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Medusoid fibril bodies: a novel type of nuclear filament of diameter 8 to 12 nm with periodic ultrastructure demonstrated in oocytes of Xenopus laevis

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Nucleolus — nuclear skeleton — protein filaments

Nuclei of oocytes of Xenopus laevis contain numerous (several thousand) bodies of diameters ranging from 0.2 to 1.0 µm which are composed of a characteristic type of filamentous structures projecting from a central dense aggregate ("medusoid fibrillar bodies"). The ultrastructure of these nuclear bodies has been studied by electron microscopy using ultrathin sections and spread preparations of nuclear contents visualized with negative and positive staining techniques. As shown in these spread preparations, the individual filaments which extend from the densely stained core material are relatively long (maximal contour length traced 2.4 µm) and show an overall diameter of 8 to 12 nm. At higher magnification these filaments reveal a periodic organization and appear as beads-on-a-string with an average center-to-center distance of approximately 12 nm. Occasionally, regions are seen in which a core thread filament of diameter 3 to 5 nm is resolved and free spheroidal particles of about 10 nm diameter lie nearby on the specimen grid, suggesting that the beaded aspect of the native 8 to 12 nm filaments reflects the close association of spheroidal 10 nm particles with the 3 to 5 nm core thread filament. The filaments are resistant to treatments with pancreatic ribonuclease, DNAse I and micrococcal nuclease but are digested with proteolytic enzymes. Notably the 3 to 5 nm core filament are also resistant to treatment with high salt buffers (2 M KCl). To these 8 to 12 nm beaded protein filaments are attached, at variable frequencies, large spheroidal particles of 18 to 30 nm diameter which morphologically resemble isolated preribosomal particles. These medusoid fibril bodies are frequent in the periphery of nucleoli, and in spread preparations are often seen in close proximity to nucleolar genes, but occur also elsewhere in the nucleoplasm. Spheroidal bodies with this characteristic type of beaded filaments have also been found in oocytes of other amphibia and in various other cell types. The possible relationship of the filaments characteristic of the medusoid fibril bodies to the karyoskeletal elements recently described in association with isolated nucleoli and their possible role in providing structural support to preribosomal particles is discussed.

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Introduction

The cell nucleus contains, within the confinements of the nuclear envelope-lamina complex shell, only few morphologically well identified and distinct structural components sui generis. These include the nucleoli which can occur in chromosomal and extrachromosomal forms and appear to be ubiquitous, except of cells devoid of rRNA synthesis such as very late stages of erythropoesis and spermiogenesis (for reviews see [3, 4]). Only in the case of amplified nucleoli these structures are fully independent and separated units of their own. Whole chromosomes are recognized as individual distinct structures only in certain types of nuclei and more commonly seem to be entangled and interconnected with loss of chromosomes definition into the chromatin mass.

Other much smaller subnuclear structures described in several types of nuclei are the perichromatin granules (40–70 nm in diameter) and the perichromatin fibrils; the ultrastructure and nature of both structures is still unclear and they are believed by some authors to simply reflect localized coilings of nascent ribonucleoprotein material probably still associated with chromatin (for recent reviews see [7, 30]). Another type of a small morphologically distinct particle is the interchromatin granule of diameter 20 to 30 nm; these particles also are widely assumed, but by no means have been convincingly shown, to represent another type of ribonucleoprotein particles [7]. In addition, a variety of "nuclear bodies" have been described in various cell types and this category includes diverse structures such as the homogeneously textured filamentous aggregates ("simple nuclear bodies") as well as complex spheroidal bodies of a granular-looking composition, with or without a finely filamentous shell or "capsule" [3, 30]. In certain cells, prominently of nervous tissue, conspicuous repeatedly paracrystalline arrays of 6 to 8 nm filaments or of tubular formations have been described [1]. These structures have only been described in thin sections and their significance and detailed organization is largely unclear. None of the various "nuclear bodies" has been successfully isolated and visualized by spreading techniques, and

biochemical data that many shed some light onto their composition are not available.

Information on the chemical composition and molecular organization of distinct nuclear components is currently available only for two major structures which have also been isolated and examined in spread prepearations using various electron microscopic staining techniques. 1. The organization of chromatin, inactive [26, 44, 45] as well as transcriptionally [11, 18, 21–23, 38] and replicationally [20] active forms, has been extensively studied. 2. In addition, we have recently described [12] a system of karyoskeletal filaments insoluble in high salt buffers and non-ionic detergents which occurs in association with the nucleolus and, as indicated by immunolocalization data, also in certain smaller perinucleolar particles throughout the nucleoplasm [17]. In the present electron microscopic study we report on the existence of a novel class of distinct spheroidal units, ranging in diameter from about 0.5 to 3 μm, which display a characteristic tumbleweed-like or medusoid organization and contain a type of protein filaments with periodical arrays of beaded structures of diameter 9 to 14 nm. These structural units which occur in the nucleolar periphery as well as elsewhere in the nucleoplasm are numerous in nuclei of Xenopus oocytes but occur also in other cells.

Materials and methods

Animals

The study was carried out with vitellogenic and post-vitellogenic (stages V and VI, according to the classification of Dumont, [9]) oocytes of Xenopus laevis females either unstimulated or induced to ovulate by the administration of hormones (Predalon 1000 IE, Organon, Munich, FRG). Pieces of ovaries were removed from the anesthetized animals (0.1% MS 222, Serva Feinbiochemica, Heidelberg, FRG) and placed in Eagle's minimal essential medium diluted 1:1 with distilled water.

