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Lampbrush-Type Chromosomes in the Primary Nucleus of the Green Alga *Acetabularia mediterranea*

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Abstract. Structures with a lampbrush-chromosome-like morphology are described in the nucleoplasm of primary nuclei of the green alga, *Acetabularia mediterranea*, by light and electron microscopy in sections of cells fixed *in situ* and in spread preparations of isolated nuclear components. These chromosomes reveal typical loops (up to 20 μm long), chromomere-like nodules (1–2 μm in diameter), and 2–4 μm large axial globules. Associations of some of these chromosomes with nucleolar structures and with the nuclear envelope are also recognized. The light microscopically identified loops are correlated with distinct fibrillogranular structures observed in the thin sections and with the very long matrix units seen in the spread preparations. The similarity of these structures to the lampbrush chromosomes of various animal cell types, all exclusively stages of meiotic prophase, is discussed as well as the possible relation of the appearance of lampbrush chromosomes to a defined phase of the vegetative growth of this alga.

Introduction

Typical lampbrush chromosomes with defined chromosomal axes, chromomeres, and thin lateral loop extensions so far have been convincingly demonstrated only in specific meiotic stages of animal cells, in particular in the diplotene of oocytes of some fishes, mollusks, insects, and amphibia (Flemming, 1882; Rückert, 1892; Gall, 1952, 1954, 1966; Callan, 1957, 1963; Callan and Lloyd, 1960; Gall and Callan, 1962; Kunz, 1967; Ribbert and Kunz, 1969; Ahmad, 1970; Baumeister, 1973; Bottke, 1973; for further references see Müller, 1974), and in the primary spermatocytes of several species of *Drosophila* (Meyer, 1963; Hess, 1966, 1968, 1973). In addition, there have been claims in the literature of the occurrence of lampbrush-like structures in the diplotene stages of a plant, the onion *Allium cernuum* (Grun, 1958), and a fungus, the basidiomycete *Coprinus* (Lu and Raju, 1970); however, these two reports did not reveal the clear structural criteria of lampbrush chromosomes as mentioned above. (Some authors have also described the appearance of special "puffed" regions in the polytene chromosomes of the suspensor cells of some *Phaseolus* species as a "lampbrush state", *cf.* Avanzi *et al.*,

1970; Nagl, 1970, 1973, 1974). In the course of our studies on the primary (giant) nucleus of the Dasycladacean green alga *Acetabularia*, we noted the regular occurrence of distinct chromosomal structures with a "lampbrush-like" morphology (Spring *et al.*, 1974). Since our present knowledge of the chromosomes in *Acetabularia*, a "classic" reference cell system in genetical and cytological studies (Hämmerling, 1953, 1963; Schweiger, 1969; Brachet and Bonotto, 1970; Werz, 1974), is still very poor (see, *e.g.*, the divergent reports of Schulze, 1939, and Puiseux-Dao, 1966, compare also the discussion in Berger *et al.*, 1974), in particular what concerns the primary nucleus (note the statement in a recent review on *Acetabularia* by Werz, 1974: "Chromosomes or DNA strands have not been localized definitively within the adult primary nucleus") we have investigated these structures in detail.

Materials and Methods

Acetabularia mediterranea cells were cultivated as described by various authors (Hämmerling, 1944; Beth, 1953; Lateur, 1963; Keck, 1964; Lateur and Bonotto, 1973). Some experiments were performed with algae kindly provided by Dr. J. Brachet (Université Libre de Bruxelles, Belgium).

a) Light Microscopy. Algae with stalk lengths from 10 to 40 mm, *i.e.*, before the stage of cap formation, were fixed for two hours in 5% glutaraldehyde (Serva, Heidelberg, Germany) made up in 0.1 M sodium cacodylate buffer (pH 7.2) at 5° C or at room temperature (see also Franke *et al.*, 1974; Spring *et al.*, 1974). After rinsing in cold (ca. 5° C) cacodylate buffer the cells were dehydrated by transfer through a series of graded ethanol solutions and embedded in Epon 812 (Serva, Heidelberg, Germany) according to the conventional techniques. 1–3 μm thick sections through the rhizoid containing the nucleus were prepared using a Reichert Om U 3 ultramicrotome using glass knives. The sections were observed in a Zeiss photomicroscope III either in phase contrast or after staining. For staining of the lampbrush chromosomes we used the hematoxylin chromosome stain according to Melander and Wingstrand (1953) which gave, in our hands, the best staining results of the very faint chromosomal structures, whereas staining with toluidine blue, methylene blue, and other basic dyes resulted only in poor colorization. Before staining, the sections had to be de-eponized by the method described by Mayor *et al.* (1961; compare also Spring *et al.*, 1974). Hydrolysis when performed was carried out in 1N HCl at 60° C, the most specific staining for chromosomal structures was found after a hydrolysis time of 8 to 14 minutes.

