I it is clear that the definition of nucleolar chromatin includes different sorts of DNA and different proportions of chromatin and RNP material in the diverse pre-rRNA gene arrangements described. In transcribed nucleolar chromatin, for example, chromatin of matrix units ("matrix chromatin") is clearly different in composition from the chromatin of nontranscribed spacer regions ("spacer chromatin"), and the different ratios of both regions in the different organisms and cell types should also result in great compositional differences such as in the ratio of histones and non-histone protein components to the total rDNA. Thus, although nucleolar chromatin is a relatively well-defined type of chromatin, it is not identical in composition in different eukaryotes, and can even be different in different cell types, nuclei or nucleolar regions of the same organism.

II. Appearance of Nucleolar Chromatin in Ultrathin Sections

The appearance of nucleoli, intrachromosomal as well as extrachromosomal ones, in electron micrographs of ultrathin sections has been sufficiently described in review articles (for references, see, e.g., Busch and Smetana, 1970; Bouteille *et al.*, 1974; Smetana and Busch, 1974; Gimenez-Martin *et al.*, 1977). Essentially, three morphological entities can be distinguished: (1) the condensed perinucleolar chromatin which is mostly inactive in transcription and may include, in many situations, nonnucleolar chromatin; (2) the transcriptionally active true nucleolar chromatin appears in various forms of fibrillogranular arrays. These include the very characteristic "nucleolonemata" (for definition see reviews quoted above) which seem to represent individual rDNA-containing chromatin strands, each containing one axis, that are heavily engaged in transcription and are associated with nascent ribonucleoprotein (pre-rRNP) material, a portion of which appears in the form of densely stained granules of 13–20 nm in diameter (Fig. 1;

Fig. 1 Electron micrograph of an ultrathin section through the nucleolus of a PtK₂ cell (permanent cell line derived from kidney epithelium of the rat kangaroo, *Potorous tridactylis*) that has been grown in "starvation medium" (only 1% fetal calf serum added; for normal culture conditions, see Osborn *et al.*, 1977). Note the "fibrillar center" (fc) and the relatively dispersed and extended nucleolonema structures. Each nucleolonema strand consists of a core of fibrillar material, including the putative rDNA chromatin axis (denoted by arrows in the insert), and 13–20 nm large densely stained granules in the more peripheral zones. Bars denote 0.5 μm and 50 nm (insert).

see reviews quoted above). Such granules often seem to be the terminal particles of fibrils laterally projecting from the chromatin axis of the nucleolonema, indicative of terminal coiling up of the pre-rRNP fibrils (see below). In other words, the nucleolonema seems to represent the relatively compacted *in situ* state of the structural complexes that appear as typical matrix units upon dispersion in low salt buffers and spreading (see below). (3) In addition, nucleoli often contain, or are associated with, masses of more or less dispersed or aggregated fibrillar material, which seem to be proteinaceous in nature, often in close association with ribonucleoprotein (rRNP) particles. In certain situations, these nucleolus–chromatin-associated protein fibrils appear to be concentrated in so-called “fibrillar centers” or fibrillar bodies (Fig. 1; see reviews mentioned above) which reveal a rather homogeneously textured ultrastructure. This aggregated appearance seems to contrast with a more dispersed state of such fibrils such as in the nucleolar “cavities” or “vacuoles” of transcriptionally very active nucleoli. A more dispersed form of these fibrillar masses may also be present in the typical *pars granulosa* of nucleoli and serve as a matrix for the attachment of rRNP particles. Nucleolus-associated fibrillar aggregates of various sizes often are increased or appear as “segregated” zones of the nucleolus after drastic reduction of the transcriptional activity of nucleolar chromatin, be it natural or induced by treatment with drugs inhibiting transcription (for references, see Busch and Smetana, 1970; Franke *et al.*, 1973; Simard *et al.*, 1974; Eckert and Franke, 1975; Gimenez-Martin *et al.*, 1977). In our opinion, the diverse phenomena observed would be best explained by the interpretation that such aggregate bodies include fibrillar aggregates of different nature. They represent either (1) the inactive nucleolar chromatin after release of the nascent RNP fibrils (cf. Scheer *et al.*, 1975, 1976a) or (2) the aggregated protein skeleton elements of the nucleolus after release of the associated RNP particles. The specificity and the forces involved in the specific association of these tangles of protein fibrils with the nucleolar chromatin proper remain to be clarified.

After complete cessation of nucleolar activity for considerable periods of time such as in mature erythrocytes of amphibia and sauropsids, in late spermiogenesis, or during extreme nuclear pyknosis, the nucleolus and the nucleolar chromatin are no longer able to be identified and seem to be lost in the uniformly appearing condensed chromatin. Therefore, it seems justified to conclude that inactive nucleolar chromatin can assume the same package into the granular structures characteristic of inactive chromatin as other portions of the genome (Franke *et al.*, 1978a; Franke and Scheer, 1978; see also be-

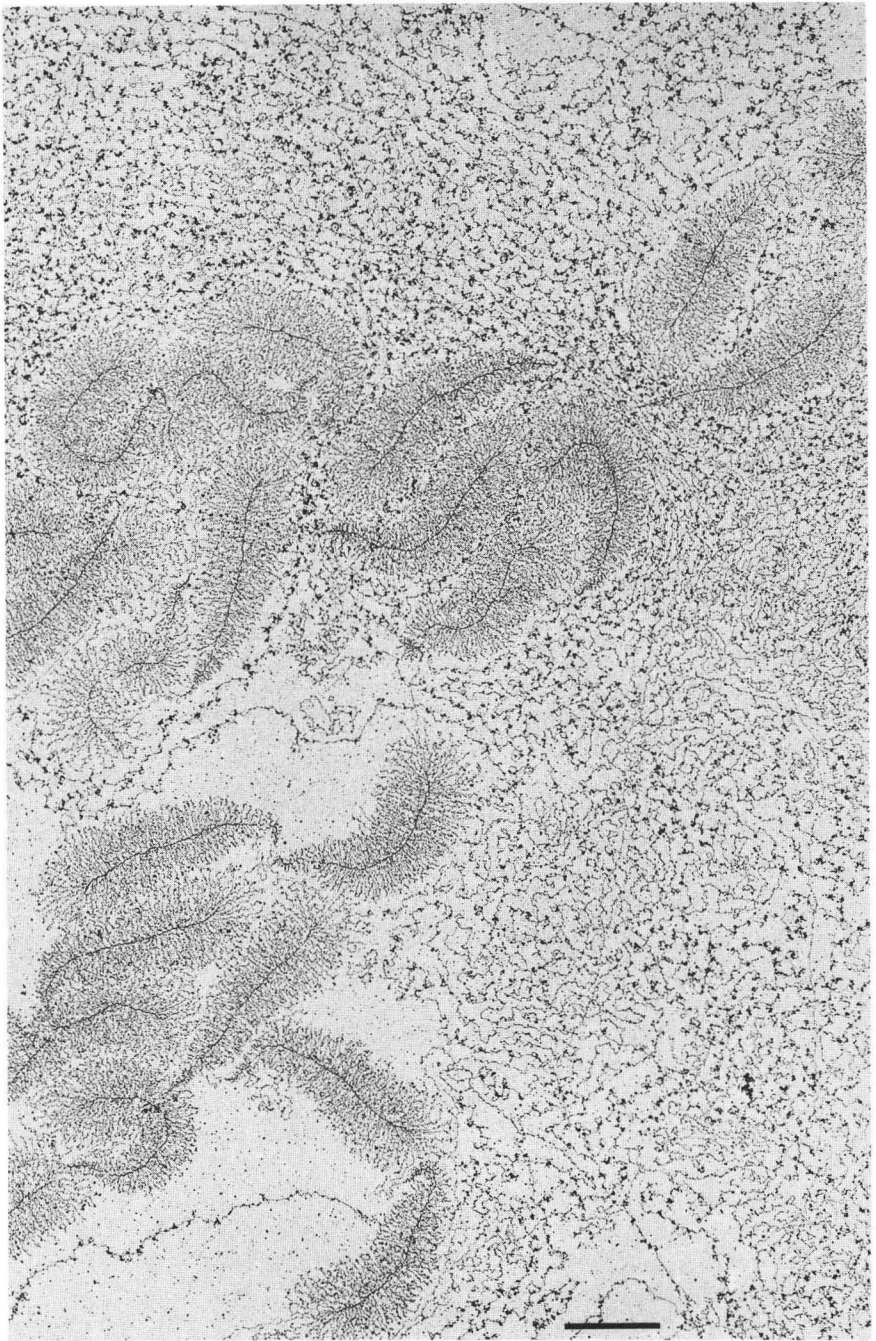
low). This is particularly suggested for the inactive stages of amplified nucleolar chromatin as described, for example, in early stages of oogenesis of some insects (e.g., Trendelenburg *et al.*, 1977; Scheer and Zentgraf, 1978).