Isolation of nuclei

Nuclei (germinal vesicles) were isolated in "5:1 medium" buffered in 10 mM Tris-HCl (*p*H 7.2) with or without addition of $MgCl_2$ (5 mM or 10 mM) as described [12, 15–17, 35–37]. In some experiments 10 mM dithioerythritol was included in all isolation and extraction media used.

Spreading preparations

After isolation of nuclei in "5:1 medium" buffered to *p*H 7.2 with 10 mM Tris-HCl, the nuclear envelopes were disrupted with fine needles and the nuclear contents allowed to disperse for 20 min in a droplet of water adjusted to *p*H 8.8 with 0.1 mM sodium borate buffer at about 10 °C or at room temperature. In some experiments 0.02% Sarkosyl [36, 37] or 0.01% Joy [23] was included in the dispersion solution. The dispersed material was centrifuged on carbon-coated grids freshly treated by the glow discharge method, in microchambers filled with 0.1 M saccharose (same buffer) containing 1% formaldehyde freshly prepared from paraformaldehyde. For positive staining, grids were stained, after centrifugation, with 1% alcoholic phosphotungstic acid (PTA) according to the method of Miller and Beatty [21, 22]. Some of these PTA-stained preparations were rotary-shadowed with Au-Pd (75%–25%) at an angle of 7° (Balzers Mikrobedampfungsanlage BA3; Balzers, Liechtenstein).

For negative staining, the grids were washed for 30 sec in 4% Photo-Flo 200 (Kodak) *p*H 8, briefly dried and immediately stained for 10 to 20 sec with 2% aqueous PTA adjusted to *p*H 7.2 with NaOH. The grids were dried by blotting on filterpaper. Alternatively, dispersed nuclear contents were prepared and negatively stained with uranyl acetate as described [12].

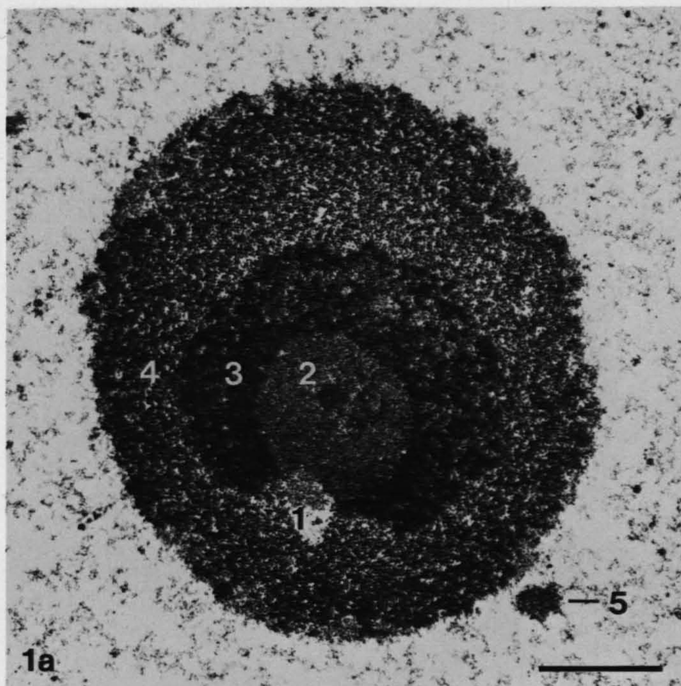
Fixation and sectioning

Oocytes, nuclei isolated in "5:1 medium" with or without $MgCl_2$ added, nuclear contents prepared therefrom and allowed to disperse for 5 min in low salt buffer A (8 mM KCl, 1 mM NaCl, 1 mM Tris-HCl, *p*H 8.8) or, for a total of 30 min, in plain 1 mM Tris-HCl buffer (*p*H 7.6), replacing the latter medium four times by low speed centrifugation in a laboratory centrifuge and subsequent resuspension by swirling, were fixed with 2.5% glutaraldehyde (0.05 M sodium cacodylate, *p*H 7.2), with or without alkali ions and $MgCl_2$ added [10]. After fixation for at least 30 min in the cold, specimens were fixed with osmium tetroxide, soaked with uranyl acetate, washed, dehydrated, embedded and sectioned as described [11, 12]. For embedding of very small specimens dehydrating was often performed using the actone-vapor method [40].

Enzymatic digestion

Enzymatic digestions were performed after spreading of nuclear content and centrifugation on grids. The enzymes used were pancreatic ribonuclease (Serva, Heidelberg; free of DNAse and proteinase) and proteinase K (Merck, Darmstadt, FRG). Enzymes were used at concentrations of 25 or 100 μg/ml and at *p*H 7.2. After centrifugation the grids were washed for 1 min, in 5:1 medium (*p*H 7.2), allowed to float on a drop of a solution of the specific enzyme solution for 10 min (room temperature), and washed again in 5:1 medium and then in the borate buffer described above. After digestion the grids were positively stained with 1% alcoholic PTA and rotary shadow-cast with metal as described.

