in nucleoli of Japanese quail oocytes and somatic cells. *Journal of Ultrastructure Research*, **64**, 377–87.
Mirre, C. & Stahl, A. (1981). Ultrastructural organization, sites of transcription and distribution of fibrillar centres in the nucleolus of the mouse oocyte. *Journal of Cell Science*, **48**, 105–26.
Moore, G. P. M., Lintern-Moore, S., Peters, H. & Faber, M. (1974). RNA synthesis in the mouse oocyte. *Journal of Cell Biology*, **60**, 416–22.
Oakberg, J. F. (1967). ^{3}H-uridine labelling in mouse oocytes. *Archives d'Anatomie Microscopique et de Morphologie Expérimentale*, **56** (Suppl. 3–4), 171–84.
Pouchelet, M., Gansmüller, A., Anteunis, A. & Robineaux, R. (1975). Mise en évidence en microscopie électronique, dans les noyaux interphasiques des cellules L929, de filaments de DNA associés aux zones fibrillaires RNA des nucléoles. *Compte Rendu de l'Académie des Sciences*, **280**, 2461–3.
Recher, L., Sykes, J. A. & Chan, H. (1976). Further studies on the mammalian cell nucleolus. *Journal of Ultrastructure Research*, **56**, 152–63.
Recher, L., Whitescarver, J. & Briggs, L. (1969). The fine structure of a nucleolar constituent. *Journal of Ultrastructure Research*, **29**, 1–14.
Royal, A. & Simard, R. (1975). RNA synthesis in the ultrastructural and biochemical components of the nucleolus of chinese hamster ovary cells. *Journal of Cell Biology*, **66**, 577–85.
Ryser, U., Fakan, S. & Braun, R. (1973). Localization of ribosomal RNA genes by high resolution autoradiography. *Experimental Cell Research*, **78**, 89–97.
Schwarzacher, H. G., Mikelsaar, A. V. & Schnedl, W. (1978). The nature of the Ag-staining of nucleolus organizer regions. *Cytogenetics and Cell Genetics*, **20**, 24–39.
Stahl, A., Luciani, J. M., Devictor, M., Capodano, A. M. & Hartung, M. (1974). Heterochromatin and nucleolar organizers during first meiotic prophase in quail oocytes. *Experimental Cell Research*, **91**, 365–71.
Stahl, A., Mirre, C., Hartung, M. & Knibiehler, B. (1980). Localisation, structure et activité des gènes ribosomiques dans le nucléole de l'ovocyte en prophase de méiose. *Reproduction, Nutrition et Développement*, **20**, 469–83.
Stahl, A., Mirre, C., Hartung, M., Knibiehler, B. & Navarro, A. (1978). Localization and structure of nucleolar organizers in the oocyte during meiotic prophase I. *Annales de Biologie Animale, de Biochimie et de Biophysique*, **18**, 399–408.
Tokuyasu, K., Madden, S. C. & Zeldis, L. J. (1968). Fine structural alterations of interphase nuclei of lymphocytes stimulated to growth activity *in vitro*. *Journal of Cell Biology*, **39**, 630–60.
Unuma, T., Arendell, J. P. & Busch, M. (1968). High resolution autoradiographic studies of the uptake of ^{3}H-5-uridine into condensed and dispersed chromatin of nuclei and granular and fibrillar components of nucleoli of Novikoff hepatoma ascites cells. *Experimental Cell Research*, **52**, 429–38.
Vagner-Capodano, A. M. & Stahl, A. (1980). Relationship of chromatin to nucleolar fibrillar center revealed by action of Actinomycin D. *Biologie Cellulaire*, **37**, 293–6.

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Transcriptional and skeletal elements in nucleoli of amphibian oocytes

Introduction

Nuclei ('germinal vesicles') of amphibian oocytes contain numerous amplified, extrachromosomal nucleoli which can be separated easily from other nuclear constituents. In very early stages of oocyte development (pachytene of the meiotic prophase) a specific and drastic increase of the relative amount of rDNA occurs in a process called amplification. The newly amplified DNA is not integrated into the chromosomes but gives rise to the formation of numerous nucleoli which occur free in the nucleoplasm, i.e. not attached to the chromosomes. The amplification of rDNA and the formation of extrachromosomal nucleoli has been studied in special detail, by both biochemical and cytological methods, in the South African clawed toad, *Xenopus laevis* (Brown & Dawid, 1968; Gall, 1969; Macgregor, 1972; Tobler, 1975). The oocytes of this species contain about 30 pg of amplified rDNA as opposed to 12 pg of chromosomal DNA (4C; haploid genome contains 3 pg). Thus, the amplification process generates $1–2 \times 10^6$ extra rRNA genes located in about 1000 free nucleoli (Buongiorno-Nardelli, Amaldi & Lava-Sanchez, 1972), in addition to the *ca.* 1800 rRNA genes located in the chromosomal nucleolus-organiser regions. Since during the extended period of oocyte growth almost all amplified rRNA genes are transcriptionally highly active, a single oocyte nucleus of *Xenopus laevis* is capable of synthesising about 300000 pre-rRNA molecules per second (Scheer, 1973; LaMarca, Smith & Strobel, 1973) as compared to 10–100 in a somatic cell nucleus. Therefore, these oocyte nuclei provide (i) a high natural enrichment of nucleolar material over other nuclear structures, and (ii) a source for isolating nucleoli in a 'pure' state, i.e. not surrounded by – and attached to – perinucleolar chromatin structures such as heterochromatin which is usually the case with somatic cell material (Rae & Franke, 1972; Smetana & Busch, 1974).

The availability of nuclear fractions highly enriched in nucleoli allows us to answer several important questions related to the architecture and biochemical composition of nucleoli. This chapter focuses on two aspects:

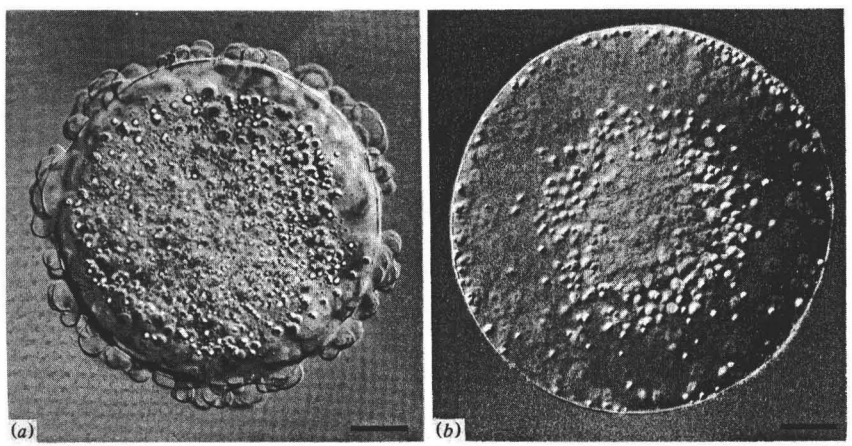
(i) The arrangement of the transcriptional units of the rRNA genes; and (ii) an 'insoluble' (residual) protein component which might serve as an architectural or skeletal framework for these nucleoli.

Morphology of nucleoli at physiological ionic strength

Nuclei can be easily isolated manually from the large oocytes of amphibia. Maturing oocytes are placed in a simple saline solution (75 mM KCl, 25 mM NaCl, buffered with 10 mM Tris-HCl to pH 7.2) and are torn open under a dissecting microscope using fine forceps so that the germinal vesicle is liberated (Fig. 1 *a*, *b*; for technical details see Callan & Lloyd, 1960; Gall, 1966). When viewed in phase or interference contrast the numerous extrachromosomal nucleoli are readily seen (Fig. 1 *a*, *b*). Especially in the nuclei of *Xenopus laevis* oocytes the densely packed amplified nucleoli (about 1000 per nucleus) represent by far the predominant structure of the nuclear interior (Fig. 1 *a*). The diameters of the nucleoli are somewhat variable and usually range from 4 to 10 μm.