III. Morphology of Transcriptionally Active Nucleolar Chromatin in Spread Preparations

When transcriptionally active nucleoli are isolated, allowed to disperse in media of low ionic strength, and are then centrifuged onto an electron microscopic grid according to the technique by Miller and co-workers (Miller and Beatty, 1969a,b; Miller and Bakken, 1972), two prominent structures are observed (Figs. 2–4). One is the nucleolar chromatin proper identified by the typical arrays of matrix units and apparent spacer regions (see below; see also Miller and Beatty, 1969a,b; Miller and Bakken, 1972). In most nucleoli, this nucleolar chromatin is intimately associated with, or is surrounded by, masses of 6–8 nm thick filaments which sometimes reveal a regular beaded substructure and often show, in highly variable frequency and pattern, attached 15–40 nm large, densely stained granules. These fibrils, some of which can be traced for several micrometers, are resistant to treatments with very low as well as high salt concentrations, sulfhydryl agents, non-ionic detergents, and various nucleases, but are digested with proteases and therefore most likely represent purely proteinaceous material. It may well be that such fibrillar structures represent the spread out state of the fibrillar masses described in ultrathin section studies (see above), including the fibrillar centers and the skeletal components of the *pars granulosa* (see above; cf. Busch and Smetana, 1970, Fig. 14b; Franke *et al.*, 1978b). These two major structural components are observed in spread preparations of nucleoli from animal as well as from plant cells, in chromosomal nucleoli as well as in amplified nucleoli (Figs. 2–4). In many preparations, the two portions are separated during the dispersion and the spreading and can be found as distinct, isolated units on the supporting grid. Small amounts of residual fibrils, however, are usually still recognized in the vicinity of the matrix units of the nucleolar chromatin (e.g., Fig. 5).

In fully active nucleoli, the nucleolar chromatin proper usually consists of two different portions.

1. The regions containing the pre-rRNA genes appear as matrix units and their chromatin axis is densely covered by transcriptional complexes. These are recognized as 10–14 nm large particles which



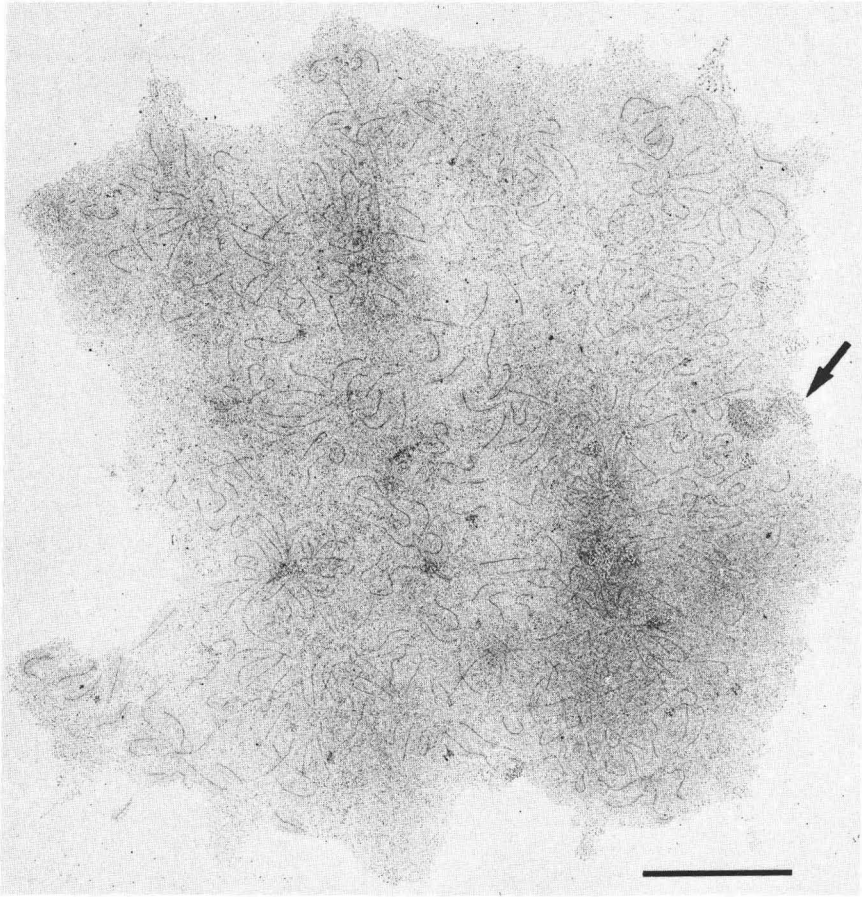
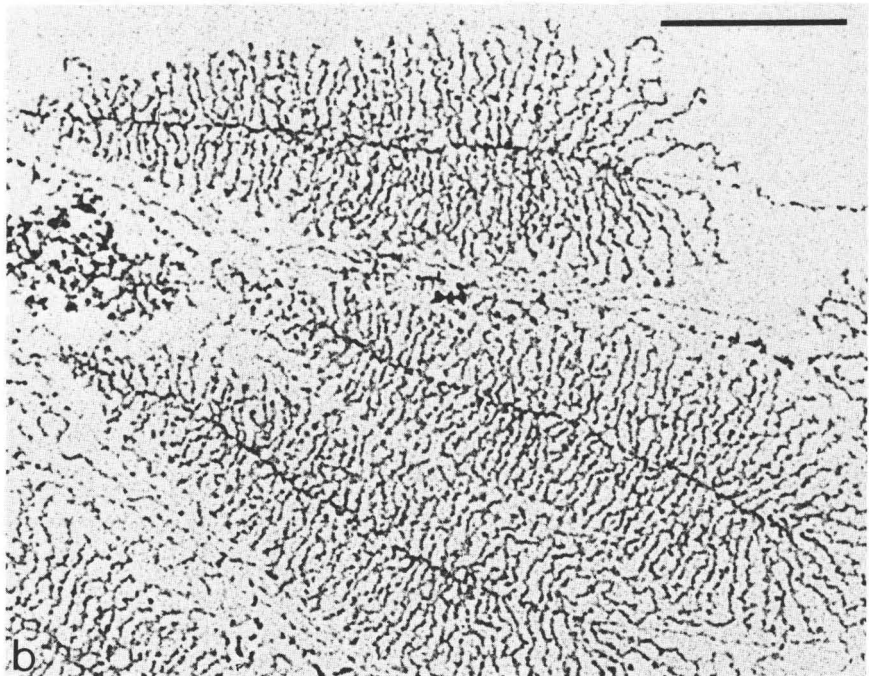
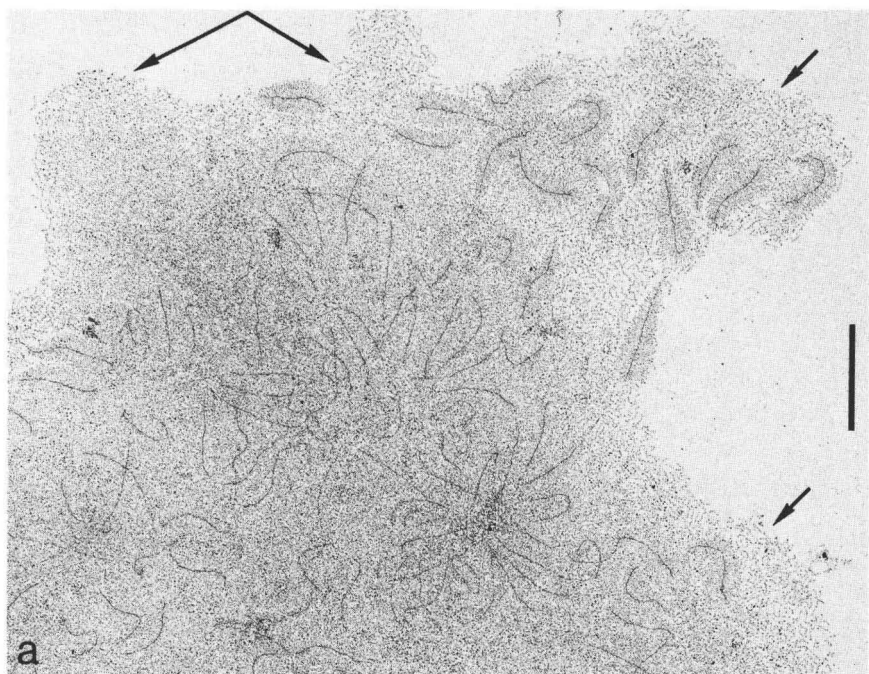


Fig. 3 Survey electron micrograph of a spread preparation of a nucleolar body isolated from the primary nucleus of the green alga, *Acetabularia mediterranea* (for details of preparation, see Spring *et al.*, 1974, 1978). Note that the nucleolar body represents a compact unit consisting of two major components, the nucleolar chromatin proper which is identified by the densely stained matrix units of the pre-rRNA genes, and the numerous filamentous masses that are preferentially accumulated in the peripheral regions (see also Fig. 5). The arrow denotes a matrix unit that is significantly larger than the usual pre-rRNA matrix. Bar denotes 5 μm .