Fig. 1. Electron micrographs of thin sections through nuclei of Xenopus laevis oocytes after isolation in "5:1 medium" without $MgCl_2$ added and fixed in glutaraldehyde **(a–e)** and through a nucleus isolated in "5:1 medium" containing 10 mM $MgCl_2$, then transferred to 1 mM Tris-HCl (*p*H 8.6) and washed repeatedly by centrifugation and resuspension (**f**; see Materials and methods). Two types of spheroidal bodies are predominant, large typical nucleoli (**a, b**; the numerals in **a** denote the different regions: 1. "Nucleolar vacuolization"; 2. Fibrillar center; 3. Pars fibrosa with nucleolonema organization; 4. Pars granulosa and nucleolar cortex; 5. Peripheral protuberances and perinucleolar medusoid fibril bodies) and the numerous densely stained 0.2 to 1.0 μm large medusoid fibril bodies occurring in the nucleolar periphery **(a, b, d)** as well as scattered throughout the nucleoplasm **(e)**. At higher magnification, the different regions of the medusoid fibril bodies are recognized, in particular the central aggregate and clusters of densely stained 18 to 30 nm granules similar to those present in the nucleolar pars granulosa (**e**; *arrow* in **c**) and the peripheral 8 to 12 nm filament structures that project into the nucleoplasm (some are denoted by *arrowheads* in **c**). The special relationship of such medusoid bodies to the nucleolar (No) periphery is seen in **d,** suggesting a derivation of such bodies from nucleoli. Upon incubation in low salt buffer the nucleolar material is swollen and dispersed but a central aggregate (CA) and small fibrillar bodies (some are denoted by *arrows*) associated with the nucleolar periphery are still recognized. Resulting from the repeated centrifugations the nucleolar residue material is more distorted here than in the low salt buffer preparations described elsewhere [12]. — Bars 1 μm **(a, b, e, f)**, 0.2 μm **(c, d)**. — 15000× **(a)**. — 9000× **(b)**. — 50000× **(c)**. — 40000× **(d)**. — 14000× **(e)**. — 16000× **(f)**.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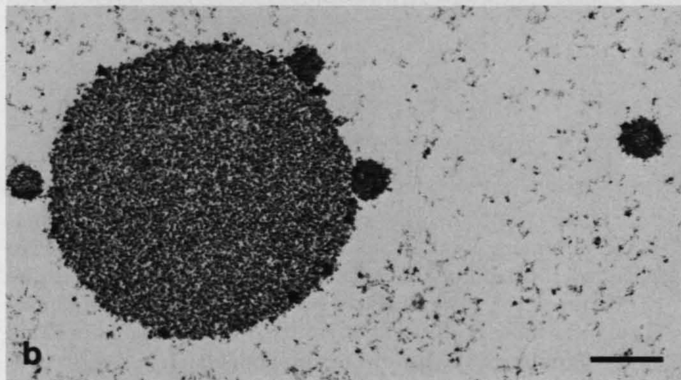
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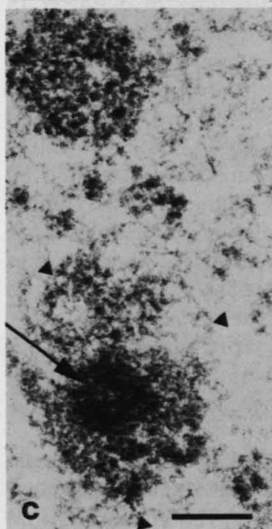
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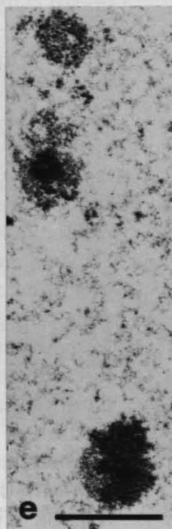
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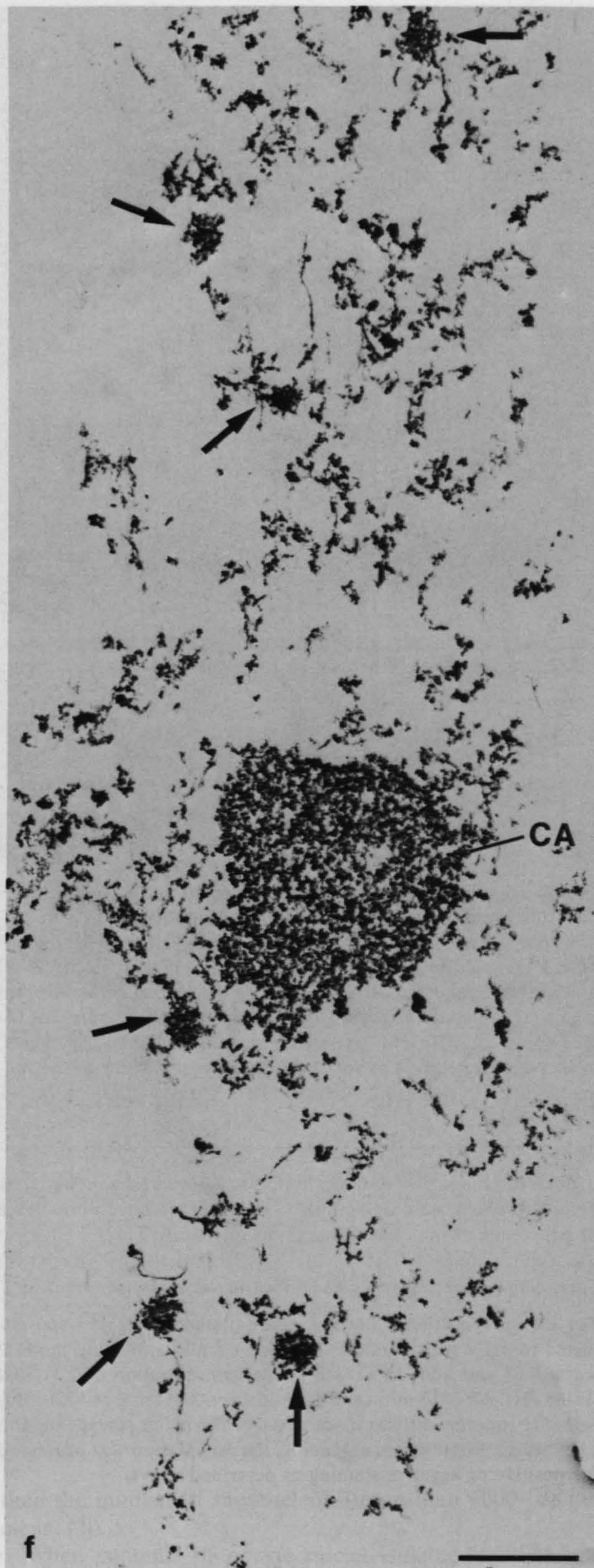
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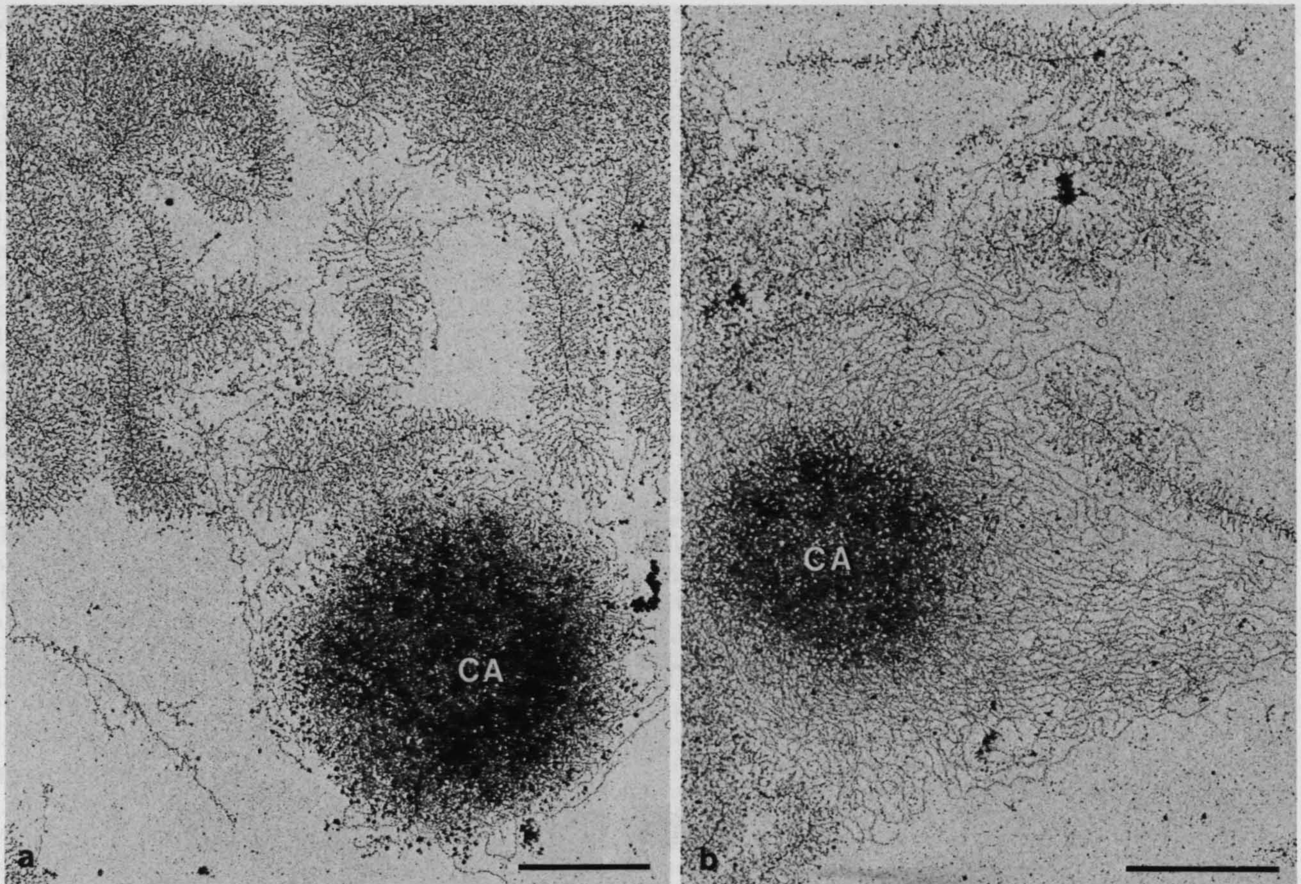