After manual isolation of the nuclei, the chromosomes were isolated and spread as described for the preparation of lampbrush chromosomes from amphibian oocytes by Gall (1966; for some details of preparation see also Spring *et al.*, 1974). In some experiments, chromosomes were also isolated using the isolation medium of Müller (1974). Both methods gave essentially the same results. Isolated chromosomes spread in such a way were observed either directly in phase contrast optics or, after staining on the slide (see above) and mounting, in bright field.

b) Electron Microscopy. Fixation and embedding procedures for electron microscopy were as previously described (Franke *et al.*, 1971, 1974). Spread and positively stained preparations of total nuclear contents and of nucleolus-free nuclear material (*i.e.*, supernatants obtained after sedimentation at 200 g for 1 minute or for

3–5 minutes at gravity) were performed according to our modification of the technique by Miller and his associates (Miller and Beatty, 1969; Miller and Bakken, 1972, 1973; see also Scheer *et al.*, 1973; Trendelenburg *et al.*, 1974; Spring *et al.*, 1974; Berger and Schweiger, 1975). Specimen grids were observed in electron microscopes Zeiss EM 10 or Siemens 101.

Results and Discussion

Light microscopic and electron microscopic studies of the primary nucleus of *Acetabularia* had hitherto revealed nucleoli, some nucleolus-associated bodies and the about 30 to 40 nm large granules that are usually accumulated in the nuclear periphery (for detailed descriptions see Franke *et al.*, 1974; Spring *et al.*, 1974; Berger *et al.*, 1975) as the only consistent morphologically defined components of the nuclear interior (see also Werz, 1974). However, at closer inspection of 1 to 3 μm thick sections with phase contrast optics we consistently noted faint, longitudinal nucleoplasmic structures (Figs. 1a and 2) that closely resembled the organisation of the lampbrush chromosomes known from meiotic stages in animal cells. These lampbrush-chromosome-like structures appeared in special clarity after removal of the embedding epoxy resin and staining with chromic hematoxylin (Figs. 1b and 4). Serial sections from such primary nuclei illustrated both the high number and the morphological variations of such chromosome-like elements (Figs. 3 and 4). Differences were in particular notable with respect to chromosomal size (length), opacity and stainability, respectively, and in their degree of lateral loop dispersion. Some of the chromosome-like structures showed close proximity to, perhaps representing association with, the nuclear envelope, the nucleolar components and sometimes to a specific paranucleolar body that occurred in some nuclei (Figs. 3 and 4; see also Fig. 9).

When regions of ultrathin sections that corresponded to the localization of lampbrush chromosome structures in adjacent 1–3 μm thick sections were examined in the electron microscope, distinct, though not conspicuous aggregations of granulofibrillar structures were noted. One could distinguish large (up to 0.8 μm in diameter), densely stained and densely packed clumps, some of which seemed to be spherical in shape (Fig. 5), that are associated, and in some regions seemingly in fibrillar connection, with more cylindrical (sausage-shaped) granulofibrillar formations. The size of the granules contained in these aggregate structures was between 20 and 25 nm (Figs. 5a and 5b), and the whole structural arrangement resembles the organisation of the granulofibrillar structures in the chromosome loops of the oocytes of *Triturus cristatus carnifex* as recently described by Malcolm and Sommerville (1974).