Fig. 2 Spread preparation of nucleolar material isolated from an oocyte of the newt, *Triturus cristatus*, at the lampbrush chromosome stage. Note the predominance of two structural components, the chromatin axis with the matrix units characterized by the dense coverage with lateral fibrils, and the tangles of 6–8 nm filaments attached with numerous densely stained granular particles of diameter 20–40 nm. It is suggested that this extended filamentous system represents the spread structures of the “fibrillar centers” and the nucleolar periphery, including the skeletal portion of the “pars granulosa.” Bar denotes 1 μm .



contain the RNA polymerase A molecules to which 7–15 nm thick pre-rRNP fibrils are attached (Figs. 6–9). The interpretation of these fibrils as nascent pre-rRNP is supported by autoradiographic demonstrations of intensive incorporation of ribonucleosides into matrix unit structures (Fig. 9c,d; cf. Miller and Beatty, 1969c; Angelier *et al.*, 1976a,b; Trendelenburg, 1977; Villard and Fakan, 1978). Characteristically, the lengths of the lateral fibrils show a gradual increase in the direction of transcription, resulting in the typical “Christmas tree” configuration (Figs. 2, 4–7, 9). However, in several types of matrix units, especially in the relatively long ones (cf. Table I), the length of the lateral fibrils remains rather constant within the terminal half of the matrix unit (this is particularly conspicuous in the matrix units of oocytes of the insects, *Dytiscus marginalis* and *Acheta domesticus*, and various mammalian cells; cf. Miller and Bakken, 1972; Trendelenburg *et al.*, 1973, 1977; Franke *et al.*, 1976b; Puvion-Dutilleul *et al.*, 1977a,b; cf. also Laird *et al.*, 1976). The end of the lateral fibrils are often accentuated by a densely stained granular particle of variable diameter, ranging from 15 to 40 nm, the “terminal knob” (see also the morphological articles listed in Table I). It is generally believed that this terminal granule reflects the terminal coiling up of the nascent pre-rRNP. Occasionally, disproportionately long lateral fibrils are observed, either as individual fibrils or in groups (e.g., Fig. 10; cf. Spring *et al.*, 1974; Franke *et al.*, 1976b; for possible interpretations, see below). The lateral pre-rRNP fibrils also often reveal a somewhat regular beaded substructure (e.g., Figs. 6e, 9a,b, and 10b; Franke *et al.*, 1976a, 1978a; Franke and Scheer, 1978), indicative of periodical differences in the package of the nascent pre-rRNP.

The lengths of the matrix units can exhibit significant differences in different organisms as well as in different nucleolar chromatin strands, and even in different regions of the same strand (cf. Table I and literature quoted therein). Nevertheless, the mean and/or median values of matrix unit lengths as well as their distribution curves are often rather characteristic for the specific organism (Table I). It is unclear at present whether occasionally observed unusually sized individual matrix units that are coaxial with rather homogeneously sized pre-rRNA matrix units represent transcriptional units of pre-rRNA genes or of other

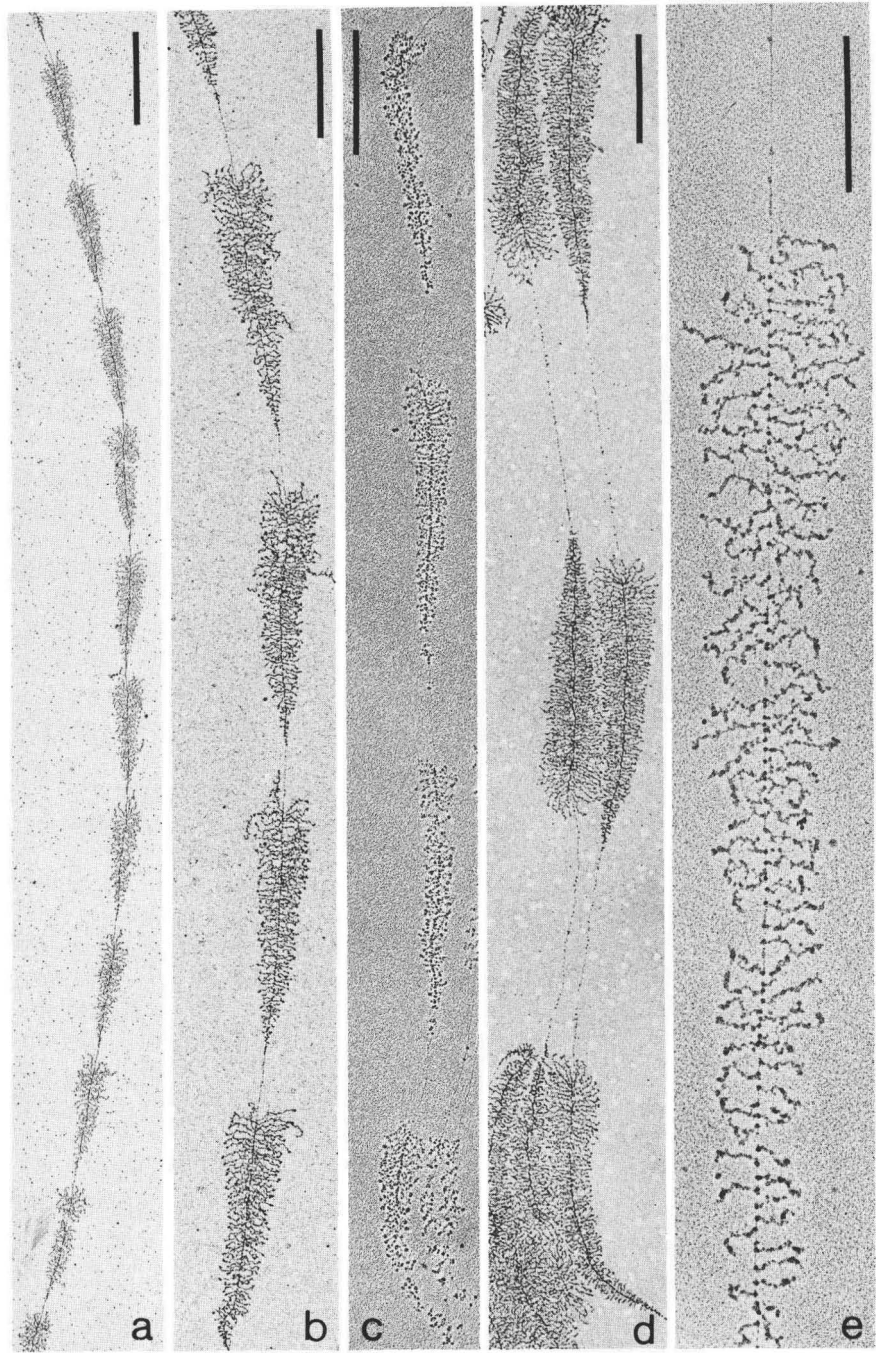
Fig. 4 Higher magnification of spread preparations of nucleolar material from the primary nucleus of *Acetabularia mediterranea* (a) and *major* (b). Partial magnification from Fig. 3 and (a) allows the identification of the matrix units containing the pre-rRNA genes and the filamentous aggregates of the nucleolar periphery (denoted by arrows); (b) shows the typical appearance of the nucleolar chromatin proper as constituted by matrix units and apparent spacer regions. Bars denote 2 μm (a) and 0.5 μm (b).



genes. In many organisms, the mean or modal values of the lengths of the matrix units show a fair correspondence to the gene length expected for the coding region of the isolated pre-rRNA of the specific organism. There are, however, notable exceptions to this correlation, including both unexpectedly long (e.g., *Dytiscus*, *Acheta*, *Amoeba*; Table I) and short (*Acetabularia exigua*, Table I) matrix units. The situation described in the amplified nucleoli of the oocytes of *Dytiscus* (35% excess of matrix unit length) and *Acheta* (ca. 100% excess) suggests that here the isolated pre-rRNA (2.8×10^6 MW; Trendelenburg *et al.*, 1973, 1976) does not represent the complete transcript but an already processed stable product (for details and general discussions of the problems of the characterization of a uniform primary and complete transcript, see Franke *et al.*, 1976b; Rungger and Crippa, 1977; Hadjiolov and Nikolaev, 1976; Reeder *et al.*, 1977, 1978; Batts-Young and Lodish, 1978).

2. Apparent spacer units usually appear as thin chromatin strands of 4–8 nm width and rather smooth contours (Figs. 5, 9, 11, and 12; cf. morphological references quoted in Table I and Franke *et al.*, 1976a, 1978a; Franke and Scheer, 1978). Their lengths can be rather homogeneous in some nucleoli and organisms, in which cases they are as characteristic for the specific type of nucleolar chromatin as the matrix unit lengths. However, comparison of spacer lengths in different organisms, different individuals of the same species, and different nucleolar chromatin strands or strand intercepts of the same cell has revealed that the sizes of the apparent spacer units can show great differences. Pronounced length differences have been found not only between different species but also, at least in some organisms, from one individual to another and even between different nucleolar chromatin strands of the same cell (e.g., Fig. 7; Miller and Beatty, 1969a,b; Scheer *et al.*, 1973, 1977; Trendelenburg *et al.*, 1974, 1976; Spring *et al.*, 1974, 1976; Berger and Schweiger, 1975c). Such heterogeneity of spacer unit lengths has not only been observed in spread preparations of transcribed chromatin but has also been demonstrated in measurements of sizes of isolated amplified rDNA rings (cf. Fig. 8; Trendelenburg *et al.*, 1976, 1977; Trendelenburg, 1977; Findly and Gall, 1978) and of rDNA spacer-containing fragments obtained after

Fig. 5 Nucleolar chromatin material in full transcriptional activity (lampbrush chromosome stage oocytes of *Triturus cristatus*) after separation of most of the nucleolus-associated nonchromatinous fibrillar material often enriched in the peripheral portions of the nucleoli (some residual filaments of this material are denoted by the arrow). Note that the nucleolar chromatin proper exclusively consists of matrix units and spacer regions. Bar denotes 2 μ m.