Fig. 2. Spread preparation of nucleolar and perinucleolar material from an mid-vitellogenic oocyte of Xenopus laevis (1-nu mutant) prepared in borate buffer and 0.01% Joy, stained with alcoholic PTA solution, not metal shadow-cast. Typical transcriptional units of nucleolar genes and interspersed spacer chromatin are seen next to distinct roundish aggregates (medusoid bodies) of filaments which are more extended, revealing individual filaments, in **b.** Note association of small (18-30 nm) granules with the filaments of these medusoid bodies. — Bars 1 μm. — 17000 × **(a).** — 19000 × **(b).**

For examination in thin sections, nuclear contents prepared as described above were treated with DNase I, micrococcal nuclease or pancreatic ribonuclease as previously described [12].

Spreading preparations of high salt extracted nuclear contents

For each preparation, 15 nuclei were isolated in 500 ml water adjusted to *p*H 9 with borate buffer. After 5 min spreading in *p*H 9 water KCl was added to reach a final concentration of 2 M KCl (same *p*H). After 10 min centrifugation at 6000 *g* in 2 M KCl solution, the supernatant was discarded and the pellet resuspended in *p*H 9 water. After 30 min spreading, the preparation was processed for positive or negative staining as described above.

Results

Thin sections

When examined in conventionally stained ultrathin sections nuclei of Xenopus oocytes were found to contain a certain type of densely stained spheroidal bodies of diameters ranging from 0.2 to 1.0 μm which appear to be scattered throughout the nucleoplasm, often enriched in the periphery of nucleoli (Fig. 1). The ultrastructural organization of these bodies was complex. Many of them appeared as mosaics of the following components: 1. A region contained densely stained granular structures of diameter 18 to 35 nm reminiscent of the granules characteristic of the outer part ("pars granulosa") of the nucleolus proper (Fig. 1). 2. In many of these bodies another region could be distinguished which was occupied by less intensely stained aggregates of filaments of diameter 8-12 nm. 3. Projecting from the central portion of these bodies we usually noticed similar-sized, extended filaments running in apparently random arrays into the surrounding nucleoplasm. 4. Usually more to the center of these bodies we sometimes identified a heavily and homogeneously stained region in which no granular particles could be discerned. However, we could not decide whether this structure was really different from that described under 1. and did not simply represent a less favourable appearance not allowing the distinction of the individual granules.

In situations in which such bodies occurred in association with the nucleolar cortex (e.g. Figs. 1a, b, d) this close relationship seemed to be mediated by fine filaments, similar to those described above under 2 and 3, connecting the bodies to certain filament aggregates present within the outer cortex of the nucleolus. The total number of such bodies per nucleus apparently was very high, much higher

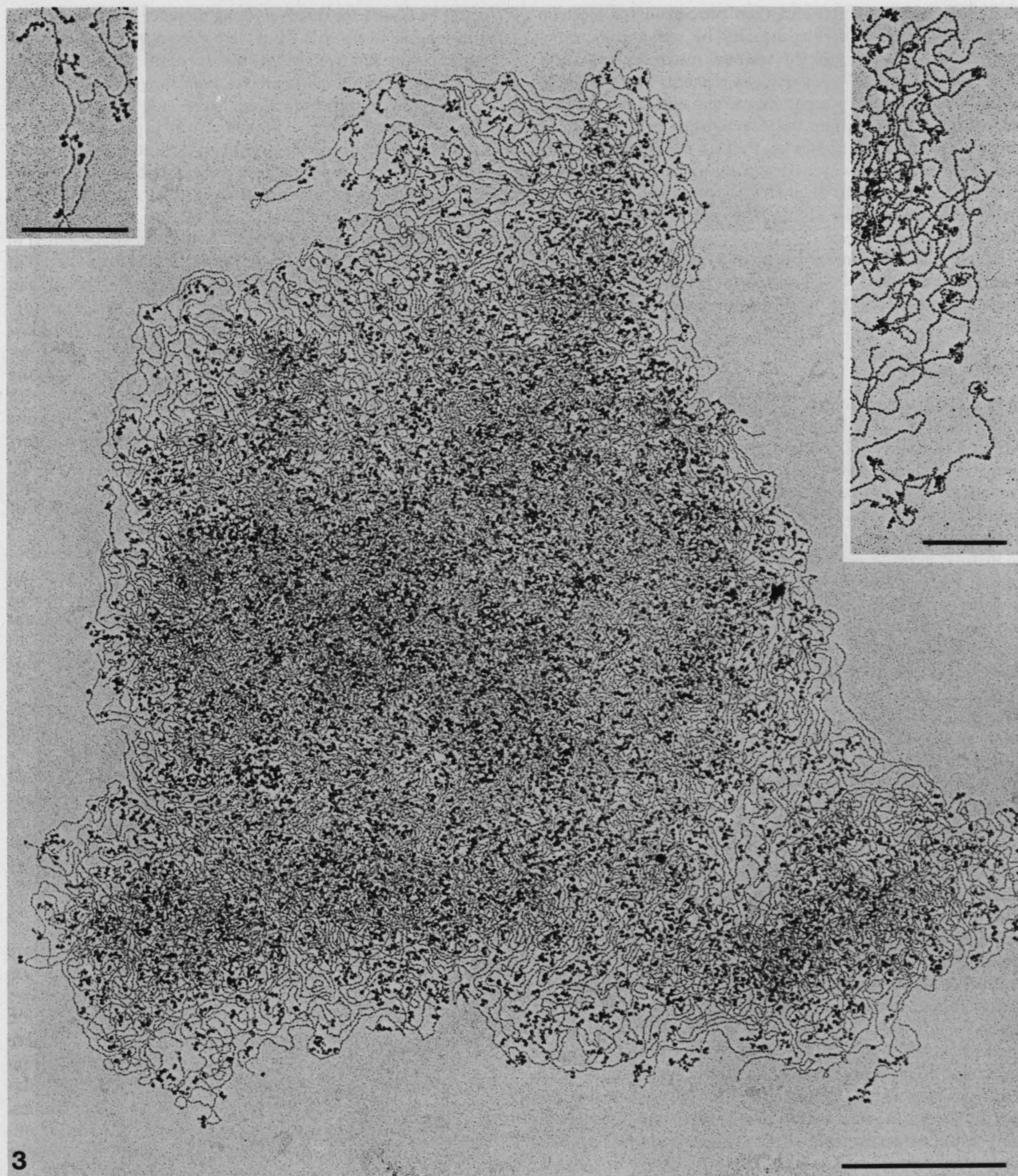


Fig. 3. Spread preparation of medusoid fibrillar body from Xenopus oocyte, positively stained and metal shadow-cast, revealing details of organization of component 8 to 12 nm filaments and 18 to 30 nm granules. Beaded aspect of this type of filament and various arrays of clusters of associated granular particles are seen in the *inserts* (top). — Bars 1 μm and 0.5 μm *(inserts)*. — 27 000 × *(left insert:* 35 000 × ; *right insert* 28 000 ×).

than the number of the nucleoli (more than 1000 per nucleus; [2]).