Ultrathin sections through isolated nuclei fixed and embedded for electron microscopy illustrate the abundance of these nucleoli which occur as separate units embedded in a finely fibrillar nucleoplasm without any detectable attachment to chromosomal material (Fig. 2 *a–d*). Usually two zones can be distinguished: a spherical dense aggregate ('fibrillar region') is surrounded by a cortical layer of 'granular material' ('pars granulosa'; for terminology

Fig. 1. Manually isolated nuclei from large oocytes of *Xenopus laevis* (*a*) and *Triturus alpestris* (*b*), photographed with Nomarski interference-contrast optics. Numerous extrachromosomal nucleoli are visible. The nuclear envelope of the *Xenopus* nucleus shows many protrusions (*a*). Scale bars, 100 μm.



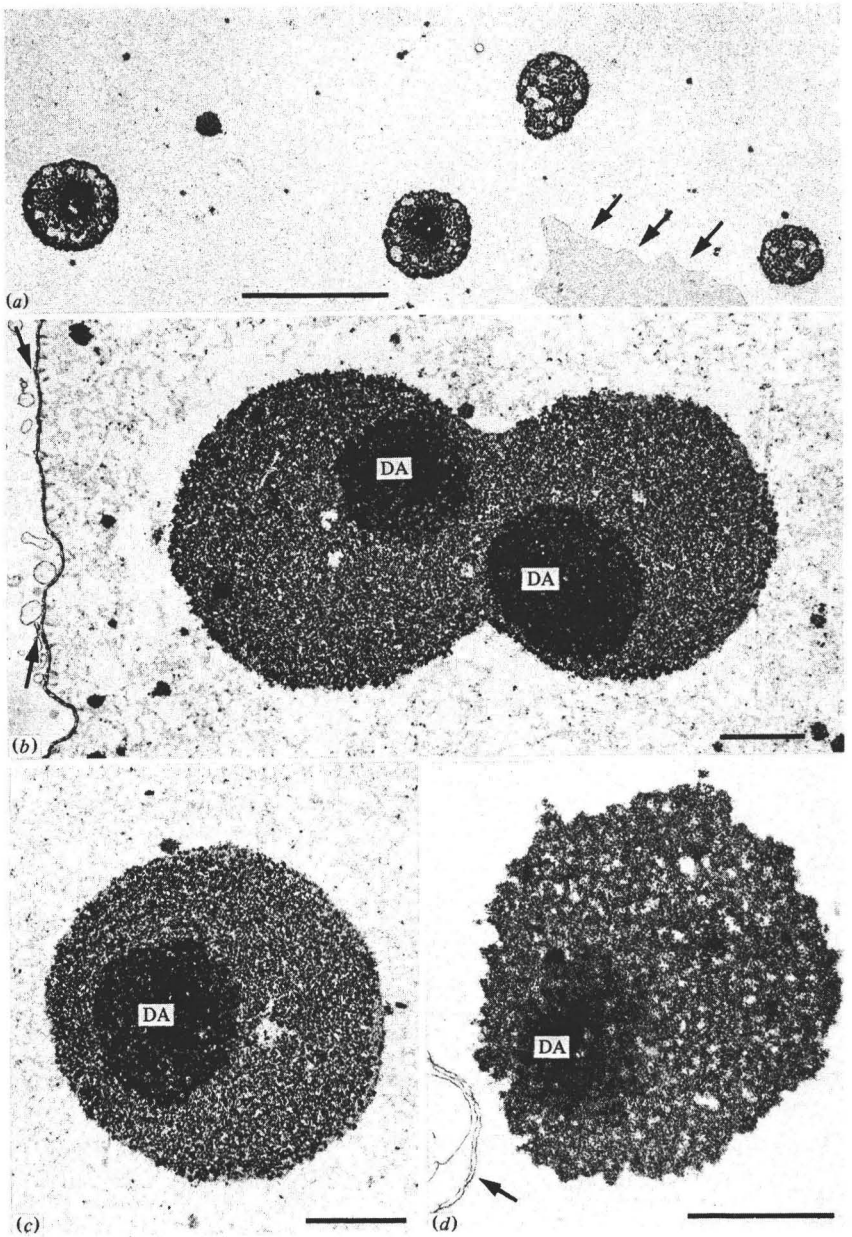


Fig. 2. Ultrathin sections showing the electron-microscopic appearance of nucleoli fixed in intact oocyte nuclei of *Xenopus laevis* (*a–c*) and *Pleurodeles waltlii* (*d*). Each nucleolus contains at least one spheroidal dense aggregate (DA) surrounded by a granular cortex. The arrows denote the nuclear envelope. Scale bars, 10 μm (*a*), 2 μm (*b*, *c*) and 1 μm (*d*).

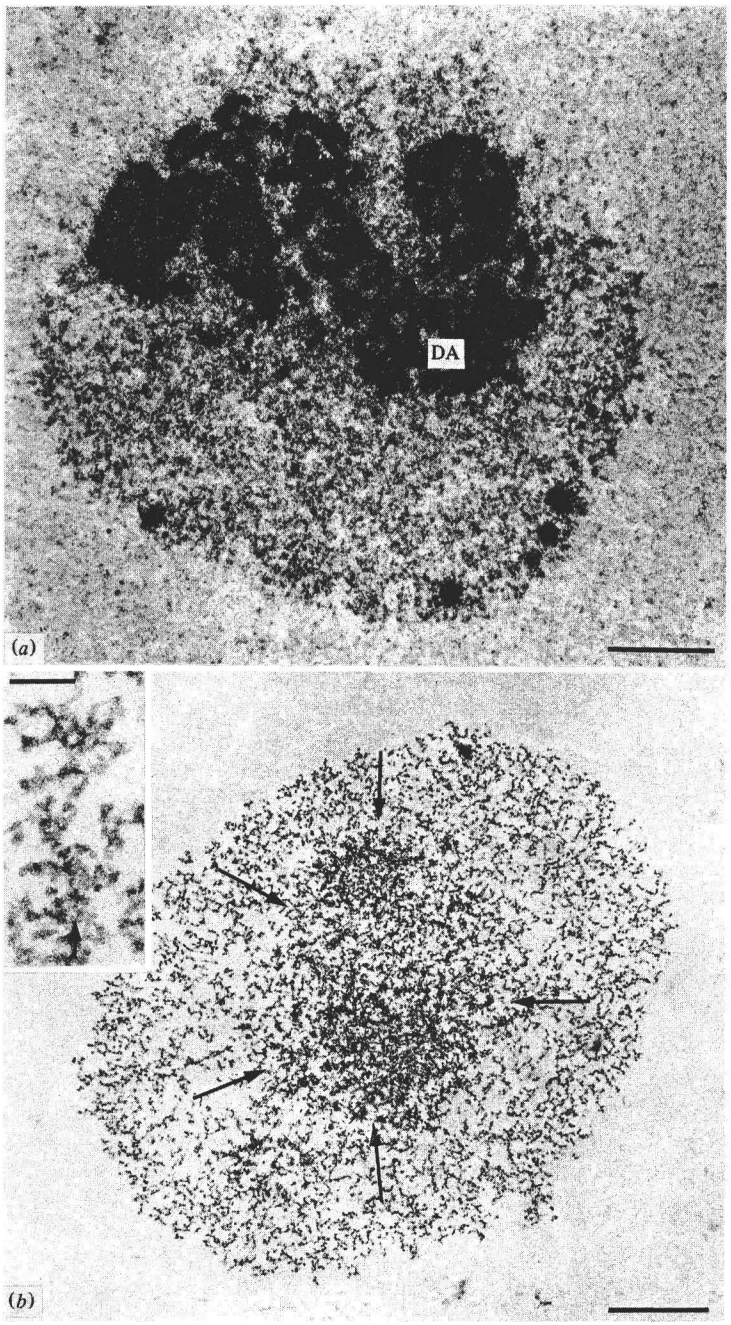


Fig. 3. Progressive unravelling of the dense aggregate (DA) induced by low-salt treatment for 30 s (*a*, nucleolus from a *Triturus alpestris* oocyte) and 20 min (*b*, nucleolus from *Xenopus laevis* oocyte). After the more extensive swelling the dense aggregate can be seen to consist of numerous closely spaced rTUs (*b*; the chromatin-containing area is encircled by arrows). The inset in *b* shows the terminal region of an rTU at higher magnification; the arrow indicates the position of the chromatin axis. The length gradient of the lateral RNP fibrils is recognised. Note the structural maintenance of the cortical material (*a*, *b*). Scale bars indicate 2 μm (*a*), 1 μm (*b*) and 0.1 μm (inset in *b*).