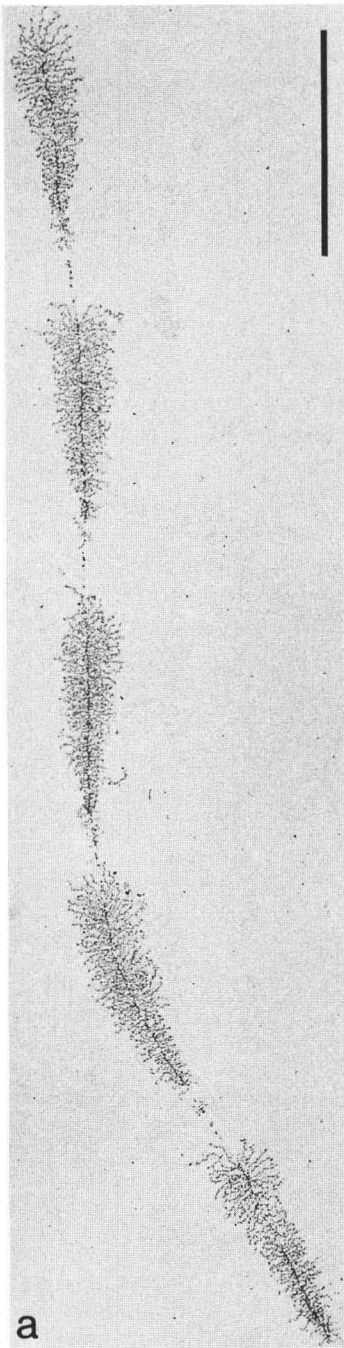


cleavage with restriction endonucleases (in *Xenopus*: Morrow *et al.*, 1974; Wellauer *et al.*, 1974, 1976a,b; Wellauer and Dawid, 1974; Wellauer and Reeder, 1975; Southern, 1975; Reeder *et al.*, 1976a,b; Buongiorno-Nardelli *et al.*, 1977; *Paramecium*: Findly and Gall, 1978; for data in mammalian rDNA, see Table I). The reason for this variability of spacer lengths in some organisms remains to be clarified.

The occurrence, in some lower eukaryotes, of genes coding for 5 S rRNA in rDNA spacer regions has already been mentioned above. Small matrix units which apparently are discontinuous with the pre-rRNA matrix units have sometimes been observed in nucleolar chromatin of various organisms (e.g., Fig. 11; cf. Scheer *et al.*, 1973, 1977; Franke *et al.*, 1976b; Rungger and Crippa, 1977; Rungger *et al.*, 1978). Such transcriptional arrays, which seem to represent independent transcriptional units, are usually rather short. They can be located immediately before the beginning of a normally sized pre-rRNA matrix unit ("prelude complexes"; Scheer *et al.*, 1973, 1977; Franke *et al.*, 1976b) or in more central regions of the apparent spacer intercept (Fig. 11; see Scheer *et al.*, 1973, 1977; Franke *et al.*, 1976b). It cannot be decided at the moment whether such structures reflect an initiation of transcription that occurs in a region corresponding to an apparent spacer unit, perhaps a rare event* (Scheer *et al.*, 1973; Franke *et al.*, 1976b; Rungger and Crippa, 1977), or whether they are due to improper termination of transcription coupled with a special sequence of processing events (Reeder *et al.*, 1977, 1978a), or whether

* This would also provide an explanation for the occasional occurrence of individual disproportionately long lateral fibrils (see Fig. 10). Alternative explanations for this observation might be: (1) a normally occurring processing step has been omitted in the specific fibril; (2) the RNP package is different in the specific fibril; (3) the RNP material of the specific fibril has been selectively unfolded and extended during the preparation.

Fig. 6 Typical appearance of strands of transcriptionally active nucleolar chromatin from newt oocytes which represent alternating tandem arrangements of matrix units and apparent spacer intercepts. (a–c) Present the repeating units in the nucleolar chromatin of *Pleurodeles waltli*, which in general is characterized by relatively short matrix units, apparent spacer units, and repeating units [mean values of 2.45, 0.55, and 3.0 μm in the nucleolus shown in (a)]. Note that the repeat unit lengths do not considerably increase in preparations that include 0.1–0.5% Sarkosyl NL-30 (not shown here) or 0.3% "Joy" (c) in the dispersion medium (for details, cf. Trendelenburg, 1977; Scheer *et al.*, 1977). The corresponding units, especially the apparent spacer intercepts, of the transcribed nucleolar chromatin of the oocytes of the other urodelan species, *Triturus cristatus* (d) and *T. alpestris* (e), are significantly longer (cf. Table I). Note the very thin axis in both matrix units and spacer regions as well as the distinctiveness of lateral RNP fibrils and their basal granules containing the RNA polymerase A molecules after treatment with Sarkosyl or in matrix units with a somewhat reduced density of transcriptional complexes. Bars denote 2 μm (a), 1 μm (b–d), and 0.5 μm (e).



these "small matrix units" reflect the transcription of pre-rRNA gene sequences translocated into intercepts corresponding to spacer units. It is interesting to note, however, that the polarity of such transcriptional units that are located in the regions corresponding to apparent spacer intercepts invariably show the same polarity as the adjacent pre-rRNA matrix units.

With the exception of the amplified palindromic rDNA units of plasmodial nuclei of *Physarum* and *Tetrahymena* macronuclei (for references, see Table I; see also Section I, page 51) and the arrangements described in the primary nucleus of *Acetabularia exigua* (Berger *et al.*, 1978), we are not aware of unequivocally demonstrated arrangements of pre-rRNA matrix units or pre-rRNA genes in opposing or alternating polarity. Transcribed chromatin strands so far shown in published micrographs and interpreted to show such "head-to-head" or "tail-to-tail" arrangements in pre-rRNA matrix units, including our own (Trendelenburg *et al.*, 1974, Fig. 4), cannot be regarded as positively identified nucleolar chromatin (see also Miller and Beatty, 1969b) but may well represent fragments of lampbrush chromosome loops in which such situations are rather common (Spring *et al.*, 1974; Angelier and Lacroix, 1975; Scheer *et al.*, 1976b; see also Scheer *et al.*, Chapter I, this volume).

When rings of amplified rDNA are isolated, a certain small proportion of them is present in a supercoiled form (Fig. 8a; Rochaix *et al.*, 1974; Rochaix and Bird, 1975; Bakken, 1975; Buongiorno-Nardelli *et al.*, 1976; Trendelenburg *et al.*, 1976). Interestingly, we have never seen indications of supercoiling in the transcribed chromatin form of such rings (Fig. 8; Trendelenburg, 1974, 1977; Trendelenburg *et al.*, 1976, 1977). It is not clear whether this is an accidental finding or whether transcription involves a transition from the supercoiled to the relaxed form.

IV. Absence of Nucleosomes in Spread Preparations of Transcriptionally Active Nucleolar Chromatin

In the nuclei of most eukaryotic organisms the major portion of the chromatin is organized into nucleosomes and appears, upon disper-

Fig. 7 Occurrence of different types of repeating units in the nucleolar chromatin of the primary nucleus of *Acetabularia mediterranea*, one type being characterized by short spacer regions (a), the other by extended spacers (b). For a detailed documentation and discussion of the heterogeneity in the patterns of transcriptional units and apparent spacer units in this organism the reader is referred to the article by Spring *et al.* (1976). Bars denote 2 μ m.