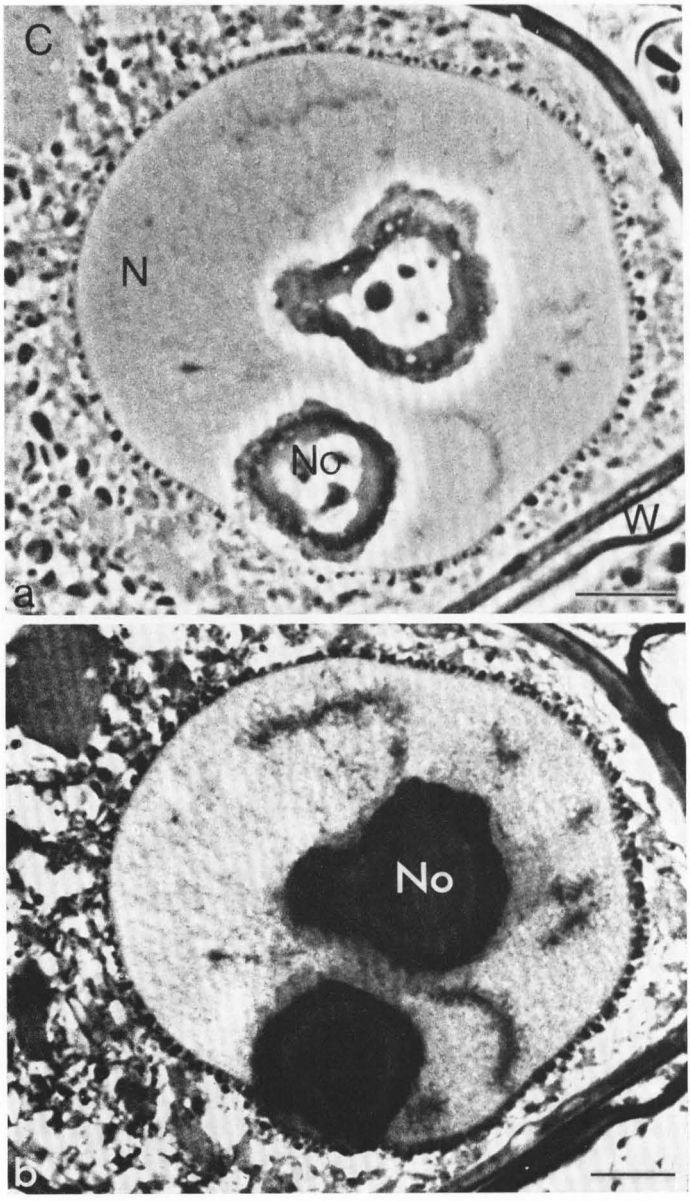

Fig. 1 a and b[1]. Light micrographs of the same 2 μm thick section through an *Acetabularia* primary nucleus fixed in situ as revealed in phase contrast optics (a) or after staining with hematoxylin after differential hydrolysis (b). Within the nucleus (*N*) one recognizes the nucleolar aggregates (*No*) and several lampbrush-type chromosomes with faint lateral loops. Note the many perinuclear dense bodies in the juxtanuclear zone. *C*, cytoplasm; *W*, cell wall