see the previous article in this book). Not infrequently, two or even more such dense aggregates can be found within a nucleolar unit, suggestive of nucleolar fusion processes (Fig. 2*b*). It should also be mentioned that, depending on the amphibian species and specific stage of oogenesis, there may be some morphological deviations from the rather compact state shown in Fig. 2 such as the occurrence of lacunar spaces or even ringlike formations (for references, see Macgregor, 1972).

Morphology of nucleoli at low ionic strength

When isolated nuclei are transferred into low-salt buffers (1 mM alkali salts or less), the components of the nucleoli swell rapidly and become much more loosely arranged. Fig. 3 shows two successive stages of this low-salt-induced dispersion and unfolding. It can be seen that the intranucleolar dense aggregate gradually unravels into a tangle of tightly packed rRNA transcription units (rTUs). Especially where rTUs are included entirely in the plane of the section, the length gradient of the nascent RNP fibrils attached to a central chromatin axis is recognised and occasionally also terminal knobs at the lateral fibrils (Fig. 3*b*) are seen. The organisation of the nucleolar cortex is still maintained under these low-salt conditions although it appears also somewhat less condensed, compared to nucleoli fixed in intact cells or nuclei at nearly physiological ionic strength.

Electron-microscopic spread preparations of such low-salt-dispersed material reveal more clearly that each central nucleolar aggregate consists of hundreds of closely spaced rTUs. When nuclei are briefly dispersed in 0.1 mM borate buffer at pH 8.5–9.0 and then centrifuged on to electron-microscope grids according to the procedure developed by Miller (Miller & Beatty, 1969; Miller & Bakken, 1972), numerous compact bodies are found with diameters corresponding to – or somewhat larger than – those of the dense aggregates seen in sectioned material (Fig. 4*a*). These bodies can be clearly identified as aggregates of transcriptionally active nucleolar chromatin by the characteristic morphology, arrangement and length of the rTUs (for further details see Miller & Beatty, 1969; Franke *et al.*, 1979). The typical pattern of tandem arrays of gene-spacer regions is recognised in the more-expanded peripheral regions of the chromatin aggregates (Fig. 4*a*). Although the rTUs are densely aggregated in the inner nucleolar masses they can be identified by the

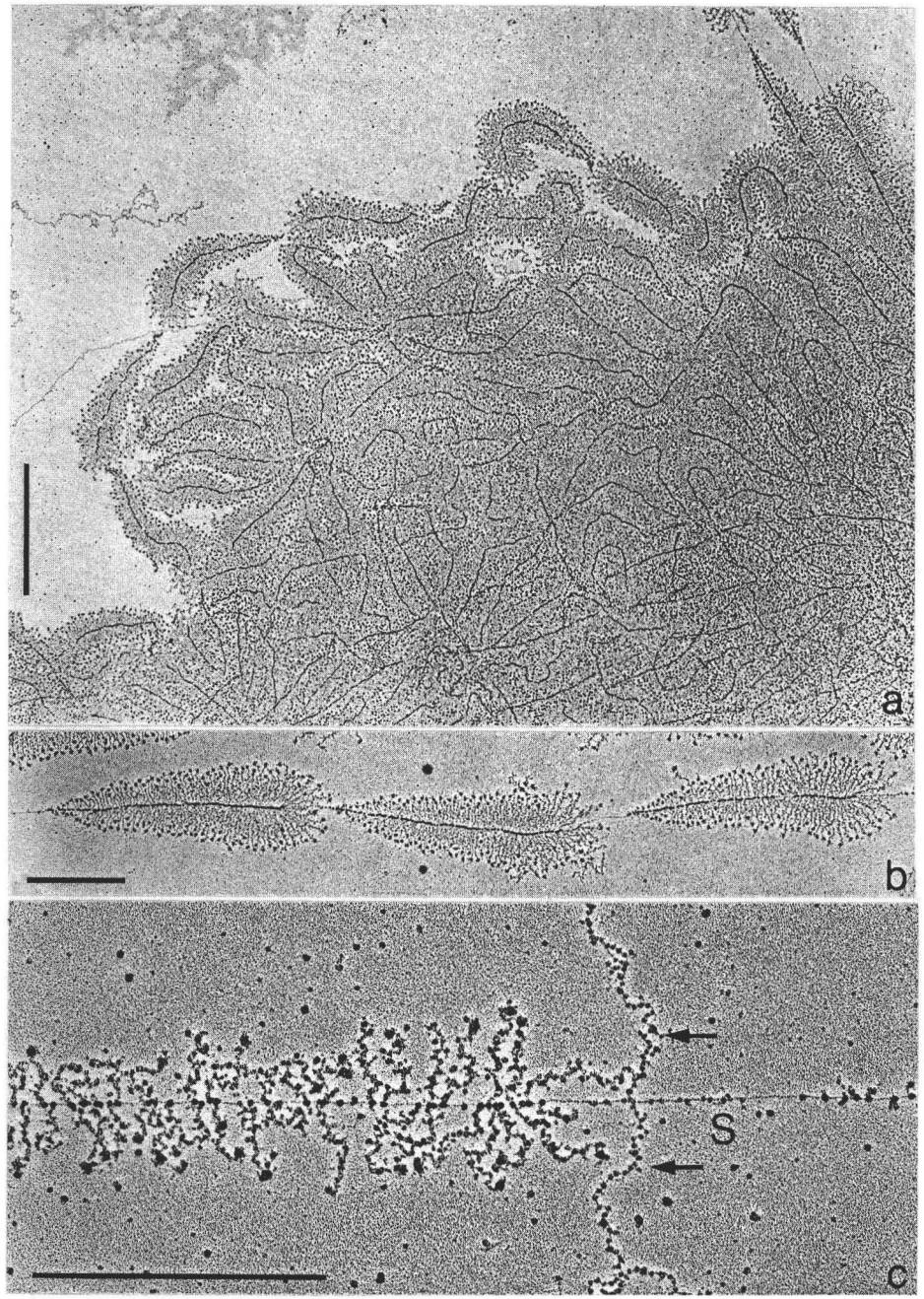


Fig. 4. Electron-microscopic spread preparation of nucleolar chromatin from oocytes of *Pleurodeles waltlii*. Brief exposure of the nucleoli to low salt concentration maintains the dense packing state of the rTUs (*a*). After more extensive spreading the characteristic tandem arrangement of the ribosomal RNA genes separated by nontranscribed spacer regions is recognised (*b*). The morphology of the nucleolar chromatin is clearly different from the

enhanced contrast of their chromatin axes, reflecting the staining of the closely spaced RNA polymerase granules. Upon further unravelling by prolonged incubation in low-salt buffer, the tandem arrangement of the rTUs separated by nontranscribed spacer regions becomes evident the more the material is dispersed and spread (Fig. 4*b*). Each rTU with an average axial length of about 2.5 μm (for heterogeneity of rTU lengths see Scheer, Trendelenburg & Franke, 1973; Scheer, Trendelenburg, Krohne & Franke, 1977) contains about 100 'transcriptional complexes' (RNA polymerase granule plus the attached nascent RNP fibril) arranged in a characteristic length gradient ('Christmas tree'), thus defining the transcriptional initiation and termination sites (Fig. 4*b*). The lateral RNP fibrils usually have terminal thickenings irrespective of their specific position within a given fibril length gradient.