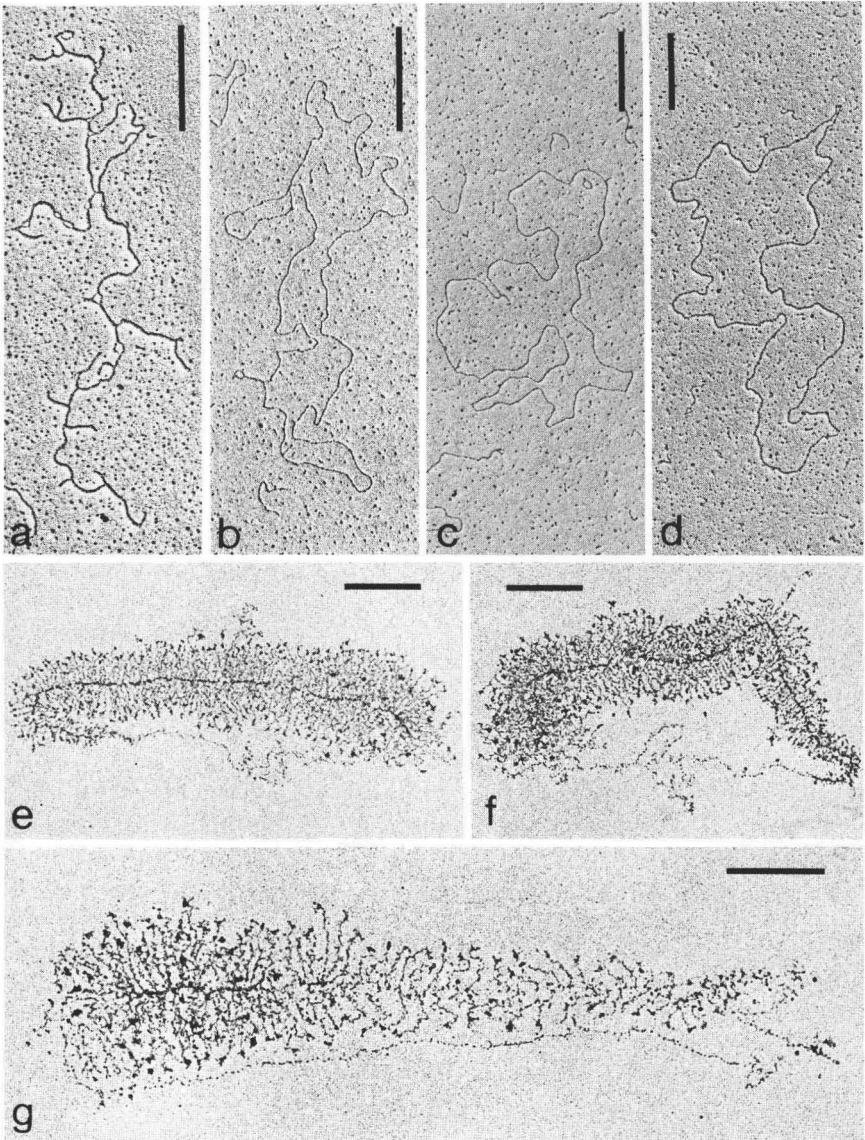
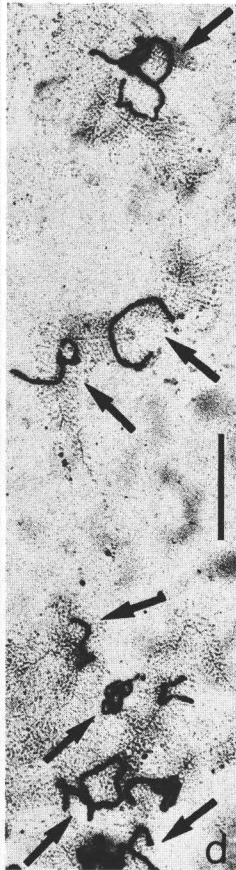
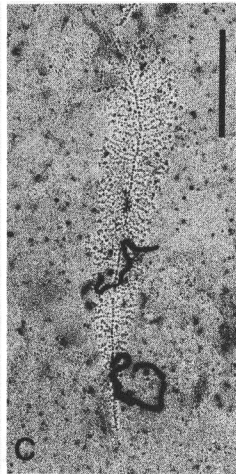
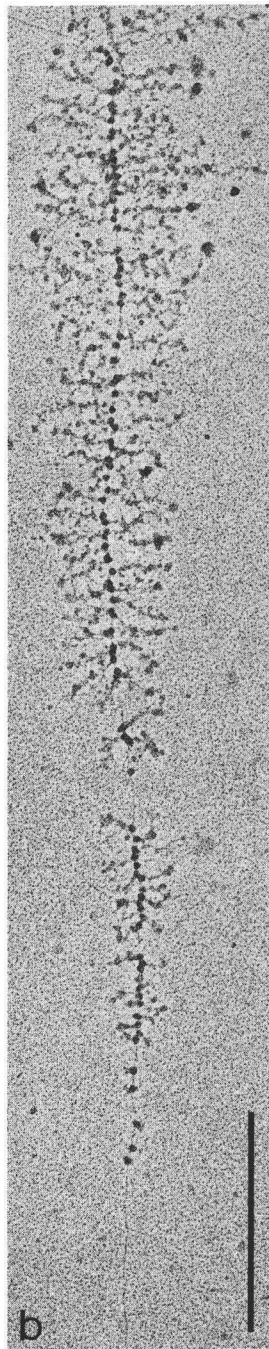
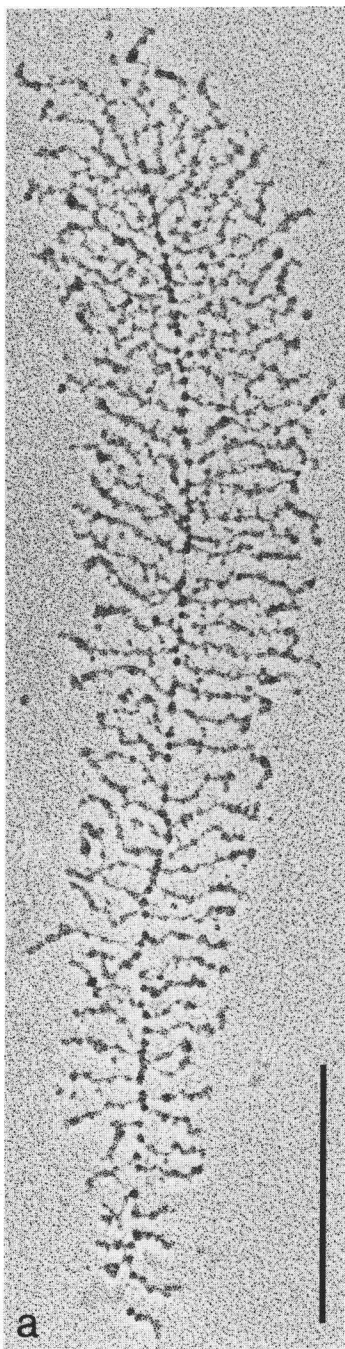


Fig. 8 Circles of amplified rDNA of oocytes of the water beetle, *Dytiscus marginalis*, demonstrated as supercoiled (a) or relaxed (b–d) rings of purified DNA (a–d) and as rings of transcriptionally active nucleolar chromatin in spread nuclear material isolated from diplotene stage oocytes (e–g). Rings containing one pre-rRNA gene and a spacer region can exhibit different contour lengths indicative of true size heterogeneity (for details, see Trendelenburg *et al.*, 1976). Such differences are observed in isolated DNA (the circles shown in Figs. 8b,c, and d, for example, have contour lengths of 7.30, 7.75, and 8.23 μm) as well as in rings of transcribed nucleolar chromatin (e–g). By contrast, rDNA of nontranscribed chromatin rings of young oocytes is packed in nucleosomes (Scheer and Zentgraf, 1978). Bars denote 0.5 μm .

sion in low salt buffers and spreading, in characteristic "beads-on-a-string" arrays (Olins and Olins, 1973, 1974; Woodcock, 1973; Oudet *et al.*, 1975; Woodcock *et al.*, 1976a). When transcriptionally active nucleolar chromatin is prepared under the conditions that allow the demonstration of nucleosomal arrays in nontranscribed chromatin strands, nucleosomal structures are not observed (e.g., Figs. 6, 8, 9, 12, and 14; Franke *et al.*, 1976a; Trendelenburg *et al.*, 1976; for further references, see Foe, 1978; Franke and Scheer, 1978). A nonnucleosomal organization is especially obvious in the matrix units (Foe *et al.*, 1976; cf. Woodcock *et al.*, 1976b) in which the RNA polymerase particles appear to be present in close packing and additional particles of a similar size are not found (both RNA polymerase molecules and nucleosomes are about 12 nm large). In addition, the axis-attached granules observed in matrix units have also been clearly identified as nonnucleosomal by their resistance to treatments with certain detergents that remove considerable portions of histones and non-histone proteins from the DNA but leave the initiated polymerase molecules attached (cf. Franke *et al.*, 1976a; Scheer *et al.*, 1977; Scheer, 1978). An absence of nucleosomal structures is also observed in apparent spacer regions which usually appear rather thin and smooth (Franke *et al.*, 1976a; Villard and Fakan, 1978). It has been suggested that 7–14 nm large particles which have occasionally been observed in spacer regions represent nucleosomes (e.g., Woodcock *et al.*, 1976b; Grainger and Ogle, 1978). On the other hand, we have shown that at least some of the sporadic spacer-associated granules are not of nucleosomal nature but rather represent RNA polymerase molecules as demonstrated by their resistance to the detergent treatments mentioned above (Franke *et al.*, 1976a; Scheer *et al.*, 1977). In addition, the frequency of granules associated with an apparent spacer region is not inversely correlated with the length of the specific spacer intercept (Franke *et al.*, 1976a) as one would expect if these particles were nucleosomes. The absence of nucleosomes in apparent matrix and spacer units is also indicated by the fair correspondence of the lengths of repeat units as observed in spread preparations of transcribed nucleolar chromatin with the lengths of the rDNA repeat units determined in isolated rDNA molecules and fragments obtained after digestion with restriction endonucleases (cf. Fig. 1 and Table I; for comparison of data, especially in *Xenopus laevis*, see Franke *et al.*, 1976a, 1978a; Scheer *et al.*, 1977; Reeder *et al.*, 1978b). From this we conclude that in transcribed nucleolar chromatin the DNA is present in an almost extended form (B) and that only very little, if any, nucleosome packaging occurs in both matrix and spacer units (cf. Foe *et al.*, 1976; McKnight *et al.*, 1978; for a report of some foreshortening in the apparent spacer



regions of *Physarum* rDNA see, however, Grainger and Ogle, 1978).