1 The scales represent 10 μm if not otherwise stated.

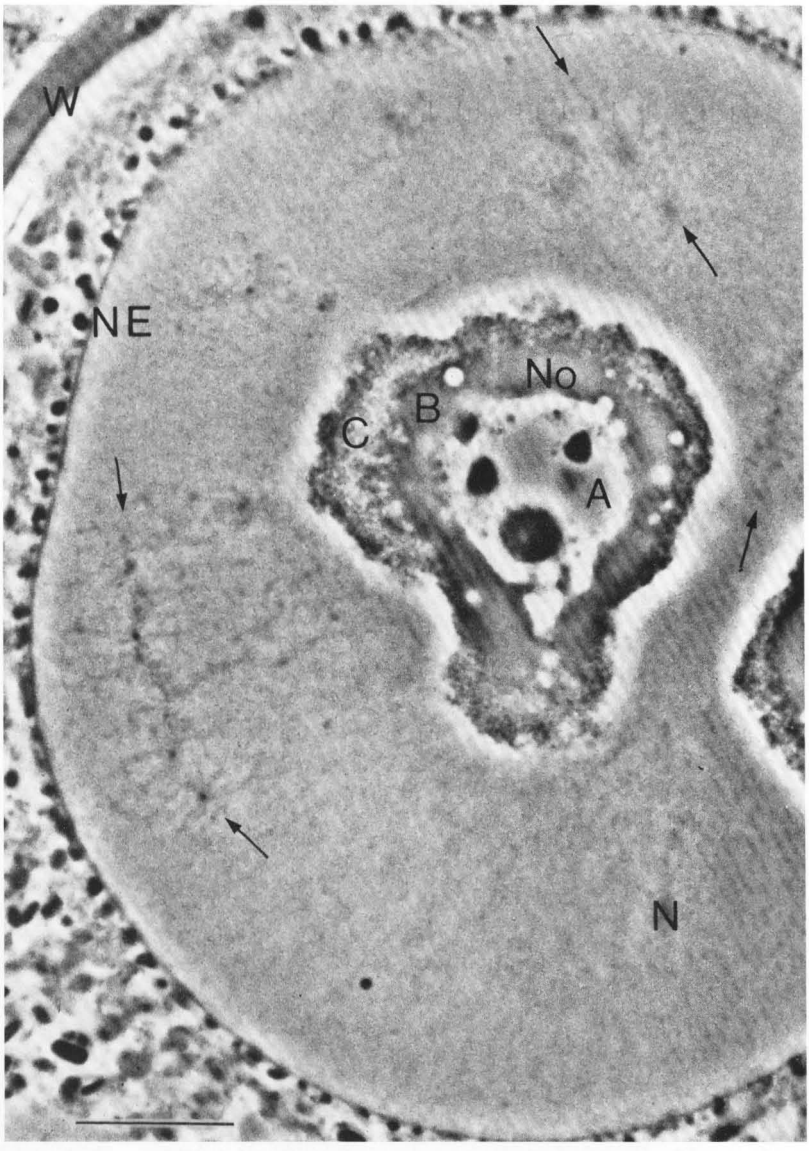

Fig. 2. Intranuclear structures of the *Acetabularia* primary nucleus shown at higher magnification (partial magnification from the section shown in Fig. 1). Within the nucleolus (*No*) one distinguishes the three different structural zones (*A*, *B*, *C*; for details see Spring *et al.*, 1974, and Berger *et al.*, 1975). The arrows denote some of the almost axially sectioned lampbrush-type chromosomes with their chromomeric knobs and the fine, extended lateral loops. *NE*, nuclear envelope; *W*, cell wall

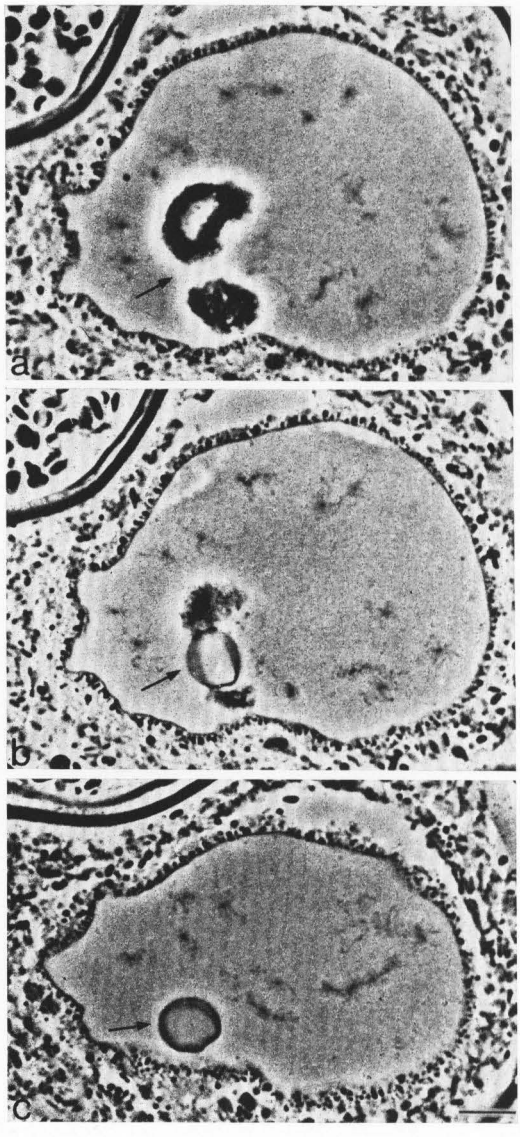

Fig. 3a—c. Phase contrast light micrographs of subsequent 2.5 μm thick serial sections through a primary nucleus of *Acetabularia mediterranea*. The corresponding position of the distinct paranucleolar body, which might serve as a marker structure, is indicated by the arrows. Note the frequency and the variation of the lampbrush-like chromosomal structures

Fig. 4a—c. A series of 2 μm thick sections as revealed after staining with hematoxylin according to the procedure described in Materials and Methods. Note again the frequency of the lampbrush chromosomes and sites suggestive of occasional associations of lampbrush-type chromosomes with some of the nucleolar aggregates