In such spread preparations the cortical component of the nucleoli is difficult to identify since it is no longer regularly associated with the chromatin material. It is possible that non-chromatin components are simply not deposited in sufficient yields on the electron-microscope grids by the low-speed centrifugation conditions used or that they are fragmented. Only rarely certain aggregates of filamentous structures are noticed in the vicinity of rTUs (Franke *et al.*, 1979) which may correspond to components of the outer 'pars granulosa' of the intact nucleolus. In any case, these observations indicate that the chromatin portion is easily detached from other non-chromatinous components of the nucleoli during the low-salt treatment and centrifugation.

The morphology of transcriptionally active nucleolar chromatin, both in gene regions and nontranscribed spacer intercepts, is different from that of the bulk of chromosomal chromatin. Transcriptionally inactive chromatin, when prepared for electron microscopy under identical conditions, invariably exhibits the characteristic 'beads-on-a-string' pattern of closely spaced nucleosomes (Olins & Olins, 1974; Oudet, Gross-Bellard & Chambon, 1975; Franke *et al.*, 1978). This holds for inactive nucleolar chromatin as well (McKnight & Miller, 1976; Scheer, 1978; Foe, 1978). By contrast, nucleolar chromatin stretches of rTUs momentarily not engaged in transcription (i.e. regions between more distantly spaced RNA polymerase granules; Scheer *et al.*, 1976) reveal a smooth, nonbeaded aspect and this is also the case in spacer regions between fully fibril-covered rTUs (Fig. 4*c*; see also Franke *et al.*, 1976, 1978, 1979). The latter can frequently be associated with irregularly distributed particles of nucleosomal size (Fig. 4*b*, *c*) which, however, have been shown to be of non-nucleosomal nature (Scheer, 1980). The smooth aspect of active nucleolar chromatin units reflects the extension of the transcribed rDNA to the length equivalent of B-conformation rDNA, at least

under the spreading conditions used. This is clearly demonstrated by the agreement of the contour lengths of repeating units (gene plus spacer unit) measured in transcribed chromatin and in isolated rDNA (Scheer *et al.*, 1977; Reeder, McKnight & Miller, 1978). On the other hand, after inactivation of the rRNA genes the rDNA-containing chromatin is rearranged and condensed into nucleosomal particles (Scheer, 1978), which, at physiological salt concentrations most likely to be present in the living oocyte, are further compacted into granular (30–50 nm diameter) arrays of supranucleosomal units (Scheer & Zentgraf, 1978; Scheer, Sommerville & Müller, 1980; Zentgraf, Müller, Scheer & Franke, 1981).

Isolation of nucleoli

Three different procedures can be used to obtain amplified nucleoli in sufficient amounts and purity suitable for biochemical analyses.

Procedure 1

Manually isolated nuclei are transferred into isolation medium with additional 5 mM $MgCl_2$. The nuclear envelope is then mechanically removed from the 'gelled nuclear contents' which contain all amplified nucleoli (Fig. 5*a*, *b*; Scheer, 1972; Krohne, Franke & Scheer, 1978*a*). Although this method does not allow the separation of nucleoli from several other structures of the nuclear interior, the nuclear contents thus prepared represent a fraction sufficiently enriched in amplified nucleoli.

Procedure 2

Nuclei of *Xenopus laevis* oocytes are isolated in bulk by the procedure of Scalenghe, Buscaglia, Steinheil & Crippa (1977). The nuclear homogenate is then stained simultaneously with two different fluorescing dyes and the nucleoli are separated and collected using the ultraviolet line of an argon ion laser for excitation illumination (fluorescence-activated particle sorting; for further details see Franke *et al.*, 1981). The purity of the nucleolar fraction obtained and the good morphological preservation of the nucleoli isolated by this procedure are demonstrated in Fig. 5*c*, *d*.

Procedure 3

Oocytes of *Xenopus laevis* are homogenised and nucleoli isolated by buoyant-density banding in Metrizamide gradients (Higashinakagawa, Wahn & Reeder, 1977).

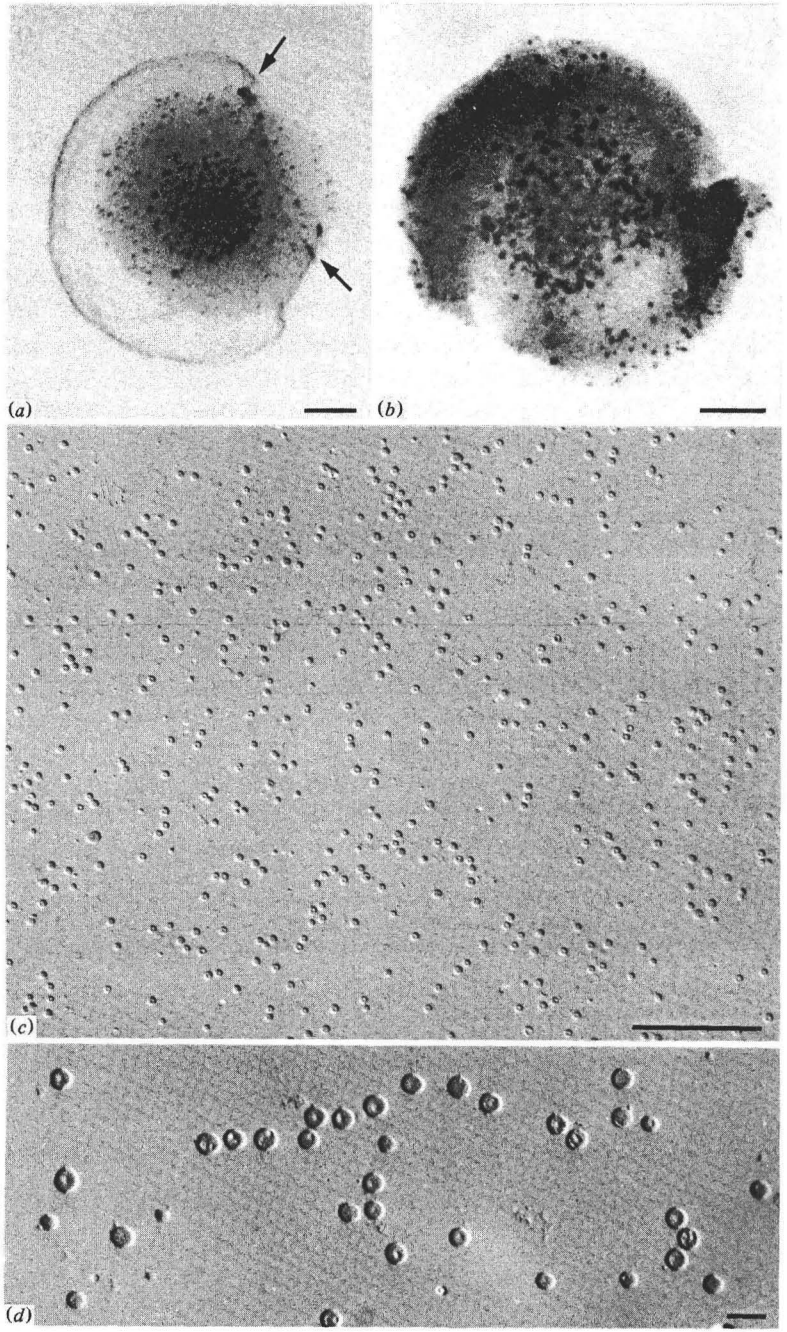


Fig. 5. Removal of the nuclear envelope from an isolated oocyte nucleus in the presence of 5 mM $MgCl_2$ (arrows in *a* denote the broken nuclear envelope) yields a 'gelled nuclear content' with all amplified nucleoli (*b*). *c* and *d* show, in interference contrast, fractions of mass-isolated nucleoli from *Xenopus laevis* oocytes using fluorescence-activated particle sorting. Scale bars, 100