The finding of an absence of considerable amounts of nucleosomes in transcriptionally active nucleolar chromatin suggests that this chromatin is arranged in a configuration different from the normal nucleosomal one. It does not mean that histones are absent in such nucleoli. Presence of histones in transcriptionally active nucleoli from stage II and III oocytes of *Xenopus laevis* has been reported by Higashinakagawa *et al.* (1977; cf. Reeder *et al.* 1978a) and is also suggested by the data of Matsui and Busch (1977) obtained in nucleolar chromatin isolated and fractionated from Novikoff hepatoma cells. Demonstration that rDNA sequences are recovered with relatively high yield in DNA fragments of 140–200 base pairs, i.e., the nucleosomal size class, upon digestion of chromatin of presumed high nucleolar transcription activity with micrococcal nuclease (Leer *et al.*, 1976; Mathis and Gorovsky, 1976; Piper *et al.*, 1976; Reeves, 1976, 1977, 1978; Reeves and Jones, 1976; Gottesfeld and Melton, 1978) is not in conflict with the morphological observations of an absence of nucleosomes.*

Digestion with micrococcal nuclease results in 140–200 base pair fragments of DNA even when the chromatin has been unfolded prior to digestion (Jackson and Chalkley, 1975; cf. Woodcock and Frado, 1978; Oudet *et al.*, 1978). Consequently, the finding of fragments of such sizes upon micrococcal nuclease digestion per se does not indicate the nucleosomal packing state but a specific pattern of arrangement of the nucleosomal histones along the DNA (for discussion, see also Franke *et al.*, 1978a; Scheer, 1978). Perhaps the transcriptionally

* In fact, the data of Reeves (1976, 1978) and Reeves and Jones (1976) seem to indicate that the amount of rDNA recovered in nucleosome-sized DNA fragments upon nuclease digestion is inversely proportional to the transcriptional activity.

Fig. 9 Details of the matrix units of pre-rRNA genes from newt oocytes [(a,c,d) *T. alpestris*; (b) *T. cristatus*] as seen in spread preparations without (a) or with (b) Sarkosyl NL-30 (0.3%) added in the "pH 9 medium" used for dispersion of the chromatin. Note the distinct RNA polymerase A containing basal particles of the individual transcriptional complexes and the somewhat regularly beaded substructure of the attached nascent RNP fibrils (a,b). Note the thin axis in regions of the matrix units that are free of lateral fibrils [(a,b); for details, see Franke *et al.*, 1976a]. Parts (c) and (d) show the silver deposits [arrows in (d)] in autoradiographs of spread preparations of nucleolar chromatin from oocytes isolated 48 hr after injection of the newts with tritiated ribonucleosides (200 μ Ci of each nucleoside per animal, injected into the body cavity, specific radioactivities: adenosine, 22 Ci/mmole; uridine, 45 Ci/mmole; guanosine, 10 Ci/mmole; cytidine, 25 Ci/mmole; see Trendelenburg, 1977). The specific enrichment of silver grains over the matrix units illustrates their nature as transcriptional structures. Bars denote 0.5 μ m (a,b) and 1.0 μ m (c,d).

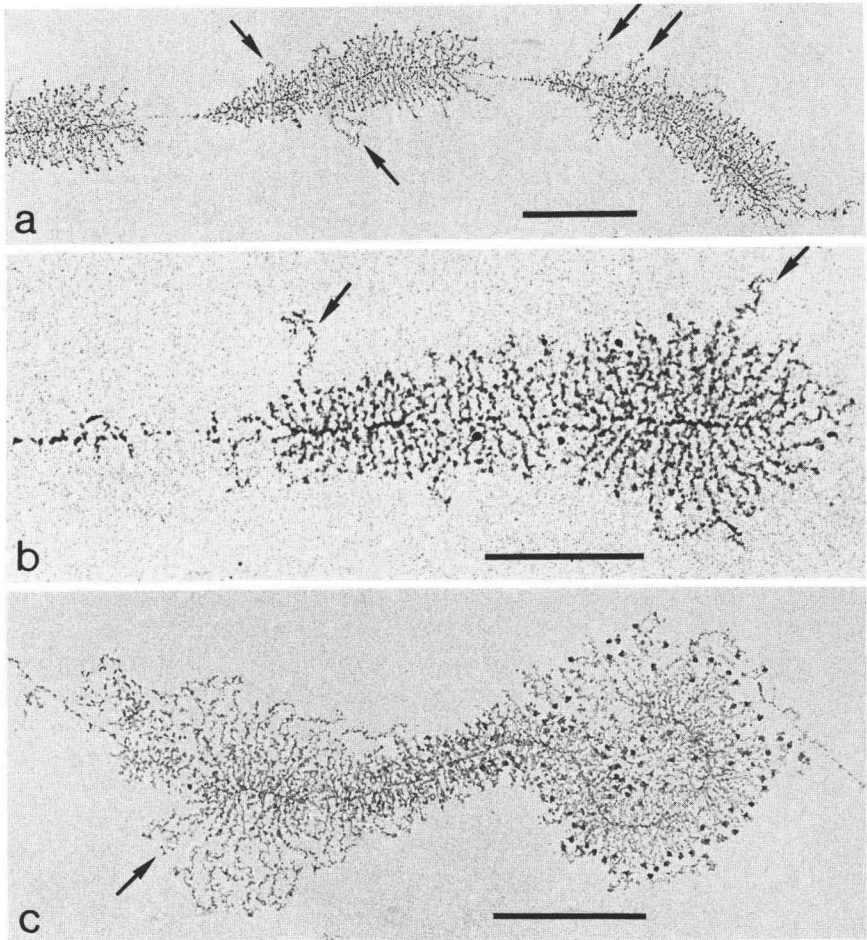
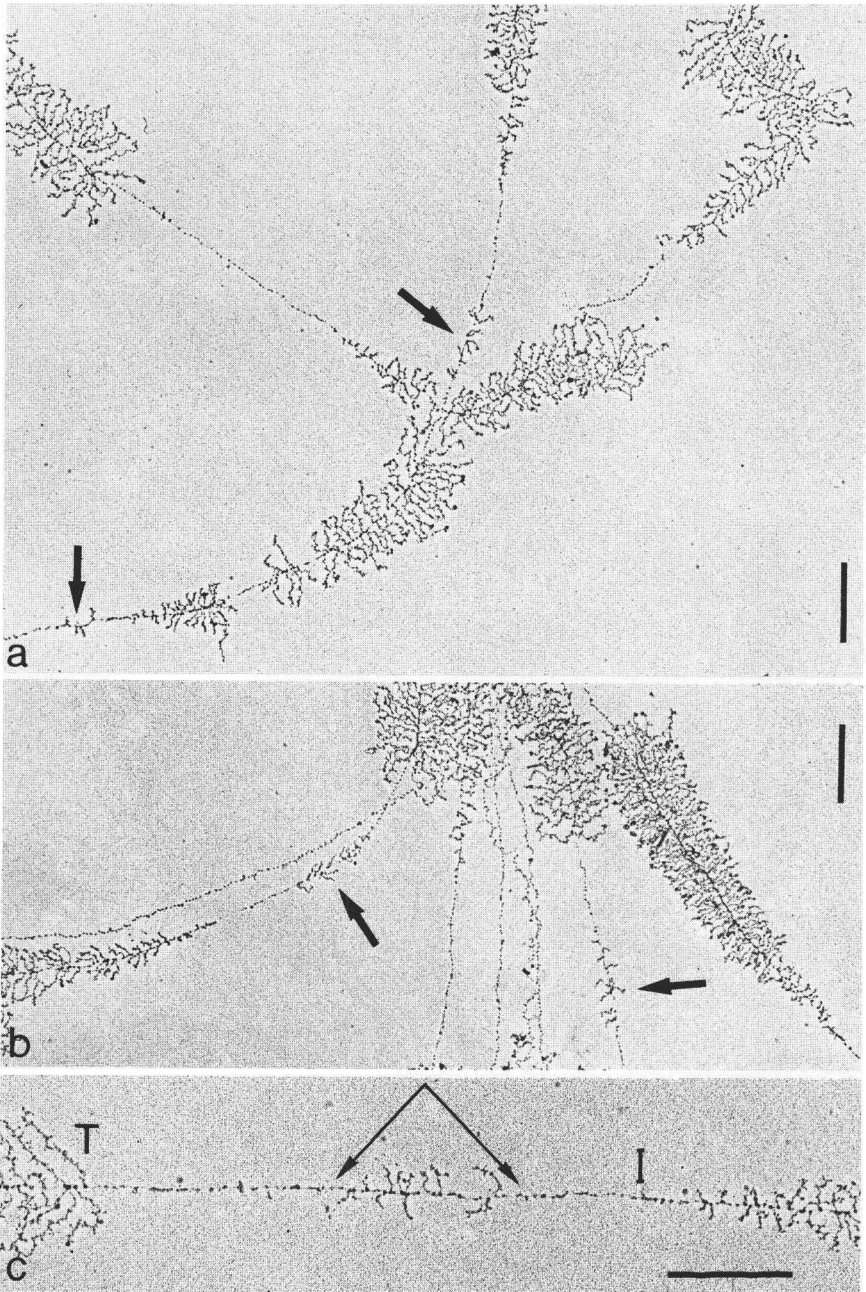


Fig. 10 Disproportionately long lateral RNP fibrils (arrows) are sometimes observed, either as individual fibrils (a,b) or as groups of fibrils (c), in matrix units of pre-rRNA genes of animal [(a) oocyte of *Pleurodeles waltli*; (b) oocyte of the house cricket, *Acheta domesticus*] and plant [(b) primary nucleus of *Acetabularia mediterranea*] cells. This could suggest that transcriptional processes may be initiated before the morphologically identified start of a matrix unit (for alternative interpretations, see text; see also Franke *et al.*, 1976b). Bars denote 1 μm (a,c) and 0.5 μm (b), respectively.