When contents of oocyte nuclei isolated in "5:1 medium" in the presence of 5 or 10 mM $MgCl_2$ were subsequently incubated in low salt buffers such as the 1 mM Tris-HCl (*p*H 7.6) buffer or the *p*H 9.0 sodium borate buffer used for spreading preparations described above (see

Materials and methods) and then processed for electron microscopy such bodies could still be recognized, especially in the periphery of the residual nucleolar structures (Fig. 1f). However, when nuclear contents were isolated without additions of stabilizing cations and directly incubated in low salt buffers the filamentous components of these small fibrillar bodies as well as the nucleolar core material were more widely dispersed and less easily identified, indicating that divalent cations contribute to the structural stability of both nucleoli (see also [12]) and medusoid fibrillar bodies.

When nuclear contents prepared as described above were treated with DNase I and/or pancreatic ribonuclease these characteristic fibrillar bodies were still identified (not shown).

Spread preparations

When nuclear contents of Xenopus oocytes were spread and prepared according to the technique developed by Miller and Beatty [21–23] and positively stained many aggregates of transcribed nucleolar genes were seen (Fig. 2a; for detailed quantitative analysis of Xenopus nucleolar genes prepared in this way, see [32, 33, 36]). Frequently, such aggregates of nucleolar genes were found lying next to distinct more or less roundish units composed of masses of a distinct type of filament emerging and projecting from a densely stained central aggregate of a interwoven filamentous web ("dense core"; Fig. 2b). In such images of filament aggregates bodies which seemingly represented the flattened out material of the fibrillar bodies more or less spheroidal in suspension and in situ, the degree of filament extension varied greatly from preparation to another and even among different nucleolar clumps on the same grid (Figs. 2a, b). The diameter of the dense core of these bodies was, in moderately spread preparations as shown in Figure 2, in the range from 1.3 to 3.0 µm. The filaments extending and projecting from the central dense core of these bodies were rather uniform in morphology and showed an overall diameter of 8 to 12 nm. They were associated, to a highly variable degree, with densely stained distinct granules of 18–30 nm diameter (e.g. Fig. 2b). Because of the resemblance of this organization to the morphology of medusae this type of nuclear body will be referred to in the following as "medusoid fibril body". A pronounced differently stained dense core was not apparent in all of the spread medusoid fibril bodies (e.g. Fig. 3). Such bodies then presented a particularly clear image of the two major structural elements of these bodies, that means the 8 to 12 nm filaments which, at higher resolution, revealed a distinctly beaded pattern (mean center to center distance 12 nm), and the densely stained 18 to 30 nm granular particles associated with these filaments (Fig. 3). These associated granules occurred both as individual granules and as serial or bunch of grapes-like arrays of a varying number of granules (inserts in Fig. 3).

The medusoid fibrillar bodies could also be demonstrated in negatively stained spread preparations (survey micrograph in Fig. 4) and here the substructure of the filaments projecting from the central core aggregate was revealed in greater detail (Fig. 5). The individual filaments could be traced for relatively long distances: Filament contour lengths of up to 2.4 µm were determined and, considering the preparative stresses involved, the original lengths of complete filaments might be even longer. Unequivocal cases of filament branching were not observed. The beaded structure of the filaments was especially clear at high magnifications of negatively stained preparations (Fig. 5b). The pictures suggested the existence of a continual filament core thread of homogeneous diameter ca. 4 nm with which particle structures of 8 to 12 nm diameter were intimately associated. Situations as those presented in the insert of Figure 5a and in Figure 5b also suggested that such individual "beads" could detach from the core thread and found free on the supporting grid (e.g. particles denoted by arrowheads in Fig. 5b). Corresponding variously-sized filament intercepts of thin and homogeneous diameter 3 to 5 nm were seen next to filament regions showing the typical "beads-on-a-string" aspect, sometimes with larger distances (10–23 nm). In addition, these filaments were associated with the much larger particles of diameters 18 to 30 nm, which occurred either individually or in clusters (Figs. 4, 5) and exhibited a spheroidal, sometimes polygonal-shaped morphology similar to that described for negatively stained preparations of preribosomal particles from various cell types ([19, 39, 41]; compare also the ribonuclease-resistant core particles described by Johnson and Kumar, [14]). In certain profile images, the attachment of these larger particles appeared to be mediated by a slender stalk (e.g. insert in Fig. 4).