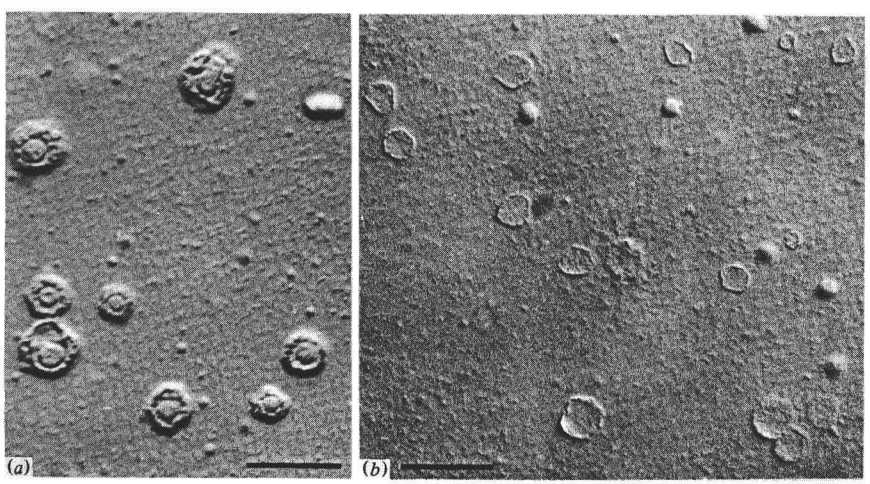
Cytochemistry of isolated nucleoli

Nucleoli isolated in the presence of millimolar concentrations of $MgCl_2$ and then transferred into saline solutions of low molality reveal, in the light microscope (phase or interference contrast), a cortical ring-like structure surrounding a central spheroidal aggregate. This appearance remains unchanged after treatment with pancreatic ribonuclease (RNase) (Fig. 6*a*). However, digestion by deoxyribonuclease (DNase) I removes the intranucleolar aggregate body but leaves the cortical structure (Fig. 6*b*). These simple cytochemical tests demonstrate the presence of DNA in the central dense aggregate, in agreement with the results described above and those obtained by other methods (Ebstein, 1969; Thiebaud, 1979).

Morphology of nucleolar residues after extraction with high-salt buffer and detergent

Extraction of isolated nucleoli by high-salt buffers (e.g. 1.0–1.5 M KCl buffered with 10 mM Tris-HCl to pH 7.4) containing nonionic detergents (1% Triton X-100 or NP-40) and relatively high concentrations of sulphydryl agents (20 mM dithiothreitol or 2-mercaptoethanol) results in the removal of the intranucleolar dense aggregate but leaves a residual fibrillar meshwork that usually appears denser in the nucleolar periphery but can also extend into the nucleolar interior (Fig. 7*a–c*). The residual nucleolar structures retain

Fig. 6. Nucleoli from *Xenopus laevis* oocytes isolated in the presence of 2 mM $MgCl_2$, after treatment with pancreatic RNase (*a*) or DNase I (*b*), followed by washing in 1 mM Tris-HCl, pH 7.2. Note the absence of the dense central aggregate after DNase digestion. The photographs were taken with Nomarski interference-contrast optics. Scale bars, 10 μm.



the size and the shape of the original nucleoli. The basic structural component left is a complex meshwork of filaments of about 4 nm diameter (Fig. 7*a–c*). Negatively stained preparations of gently homogenised nucleolar structures resistant to high-salt treatment show a meshwork composed of 3–5-nm-thick filaments which frequently seem to be coiled locally into nodules of various sizes (Fig. 7*e*). Protein-depleted rDNA is visible as very fine filaments consisting of numerous laterally aggregated DNA strands extending through the central regions of the nucleolar residues (Fig. 7*c*, *d*). DNase treatment removes these filaments. Since RNase digestion prior to extraction with high-salt buffers does not alter the morphology of the nucleolar residues, it can be concluded that the filament meshwork resistant to treatment with high-salt buffers, DNase, RNase and detergent represents a proteinaceous 'skeletal' component of the nucleolus.

Chemical nature of the nucleolar residual structures

DNA has not been detected in nucleolar residual material extracted with high-salt buffers and treated with nucleases. When RNA is extracted from manually isolated nuclei of *Xenopus laevis* oocytes and analysed on 1.5% agarose gels (for technical details see Scheer, 1973; Scheer *et al.*, 1973; Franke *et al.*, 1981) three major bands corresponding to the 40s pre-rRNA and the nuclear forms of 28s and 18s rRNAs are revealed (Fig. 8*a*). A similar pattern is observed after extraction of the RNA from 'gelled nuclear contents', with the only exception that the amount of 18s rRNA is drastically reduced and sometimes even no longer detectable. This is in agreement with observations of a selective removal of 18s rRNAs made in various other cells (e.g., Penman, 1966; Kumar, 1970; Eckert, Kaffenberger, Krohne & Franke, 1978).

A gel-electrophoretic analysis of the RNA extracted from high-salt-resistant nucleolar residues and from the supernatant fractions, i.e. from material not sedimentable at 3500 $g \times 30$ min, is shown in Fig. 8*b*. The pre-rRNA and the 28s rRNA are completely recovered in the supernatant fractions (track 1) whereas practically no RNA is detectable in the pellets of nucleolar residual structures (track 2). Therefore, it is concluded that no substantial amounts of RNA or ribonucleoproteins are associated with the proteinaceous nucleolar skeletons in a mode resistant to high-salt extraction.

The only macromolecules positively identified in the nucleolar residual filaments are proteins. Proteins present in manually isolated nuclei, 'gelled nuclear contents' and high-salt-extracted nuclear contents are demonstrable by SDS-polyacrylamide gel electrophoresis. Fig. 9*a* shows a remarkable simplification of the polypeptide pattern between the starting material, i.e. total isolated nuclei (track 4), and the high-salt-extracted (1 M KCl) nuclear