Fig. 11 Appearance of small groups of lateral fibrils (arrows) in axial intercepts that are located between normal matrix units of pre-rRNA genes and therefore correspond to apparent spacer regions (e.g., nucleoli from late lampbrush stage oocytes of the Alpine newt, *Triturus alpestris*). These groups of lateral fibrils may occur either in association with the beginning of a normal pre-rRNA matrix unit [“prelude complexes”; e.g., arrow in the lower left of (a); cf. Scheer *et al.*, 1973, 1977] or in more central parts of an apparent spacer region [b,c, the pair of arrows in (c) denotes such a typical array; T and I in (c) denote the putative sites of termination and initiation of the adjacent pre-rRNA matrix units]. Bars denote 0.5 μm .



active nucleolar chromatin is, for example, by interaction with other proteins, in a similar unfolded state as that experimentally induced by moderate treatment with urea (e.g., Olins *et al.*, 1977; Woodcock and Frado, 1978).

V. Appearance of Nucleolar Chromatin in States of Reduced Transcriptional Activity and after Complete Inactivation

The observation that normally appearing matrix units, densely covered with transcriptional complexes, occur in spread nucleoli from somatic cells that are not expected to be at maximal transcriptional activity in all pre-rRNA genes (Miller and Bakken, 1972; Puvion-Dutilleul *et al.*, 1977a,b) suggests that some genes may be active and maximally set with active polymerase molecules whereas other genes may be silent. Such a selective use of pre-rRNA genes has been demonstrated in special detail in studies of the activation of nucleolar chromatin as it naturally occurs in early embryogenesis of various insects (*Drosophila*: McKnight and Miller, 1976; *Oncopeltus*: Foe, 1978) and during early oogenesis of amphibia (Scheer *et al.*, 1976a) (rRNA synthesis during amphibian oogenesis: Scheer *et al.*, 1976a; Scheer, 1973; Denis, 1977) and some insects (*Dytiscus* and *Acheta*: Trendelenburg *et al.*, 1977; Scheer and Zentgraf, 1978). Such studies have shown that in early stages of activation some genes are covered with transcription complexes whereas others show variable degrees of fibril coverage, or are completely free of fibrils. These observations suggest an independent control of adjacent genes of the same category (McKnight and Miller, 1976; Scheer *et al.*, 1976a), a finding that is hard to explain on the basis of our current concepts of the regulation of gene activity. Another significant observation is that reported by Foe (1978; cf. Foe *et al.*, 1976) who showed that, during activation in *Oncopeltus* embryos, regions of nucleolar chromatin that correspond to matrix units appear smooth and nonnucleosomal before an association with transcriptional complexes takes place. During both activation and inactivation of nucleolar chromatin, a pronounced "cooperativity" of the transcriptional complexes is observed. This is recognized by a dense package of polymerase particles and nascent pre-rRNP fibrils in some regions of a gene, in contrast to other intercepts of the same transcriptional unit that are fibril-free (McKnight and Miller, 1976; see also Scheer *et al.*, 1976a).

Inactivation of nucleolar chromatin as observed during late stages of oogenesis (Scheer, 1973; Reeves, 1978) or after treatment of fully

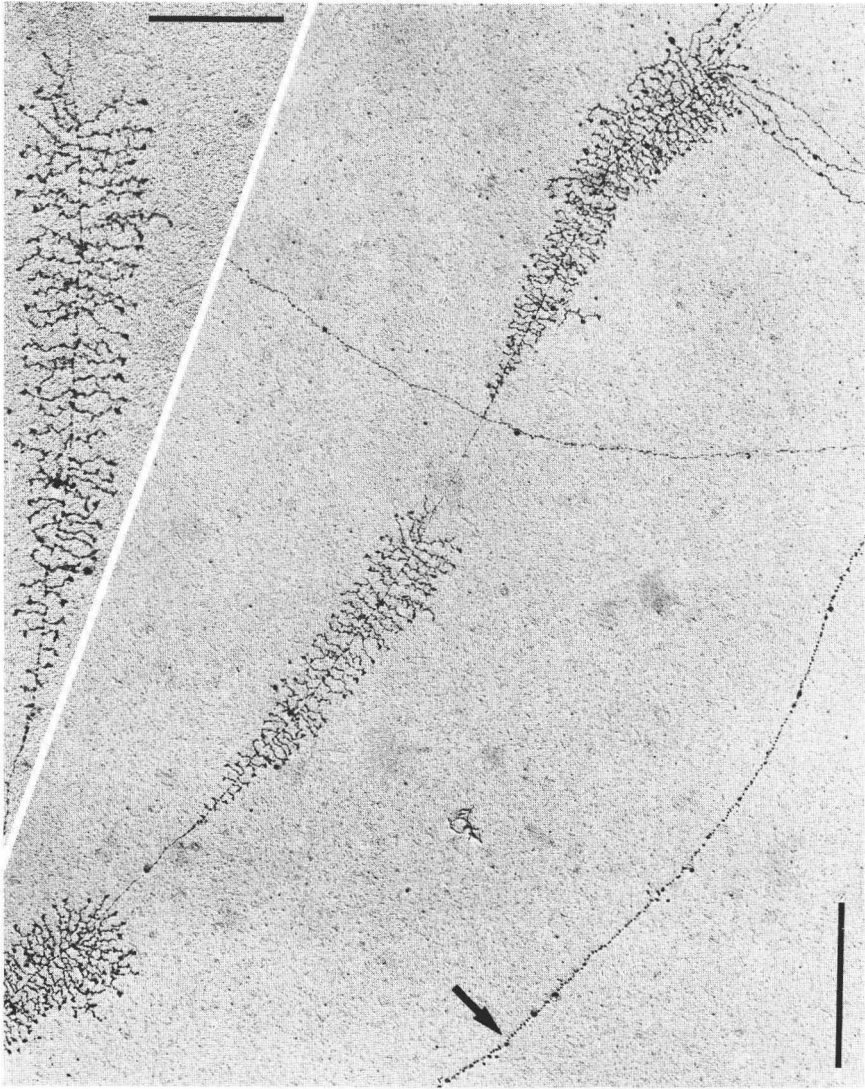
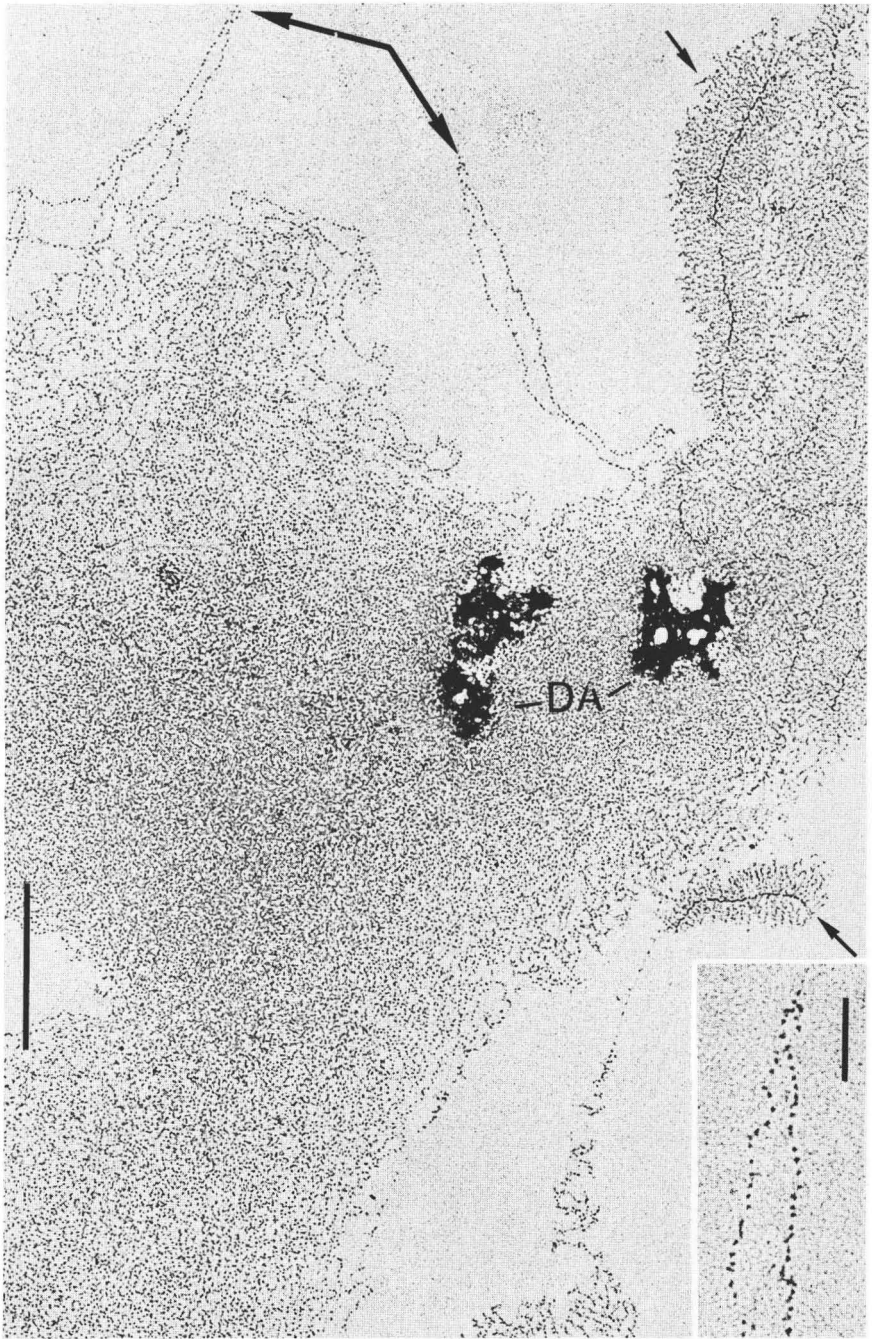