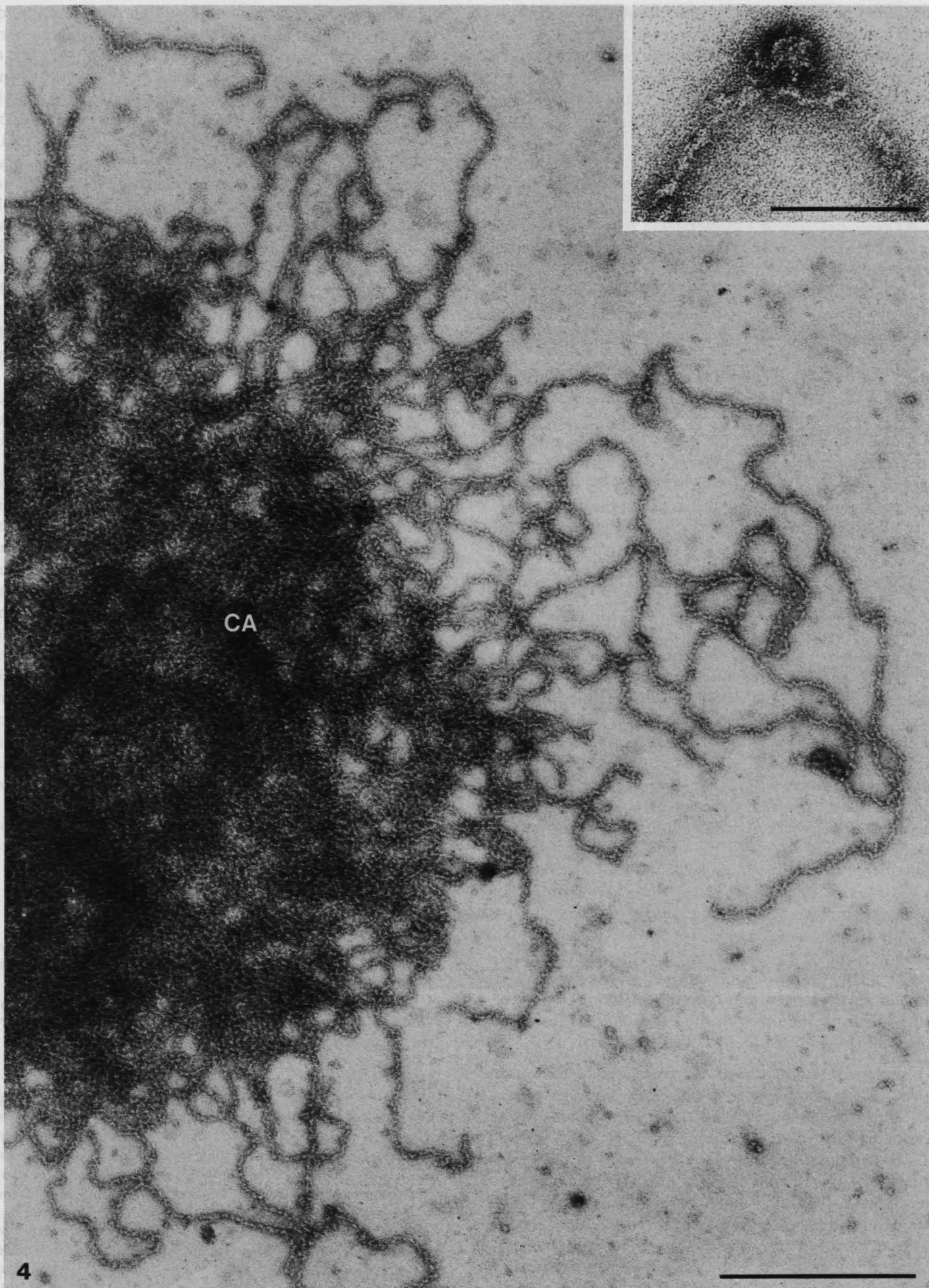
After treatment with ribonuclease and DNase the characteristic 8 to 12 nm filaments were still recognized but they were not observed after prolonged treatment of the spread material on the grid with proteinase K (data not shown).

After extraction in buffer solutions containing 2 M KCl and spreading of the nuclear content the fibrils in the outer part of the medusoid bodies were partly disassembled, revealing relatively extended regions of core thread filaments of 3 to 5 nm (not shown).

Discussion

The medusoid fibril bodies described in this study are clearly different from other types of nuclear bodies described so far [1, 3, 7]. In particular the characteristic major ultrastructural component present in the medusoid bodies, the beaded 8 to 12 nm filaments projecting from the dense central aggregate, allows to distinguish this structure from other nuclear and cytoplasmic filaments. Since similar filament arrays, often also arranged in medusoid fibril bodies, have also been observed in oocytes from various other amphibia, in diverse somatic animal cells and in vegetative nuclei of the green alga, Acetabularia mediterranea (W. W. Franke, S. Moreno, U. Scheer, M. F. Trendelenburg, G.

Fig. 4. Survey electron micrograph showing one half of a spread medusoid fibril body negatively stained with sodium phosphotungstate. Note the many filaments which project from the dense central aggregate (CA). Higher magnification of an associated particle is shown in the insert. — Bars 0.5 µm and 0.1 µm *(insert)*. — 68000 × *(insert:* 240000 ×).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Krohne and H. Spring, unpublished results), such periodically organized nuclear filaments seem to be more widespread and may well be general components of the eukaryotic nucleus. We think that the medusoid fibril bodies described here are probably identical to the dense "differentiated regions" mentioned from low power electron micrographs of partially spread nucleolar cortices from oocytes of Xenopus laevis (Fig. 11 in [21]). They might also correspond to the nuclear bodies described by light microscopy and electron microscopy of sections as "electron-dense aggregates", "small aggregates", "large granular aggregates", "micronucleoli", "nucleolus-like bodies" in oocytes of amphibia as well as in various other cell types [5, 24, 27, 28, 34, 35, 42]. However, the characteristic and diagnostic feature of the medusoid bodies described here, that means the periodic "beads-on-a-string" organization of the 8 to 12 nm filaments, has not been recognized in these studies and, therefore, a direct correlation cannot be made. We can also not decide whether the medusoid fibril bodies described in this study are related to the much larger (several μm in diameter) granular and fibrillar dense bodies described to be associated with nucleoli from oocytes of hibernating leopard frogs, Rana pipiens [43]. The medusoid fibril bodies and their characteristic "beaded" 8 to 12 nm filaments are frequently seen in association with the nucleolar periphery but they are not restricted to the nucleolus. These filaments may be identical to the masses of granule-bearing filaments occasionally reported in spread preparations of nucleolar cortex material (e.g. Fig. 4.14b in [11]). Our observations made in negative stained preparations and also in high salt treated nuclear contents suggest that these 8 to 12 nm filaments are composed of two subunit elements, a 3 to 5 nm core thread filament and periodically attached about 10 nm large spheroidal particles. The core thread filament may correspond to the thin filaments resistent to extractions in high salt buffers which have been found in association with isolated nucleoli [12]. It is obvious that direct comparison of the filaments in the two structures, the karyoskeletal filaments observed in residual nucleolar fractions ([6, 12]; for filamentous components isolated from nucleoli without extraction in high salt buffers see [8]) and the medusoid fibril bodies (this study) has to wait for biochemical and immunological data, using also antibodies to the major polypeptide of M_r 145000 found in karyoskeletal fractions from nucleoli [12, 17]. Moreover, it will have to be examined whether these filaments, in particular the thin core thread filaments, are related to some of the other fibrillar components described in nucleoli fixed in situ (e.g. [4, 13, 25, 29, 31]).