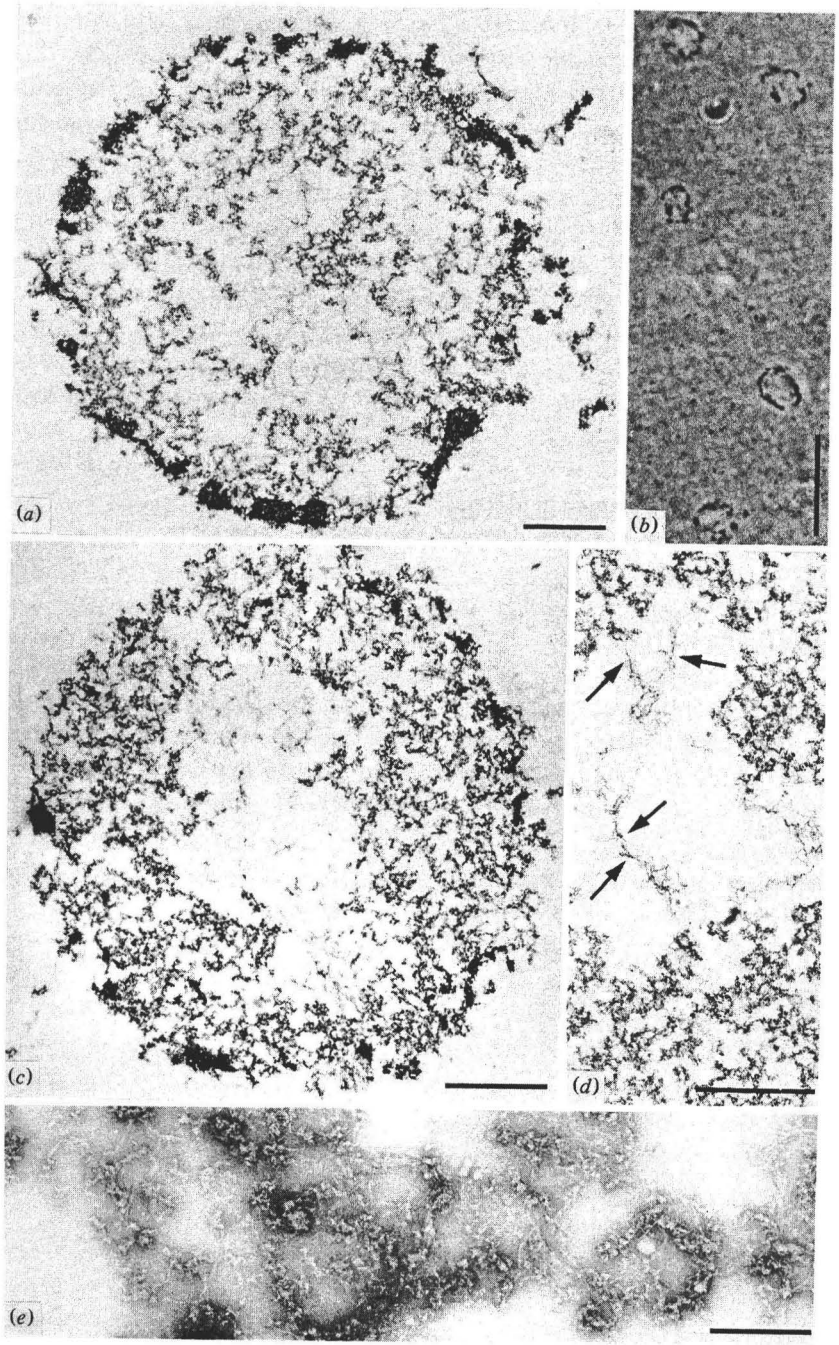


Fig. 7. Morphology of the high-salt-resistant nucleolar skeleton structure in ultrathin sections (*a*, *c*, *d*), after negative staining (*e*) and in light-microscope whole-mount preparations (*b*). The skeletal residues are composed of a relatively loose filament meshwork with denser compaction in the periphery (*a*, *c*). These peripheral aggregates can also be visualised in the light microscope (*b*). The arrows in *d* denote tangles of deproteinized DNA found in more central regions of nucleolar residues. In negatively stained preparations individual filaments of the nucleolar skeleton are recognised with diameters ranging from 3 to 5 nm (*e*). Scale bars, 1 μm (*a*, *c*), 10 μm (*b*), 0.1 μm (*d*) and 0.2 μm (*e*).

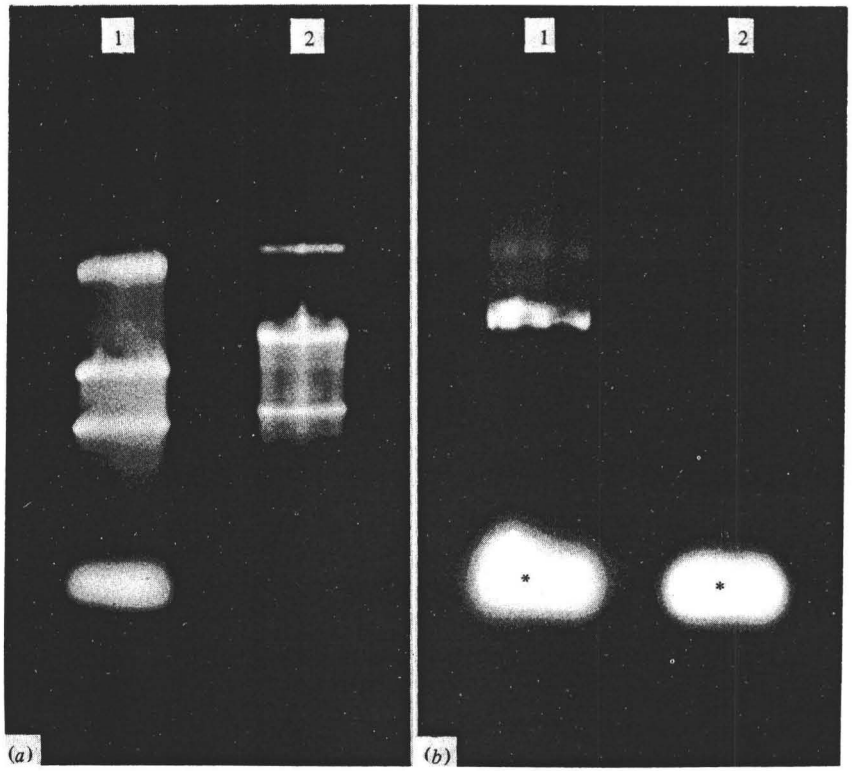
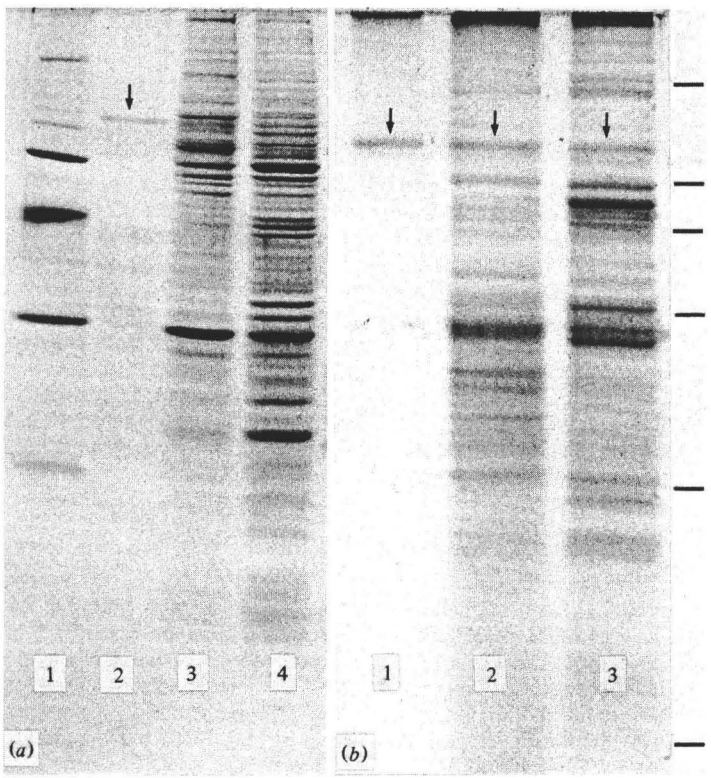


Fig. 8. Gel-electrophoretic analysis of RNA (1.5% agarose gels) from manually isolated nuclei (*a*) and high-salt-extracted nuclear contents (*b*) of *Xenopus laevis* oocytes. Nuclear RNA is found almost exclusively in 3 bands: 40s pre-rRNA, nuclear 28s and 18s rRNAs (*a*, track 2, from top to bottom). Marker RNAs in track 1 represent, from top to bottom, tobacco mosaic virus RNA, *Escherichia coli* rRNA and tRNA. After high-salt extraction of nuclear contents the RNA was analysed separately from pelletable (mainly nucleolar skeletons; Fig. *b*, track 2) and supernatant (track 1) material. The pre-rRNA and 28s rRNA is recovered almost quantitatively in the non-sedimentable (3500 *g* × 30 min) fraction. Asterisks denote tRNA added as carrier. The gels were stained with ethidium bromide and photographed under ultraviolet illumination.

contents containing the nucleolar residual structures (track 2). The major polypeptide present in the latter fraction has an apparent molecular weight of 145000 (denoted by the arrow in Fig. 9*a*, track 2). That this protein is indeed a component of nucleolar residues is demonstrated in Fig. 9*b*. Here the polypeptide pattern of isolated nucleoli (obtained by using fluorescence-activated particle sorting) is shown as the starting material for the preparation