Fig. 12 Spread preparation of nuclear contents of a large (maturing) oocyte of *Pleurodeles waltli*, showing a smooth and very thin strand of transcribed nucleolar chromatin besides a typical "beaded" strand that apparently is not transcribed (arrow). Note that the thin and nonnucleosomal appearance is seen in short fibril-free regions within the matrix units (one is shown at higher magnification in the insert) as well as in apparent spacer intercepts and occasionally also in regions of some strands which are not associated with matrix units (e.g., the individual strand that crosses the heavily transcribed strand). Bars denote 1 and 0.5 μm (insert).



active nucleoli with inhibitor drugs such as actinomycin D and fluorouridine (e.g., Scheer *et al.*, 1975; Franke *et al.*, 1976a; Rungger and Crippa, 1977; Rungger *et al.*, 1978) resulted in the progressive release of lateral fibrils from the transcriptional units.* The pattern observed suggests that (1) adjacent transcriptional units show different resistance (some individual fully covered matrix units often can be observed even in otherwise largely inactive nucleoli; see Scheer *et al.*, 1975, 1976a). (2) Matrix units can either be evenly "diluted" (Fig. 14b) or show pronounced cooperativity of fibril release, resulting in the characteristic appearance of "gaps" (Scheer *et al.*, 1975, 1976a; Franke, 1977).

During the inactivation of nucleolar chromatin, intercepts of matrix units are observed that are free of lateral fibrils but are still thin and show a smooth, nonnucleosomal organization (e.g., Figs. 9b, 12, and 14; Franke *et al.*, 1976a; Scheer, 1978; Franke and Scheer, 1978). Extended fibril-free regions in between normally looking matrix units are seen that contain whole nontranscribed pre-rRNA genes; such regions also show a nonnucleosomal appearance (Franke *et al.*, 1976a, 1978a). After long periods of inactivation, however, the proportion of fibril-free regions of nucleolar chromatin with typical nucleosomal "beads-on-a-string" arrays increases (Figs. 12-14; cf. Franke *et al.*, 1976a, 1978a; Franke and Scheer, 1978; Scheer, 1978). This leads to the appearance of aggregates of chromatin strands that are almost entirely contained in nucleosomelike granules and, under appropriate

* After release from their template-containing chromatin strand the nascent and mature pre-rRNP fiber material can remain, for a considerable period, within the confinements of the nucleolar body (e.g., Scheer *et al.*, 1975; Eckert and Franke, 1975). While considerable amounts of unprocessed pre-rRNA have been found after inhibition with actinomycin D in some cells such as amphibian oocytes (Scheer *et al.*, 1975) and *Tetrahymena* (Eckert *et al.*, 1975), processing of pre-rRNA has been reported under drug-induced inhibition of transcription in other cells (Busch and Smetana, 1970; Hadjiolov and Nikolaev, 1976). It is not at all clear whether released incomplete pre-rRNA chains can be used for the formation of functional ribosomal RNA's.

Fig. 13 Spread preparation of a nucleolus of an oocyte (mid to late lampbrush stage) of the Alpine newt, *Triturus alpestris*, after 45 min treatment with actinomycin D (Scheer *et al.*, 1975). Note that most of the nucleolar chromatin is recognized in the form of aggregated strands that are free of lateral fibrils and show a conspicuously beaded appearance (this is especially clear in peripheral regions of the nucleolus: pair of thick arrows in upper left, and insert). Some matrix units are still identified (short arrows) which indicates the selectively higher stability of transcriptional structures in some of the pre-rRNA genes. DA, dense aggregates of heavily stained material are frequently observed in spread preparations of such drug-inactivated nucleoli. Bars denote 1 and 0.25 μm (insert).

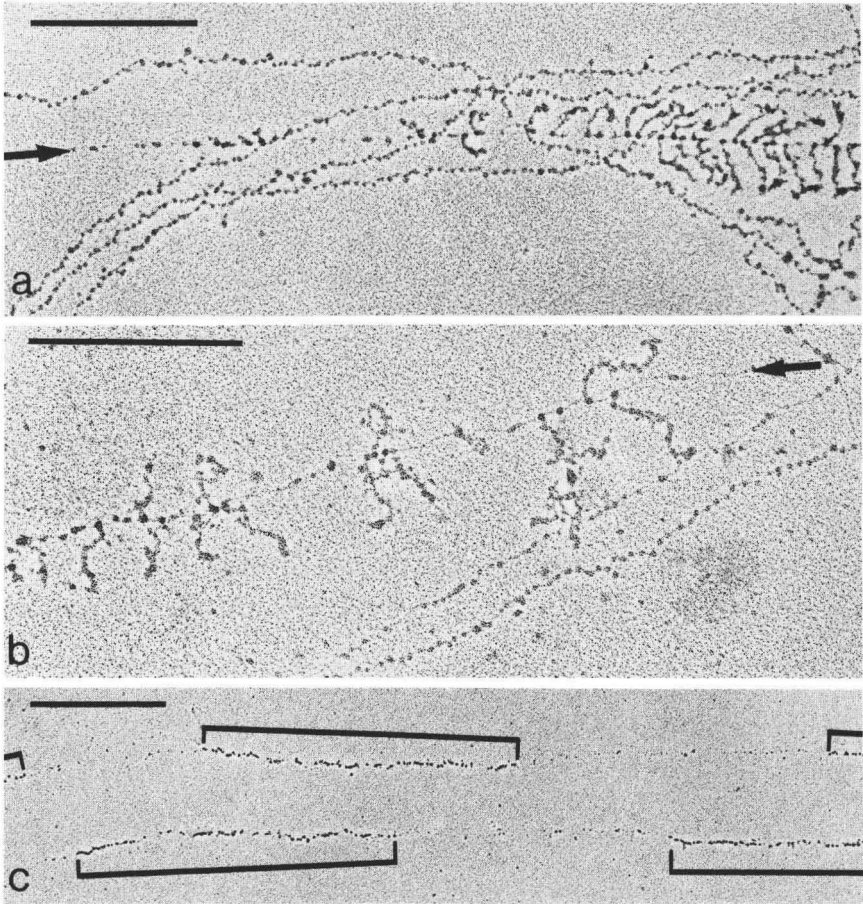


Fig. 14 Details of the appearance of transcribed and nontranscribed strands of nucleolar chromatin in spread preparations of nucleoli from oocytes [(a,b) maturing stages of *Triturus alpestris*; (c) mid lampbrush chromosome stage of *Xenopus laevis*]. Note the thin axis of the matrix unit-containing strands (arrows), besides fibril-free chromatin strands of a typical nucleosome-like beads-on-a-string appearance. (c) Presents the stability and distinctiveness of particles containing initiated RNA polymerase A molecules after extensive treatment with Sarkosyl NL-30 (for details, see Scheer *et al.*, 1977; Scheer, 1978) which sometimes results in the loss of (visualization of) lateral RNP fibrils (regions corresponding to matrix units are identified by the attached polymerase granules and are denoted by the brackets). Bars denote $0.5 \mu\text{m}$ (a,b) and $1 \mu\text{m}$ (c).

conditions, also in the 18–28 nm large granular structures (cf. Sheer, 1978, Fig. 4) that apparently represent a supranucleosomal order of package of nucleosomal chromatin (cf. Zentgraf *et al.*, 1975, 1978; Franke *et al.*, 1976a; Kiryanov *et al.*, 1976; Renz *et al.*, 1977). Thus “long-term inactive” nucleolar chromatin is structurally indistinguishable from other inactive and condensed chromatin.

Note added in proof: Two articles have appeared on the reconstitutions of nucleolar chromatin by microinjection of purified rDNA into oocytes nuclei of *Xenopus laevis*. In one experiment natural rDNA circles isolated from ovaries of an insect, *Dytiscus marginalis*, were injected and, in electron micrographs spread preparations of contents of the injected *Xenopus* oocyte nuclei, both forms—transcriptionally active and inactive rings of chromatin—were observed. Besides normally appearing transcriptional units of the *Dytiscus* type, a variety of abnormal forms of arrays of transcriptional complexes were seen, including transcriptional events in regions corresponding to normally nontranscribed apparent spacers of *Dytiscus* rDNA [Trendelenburg, M. F., Zentgraf, H., Franke, W. W., and Gurdon, J. B. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3791–3795]. In the other experiment, circular DNA of a plasmid containing a large portion of the repeating unit of *Xenopus laevis* rDNA was injected into *Xeopus* oocyte nuclei and shown to be present either in transcriptionally inactive, nucleosome-packed and foreshortened form or in rings containing densely spaced, normally appearing arrays of transcriptional complexes with DNA in an extended, nonnucleosomal configuration [Trendelenburg, M. F., and Gurdon, J. B. (1978). *Nature* **276**, 292–294]. Most of the nontranscribed native spacer intercept was devoid of nucleosomes, in contrast to the intercept containing the plasmid DNA sequences that was packed in nucleosome-sized beads. A detailed analysis of the histone composition of a fraction enriched in rDNA-chromatin from macronuclei of *Tetrahymena pyriformis* has been presented by R. W. Jones (1978). *Biochem. J.* **173**, 155–164) who reported a drastically reduced histone:DNA ratio in nucleolar chromatin, as compared to the remaining bulk of macronuclear chromatin, both in growing and stationary cells.

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