The structural similarity of the periodical organization of the nuclear filaments described here with the uromucoid filaments precipitated by dialysis of human urine against solutions of elevated ionic strength (e.g. 0.5–1.0 M NaCl) is striking [8]; however, the absence of detectable amounts of glycoproteins in the contents of the oocyte nucleus (J. Stadler and G. Krohne, not shown) as well as the mode of preparation at very low salt clearly demonstrate that this nuclear protein structure is unrelated to uromucoid.

As we have shown here the filaments of the medusoid bodies can be associated with 18–30 nm granules densely stained and similar in size to the preribosomal granules described in situ [29]. The structures described are not altered after treatment with DNAse and Thiébaud [42] has reported that the light microscopically identified "micronucleoli" present in Xenopus oocyte nuclei do not stain with sensitive DNA stains, in contrast to "true" nucleoli. Our observation that treatment with ribonuclease results in disorganization and disappearance of the 18 to 30 nm granules but leaves the 8 to 12 nm filaments practically unaltered further indicates that both components of these filaments the 3 to 5 nm core thread and the 10 nm beads are not structurally maintained by RNA (see also the nucleolar "filaments" described in [29]). From these observations we suggest that the filaments are predominantly, if not exclusively proteinaceous elements. Clark and Rosenbaum [5] have discussed a possible relationship of certain "granular aggregates", which morphologically are similar to the medusoid fibril bodies described here, to the breakdown of actin filaments in the nucleus. Analytical biochemical work and immunological experiments are currently performed in our laboratory to characterize the major proteins present in this structure.

The function of the medusoid fibril bodies and the constituent 8 to 12 nm filaments is not known. The similarity of the associated 18 to 30 nm particles with preribosomal granules of the nucleolar cortex as well as the demonstration that uridine incorporation appears in RNA in these bodies with similar kinetics as in the nucleolar pars granulosa [34, 35] may suggest that 1. the medusoid bodies are subunits of the nucleolar cortex or at least closely related to nucleolar cortex material, 2. the free nucleoplasmic medusoid bodies are derived from nucleolar cortex material [5], and 3. the 8 to 12 nm filament structures described provide structural support for the attachment of preribosomal particles and thus may be involved in the storage and intranuclear translocation of ribosomal precursor material. Experiments examining this possible function are underway.

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Fig. 5. Higher magnification of negatively stained spread preparations of the peripheral portion of a medusoid fibril body (same preparation as in Fig. 4), revealing the periodic (beaded) ultrastructure of these filaments. In some regions these filaments show a thin (3–5 nm) core filament (denoted by *arrows* in the *insert*) and are accompanied by "free" spheroidal 8 to 12 nm particles (some are denoted by *arrowheads* in the *insert* of **a** and in **b**). *White arrows* in **a** and the *black arrow* in **b** denote 18 to 30 nm large particles which structurally resemble preribosomal particles. The central aggregates of the medusoid bodies are oriented to the left margins. — Bars 0.1 μm. — 160000 × (**a** and *insert*). — 150000 × **(b).**

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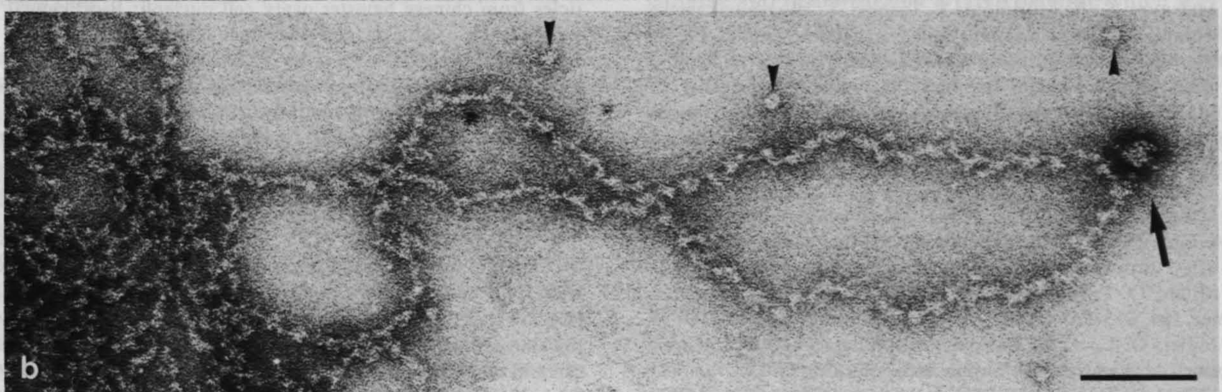

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