Fig. 9. (*a*) SDS-polyacrylamide gel electrophoresis of proteins of isolated nuclei from *Xenopus laevis* oocytes (7 nuclei, track 4), nuclear contents (from 31 nuclei, track 3) and nuclear contents extracted with 1 M KCl, 1% Triton X-100, 10 mM Tris-HCl, pH 7.4 (from 450 nuclei, track 2). Note the enrichment of a single polypeptide (arrow) with an apparent molecular weight of 145000. Reference proteins (track 1) are, from top to bottom, myosin, β-galactosidase, phosphorylase, bovine serum albumin, actin and chymotrypsinogen. (*b*) SDS-polyacrylamide gel electrophoresis of proteins of isolated nucleoli from *Xenopus laevis* oocytes (track 3) and after a single (track 2) and repeated (track 1) extraction with high-salt buffer. The arrows indicate the polypeptide with a molecular weight of 145000 which is enriched in the nucleolar skeleton fractions. Bars denote the position of the same reference proteins as in *a*, track 1.



of nucleolar skeletons (track 3), and after a single (track 2) and repeated (track 1) extraction by high-salt buffer containing 1% Triton X-100. The specific enrichment of a polypeptide band of molecular weight 145000 is evident (denoted by the arrows in Fig. 9*b*). A second protein component with an apparent molecular weight of *ca.* 65000 often present in the nucleolar skeleton fractions (Fig. 9*b*, track 1) is also noticed in other nuclear subfractions and therefore is considered not to be specific to the nucleolar residues. When analysed by two-dimensional gel electrophoresis the 145000 molecular weight protein focuses as a single polypeptide spot at an isoelectric pH of about 6.15 (see Franke *et al.*, 1981).

Summary and conclusions

The amplified extrachromosomal nucleoli of *Xenopus laevis* oocytes contain a proteinaceous fibrillar meshwork composed of filaments 3–5 nm thick that is insoluble in low- and high-salt buffers containing 2–5 mM $MgCl_2$, DNase, RNase and nonionic detergents. This skeletal meshwork extends throughout the nucleoli with an apparently higher filament packing density in the peripheral region (nucleolar cortex). Its biochemical composition seems to be relatively simple since fractions of nucleolar residue material do not contain considerable amounts of nucleic acids and show specific enrichment of only a single acidic protein which appears as a polypeptide of molecular weight 145000 and an apparent pI value, under denaturing conditions, of 6.15.

The nucleolar chromatin is surrounded by – and partly interdigitated with – this 'skeletal' nucleolar protein meshwork. It is usually embedded in the inner regions of the fibrillar framework and forms the spherical 'dense aggregate' visible in intact nucleoli. Dispersal of nucleoli in solutions of low molarity, followed by centrifugation onto electron-microscope grids, results in dispersal of the nucleolar chromatin and allows, in transcriptionally active stages, the visualisation of individual chromatin units. These units, depending on the specific spreading conditions, appear either as compact aggregates or as loose tangles of transcriptionally active ribosomal RNA genes. Although still not rigorously shown it is likely that the ribosomal precursor particles (Roger, 1968) are attached to the more peripheral regions of the nucleolar skeleton ('pars granulosa') where processing events, association with specific ribosomal proteins and transitory storage occur and from which the uni-directional nucleocytoplasmic translocation starts (see also Franke & Scheer, 1974). In any case, this putative association between the nucleolar skeleton and rRNA nucleoprotein complexes is unstable in the presence of 1 M KCl.

The fibrillar skeletal meshwork of the amplified nucleoli is different, both by structural and biochemical criteria, from all other nuclear residual protein

structures so far described. This category of nuclear structures, often collectively termed 'non-nucleoproteinaceous architectural components', include a variety of preparations such as residual nuclear membrane and pore complex-lamina fractions (Aaronson & Blobel, 1975; Gerace, Blum & Blobel, 1978; Krohne *et al.*, 1978*a*, *b*; Gerace & Blobel, 1980), chromosomal and nuclear 'scaffolds' (Adolph, Cheng & Laemmli, 1977; Adolph, 1980), and socalled nuclear matrix structures (for references see Comings, 1978; Berezney, 1979). Therefore it is concluded that the insoluble protein structure described here represents a skeletal meshwork specific for the nucleolus. It remains to be clarified whether identical structures occur in nucleoli of somatic cells as well and represent a universal component generally involved in the structural and functional organisation of nucleoli.

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References

Aaronson, R. P. & Blobel, G. (1975). Isolation of nuclear pore complexes in association with a lamina. *Proceedings of the National Academy of Sciences of the USA*, **72**, 1007–11.

Adolph, K. W. (1980). Organization of chromosomes in HeLa cells: isolation of histone-depleted nuclei and nuclear scaffolds. *Journal of Cell Science*, **42**, 291–304.

Adolph, K. W., Cheng, S. M. & Laemmli, U. K. (1977). Role of nonhistone proteins in metaphase chromosome structure. *Cell*, **12**, 805–16.

Berezney, R. (1979). Dynamic properties of the nuclear matrix. In *The Cell Nucleus*, vol. **7**, ed. H. Busch, pp. 413–56. New York: Academic Press.

Brown, D. D. & Dawid, I. B. (1968). Specific gene amplification in oocytes. *Science*, **160**, 272–80.

Buongiorno-Nardelli, M., Amaldi, F. & Lava-Sanchez, P. A. (1972). Amplification as a rectification mechanism for the redundant rRNA genes. *Nature New Biology*, **238**, 134–7.

Callan, H. G. & Lloyd, L. (1960). Lampbrush chromosomes of crested newts *Triturus cristatus* (Laurenti). *Proceedings of the Royal Society*, B **243**, 135–212.

Comings, D. E. (1978). Compartmentalization of nuclear and chromatin proteins. In *The Cell Nucleus*, vol. **4**, ed. H. Busch, pp. 345–71. New York: Academic Press.

Ebstein, B. S. (1969). The distribution of DNA within the nucleoli of the amphibian oocyte as demonstrated by tritiated actinomycin D radioautography. *Journal of Cell Science*, **5**, 27–44.

Eckert, W. A., Kaffenberger, W., Krohne, G. & Franke, W. W. (1978). Introduction of hidden breaks during rRNA maturation and ageing in *Tetrahymena pyriformis*. *European Journal of Biochemistry*, **87**, 607–16.

Foe, V. E. (1978). Modulation of ribosomal RNA synthesis in *Oncopeltus fasciatus*: an electron microscopic study of the relationship between

changes in chromatin structure and transcriptional activity. *Cold Spring Harbor Symposium of Quantitative Biology*, **42**, 723–40.

Franke, W. W., Kleinschmidt, J. A., Spring, H., Krohne, G., Grund, C., Trendelenburg, M. F., Stoehr, M. & Scheer, U. (1981). A nucleolar skeleton of protein filaments demonstrated in amplified nucleoli of *Xenopus laevis*. *Journal of Cell Biology*, **90**, 289–99.

Franke, W. W. & Scheer, U. (1974). Pathways of nucleocytoplasmic translocation of ribonucleoproteins. *Symposium of the Society of Experimental Biology*, **28**, 249–82.

Franke, W. W., Scheer, U., Trendelenburg, M. F., Spring, H. & Zentgraf, H. (1976). Absence of nucleosomes in transcriptionally active chromatin. *Cytobiologie*, **13**, 401–34.

Franke, W. W., Scheer, U., Trendelenburg, M. F., Zentgraf, H. & Spring, H. (1978). Morphology of transcriptionally active chromatin. *Cold Spring Harbor Symposium of Quantitative Biology*, **42**, 755–72.

Franke, W. W., Scheer, U., Spring, H., Trendelenburg, M. F. & Zentgraf, H. (1979). Organization of nucleolar chromatin. In *The Cell Nucleus*, vol. **7**, ed. H. Busch, pp. 49–95. New York: Academic Press.

Gall, J. G. (1966). Techniques for the study of lampbrush chromosomes. In *Methods in Cell Physiology*, vol. **2**, ed. D. M. Prescott, pp. 37–60. New York: Academic Press.

Gall, J. G. (1969). The genes for ribosomal RNA during oogenesis. *Genetics* (Supplement), **61**, 121–32.

Gerace, L. & Blobel, G. (1980). The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell*, **19**, 277–87.

Gerace, L., Blum, A. & Blobel, G. (1978). Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. *Journal of Cell Biology*, **79**, 546–66.

Higashinakagawa, T., Wahn, H. & Reeder, R. H. (1977). Isolation of ribosomal gene chromatin. *Developmental Biology*, **55**, 375–86.

Krohne, G., Franke, W. W. & Scheer, U. (1978*a*). The major polypeptides of the nuclear pore complex. *Experimental Cell Research*, **116**, 85–102.

Krohne, G., Franke, W. W., Ely, S., D'Arcy, A. & Jost, E. (1978*b*). Localization of a nuclear envelope-associated protein by indirect immunofluorescence microscopy using antibodies against a major polypeptide from rat liver fractions enriched in nuclear envelope-associated material. *Cytobiologie*, **18**, 22–38.

Kumar, A. (1970). Ribosome synthesis in *Tetrahymena pyriformis*. *Journal of Cell Biology*, **45**, 623–34.

LaMarca, M. J., Smith, L. D. & Strobel, M. C. (1973). Quantitative and qualitative analysis of RNA synthesis in stage 6 and stage 4 oocytes of *Xenopus laevis*. *Developmental Biology*, **34**, 106–18.

MacGregor, H. C. (1972). The nucleolus and its genes in amphibian oogenesis. *Biological Reviews*, **47**, 177–210.

McKnight, S. L. & Miller, O. L. (1976). Ultrastructural patterns of RNA synthesis during early embryogenesis of *Drosophila melanogaster*. *Cell*, **8**, 305–19.

Miller, O. L. & Bakken, A. H. (1972). Morphological studies of transcription. *Acta Endocrinologica* (Supplement), **168**, 155–77.

Miller, O. L. & Beatty, B. R. (1969). Visualization of nucleolar genes. *Science*, **164**, 955–7.

Olins, A. L. & Olins, D. W. (1974). Spheroid chromatin units (v bodies). *Science*, **183**, 330–2.

Oudet, P., Gross-Bellard, M. & Chambon, P. (1975). Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell*, **4**, 281–300.
Penman, S. (1966). RNA metabolism in the HeLa cell nucleus. *Journal of Molecular Biology*, **17**, 117–30.
Rae, P. M. M. & Franke, W. W. (1972). The interphase distribution of satellite DNA-containing heterochromatin in mouse nuclei. *Chromosoma*, **39**, 443–56.
Reeder, R. H., McKnight, S. L. & Miller, O. L. (1978). Contraction ratio of the nontranscribed spacer of *Xenopus* rDNA chromatin. *Cold Spring Harbor Symposium of Quantitative Biology*, **42**, 1174–7.
Rogers, M. E. (1968). Ribonucleoprotein particles in the amphibian oocyte nucleus. *Journal of Cell Biology*, **36**, 421–32.
Scalenghe, F., Buscaglia, M., Steinheil, C. & Crippa, M. (1978). Large scale isolation of nuclei and nucleoli from vitellogenic oocytes of *Xenopus laevis*. *Chromosoma*, **66**, 299–308.
Scheer, U. (1972). The ultrastructure of the nuclear envelope of amphibian oocytes. IV. On the chemical nature of the nuclear pore complex material. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **127**, 127–48.
Scheer, U. (1973). Nuclear pore flow rate of ribosomal RNA and chain growth rate of its precursor during oogenesis of *Xenopus laevis*. *Developmental Biology*, **30**, 13–28.
Scheer, U. (1978). Changes of nucleosome frequency in nucleolar and non-nucleolar chromatin as a function of transcription: an electron microscopic study. *Cell*, **13**, 535–49.
Scheer, U. (1980). Structural organization of spacer chromatin between transcribed ribosomal RNA genes in amphibian oocytes. *European Journal of Cell Biology*, **23**, 189–96.
Scheer, U., Sommerville, J. & Müller, U. (1980). DNA is assembled into globular supranucleosomal chromatin structures by nuclear contents of amphibian oocytes. *Experimental Cell Research*, **129**, 115–26.
Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1973). Transcription of ribosomal RNA cistrons. *Experimental Cell Research*, **80**, 175–90.
Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1976). Regulation of transcription of genes of ribosomal RNA during amphibian oogenesis. *Journal of Cell Biology*, **69**, 465–89.
Scheer, U., Trendelenburg, M. F., Krohne, G. & Franke, W. W. (1977). Lengths and patterns of transcriptional units in the amplified nucleoli of *Xenopus laevis*. *Chromosoma*, **60**, 147–67.
Scheer, U. & Zentgraf, H. (1978). Nucleosomal and supranucleosomal organization of transcriptionally inactive rDNA circles in *Dytiscus* oocytes. *Chromosoma*, **69**, 243–54.
Smetana, K. & Busch, H. (1974). The nucleolus and nucleolar DNA. In *The Cell Nucleus*, vol. **1**, ed. H. Busch, pp. 73–147. New York: Academic Press.
Thiebaud, C. H. (1979). The intra-nucleolar localization of amplified rDNA in *Xenopus laevis* oocytes. *Chromosoma*, **73**, 29–36.
Tobler, H. (1975). Occurrence and developmental significance of gene amplification. In *The Biochemistry of Animal Development*, vol. **3**, ed. R. Weber, pp. 91–142. New York: Academic Press.
Zentgraf, H., Müller, U., Scheer, U. & Franke, W. W. (1981). Evidence for the existence of globular units in the supranucleosomal organization of chromatin. In *International Cell Biology 1980–81*, ed. H. Schweiger, pp. 139–51. Berlin: Springer–Verlag.

HARRIS BUSCH, MICHAEL A. LISCHWE, JOANNA MICHALIK, PUI-KWONG CHAN and ROSE K. BUSCH

Nucleolar proteins of special interest: silver-staining proteins B23 and C23 and antigens of human tumour nucleoli

Introduction

Although the major role of the nucleolus in the production of ribosomes was established over a decade ago (Busch & Smetana, 1970), the excitement about the opportunities for evaluating mechanisms for controls of the rDNA genes has increased with the development of immunological and other analytical techniques, DNA sequencing methods and other advances. Now that important fragments of rDNA have been cloned, it should not be long before the macromolecules involved in nucleolar structure and the promoters and their associated control sequences are defined (Busch, 1978).

Specific nucleolar macromolecules have been isolated and the U3 RNAs have been sequenced by older cleavage techniques (Brownlee, Sanger & Barrell, 1968) and ladder methods (Maxam & Gilbert, 1977). Some of the special nucleolar proteins such as protein B23 and C23 have been more satisfactorily defined with respect to NOR (nucleolar-organising region) localisation and their functional relationships to cellular activities. A continuing study has been made of the specific elements of the nucleolar substructure as well as the nucleolar products. Accordingly, at this time, the stage is set for definitive analysis of the control of nucleolar function and their responses to the demands of the cell.

NOR proteins

One of the key questions is why do certain proteins seek rDNA and in the same vein, how do specific macromolecules localise in the nucleolar structure. Many cytologists have followed the work of Heitz (1933) and McClintock (1934) who initially defined the NOR region which has now been recognised as being the rDNA locus in the genome. This region was assigned the function of formation of the nucleolus although it is clear that DNA *per se* cannot subserve such a function. Accordingly, it seems evident that rDNA must have the property of binding specifically with both structural and