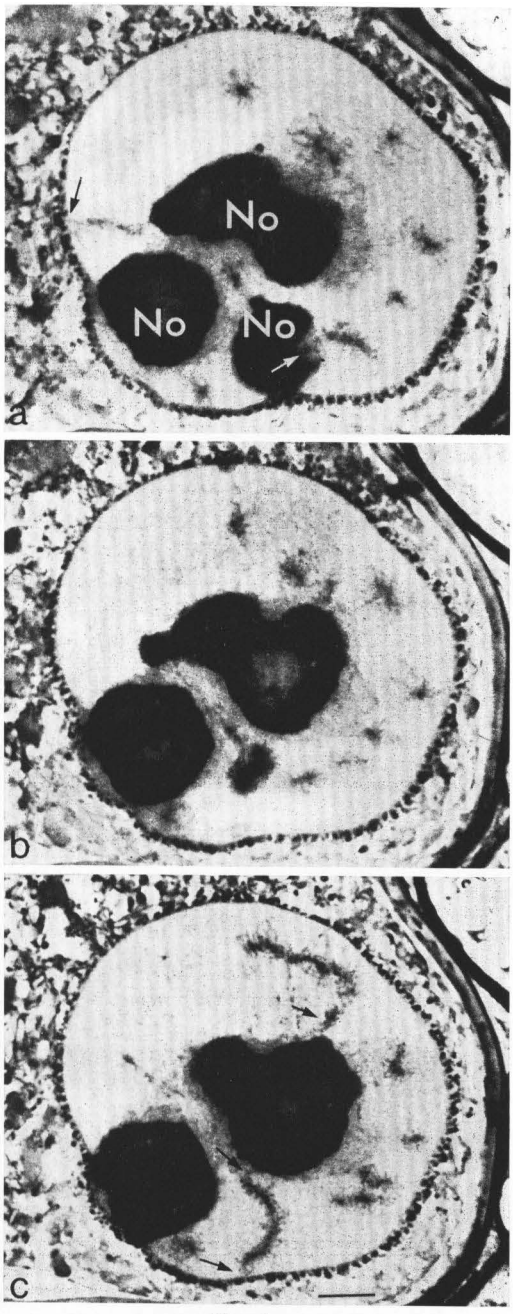

Fig. 4a—c

[*No*; *e.g.* denoted by the white arrow in (a) and the upper two arrows in (c)] and with the nuclear envelope (arrows in a and c). These micrographs have been printed with unusually high contrast in order to demonstrate the chromosomal structures, at the expense of the visibility of nucleolar details

When the contents of the primary nuclei of *Acetabularia mediterranea* were prepared in exactly the same way as Gall and Callan and others have used in the classic demonstrations of lampbrush chromosomes in animal oocytes (Gall, 1952, 1954, 1966; Callan, 1966; for further references see Müller, 1974) we found numerous small chromosomal elements many of which exhibited a lampbrush-like morphology (Figs. 6–8). The total number of chromosomes per primary nucleus has not yet been exactly determined, some uncertainty being due to possible chromosomal fragmentation during the preparation, but seems to approach, or even exceed, the figure of 20 chromosomes reported by Schulze (1939) for the cyst nuclei. (It is still not clear whether the cyst nuclei contain the haploid or the diploid genome; *cf.* Puiseux-Dao, 1966; Green, 1973; Berger *et al.*, 1975). The longest chromosomal elements measured were about 30 μm, the shortest 4 μm. Thus, they are much smaller than the typical lampbrush chromosomes of, for example, the amphibian oocytes. On the chromosomal axes one could frequently recognize distinct (about 0.7 to 1.2 μm large) nodules, sometimes with regular spacings, which appear to be equivalent to chromomeres (Figs. 7a, 7c, 8). In addition, some of the chromosomes showed larger globules, about 2 to 4.5 μm in diameter (*e.g.* Figs. 7a, 8), sometimes in telomeric or subtelomeric positions, which resemble the similar structures described in association with lampbrush chromosomes of amphibian, insect and mollusk oocytes (the "spheres", the "axial granules", or the "suspended granules" *sensu* Callan, 1966; see also Ragghianti *et al.*, 1972; compare also the "sphere-like globules" and the "telomeres" of Müller, 1974; the "lichtbrechende Kugeln" of Bottke, 1973; and the "α- und β-Kugeln" described by Kunz, 1967), some plant meiotic chromosomes (*e.g.* McClintock, 1934; Bianchi and Vetturini, 1969), but could also well represent chromosome-bound nucleoli (compare Macgregor and Kezer, 1973; Kezer and Macgregor, 1973). Distinct lateral loops were frequently recognized (Figs. 6–8) some of which could be traced to total loop lengths of 15 to 20 μm. Again one has to note that

Fig. 5a and b. Electron micrographs of ultrathin sections through regions of the primary nucleus of *Acetabularia mediterranea* which were known from adjacent 1–3 μm thick sections to contain lampbrush-type chromosomal structures. At the position of such lampbrush-chromosomes one identifies aggregates consisting of large, heavily stained clumps (some are denoted by the thick arrows) which probably represent chromomeres, very loosely packed fibrillogranular threads, and more cylindrical ("sausage-shaped"), deeply stained formations (some are indicated by the thin arrows and the arrowheads) with distinct peripheral granules about 22 nm in diameter that might correspond to coiled configurations of (and at) the lateral fibrils of the "matrix units" observed in spread preparations of nuclear components (see also Fig. 10). *No*, nucleolar periphery. The scales indicate 1 μm

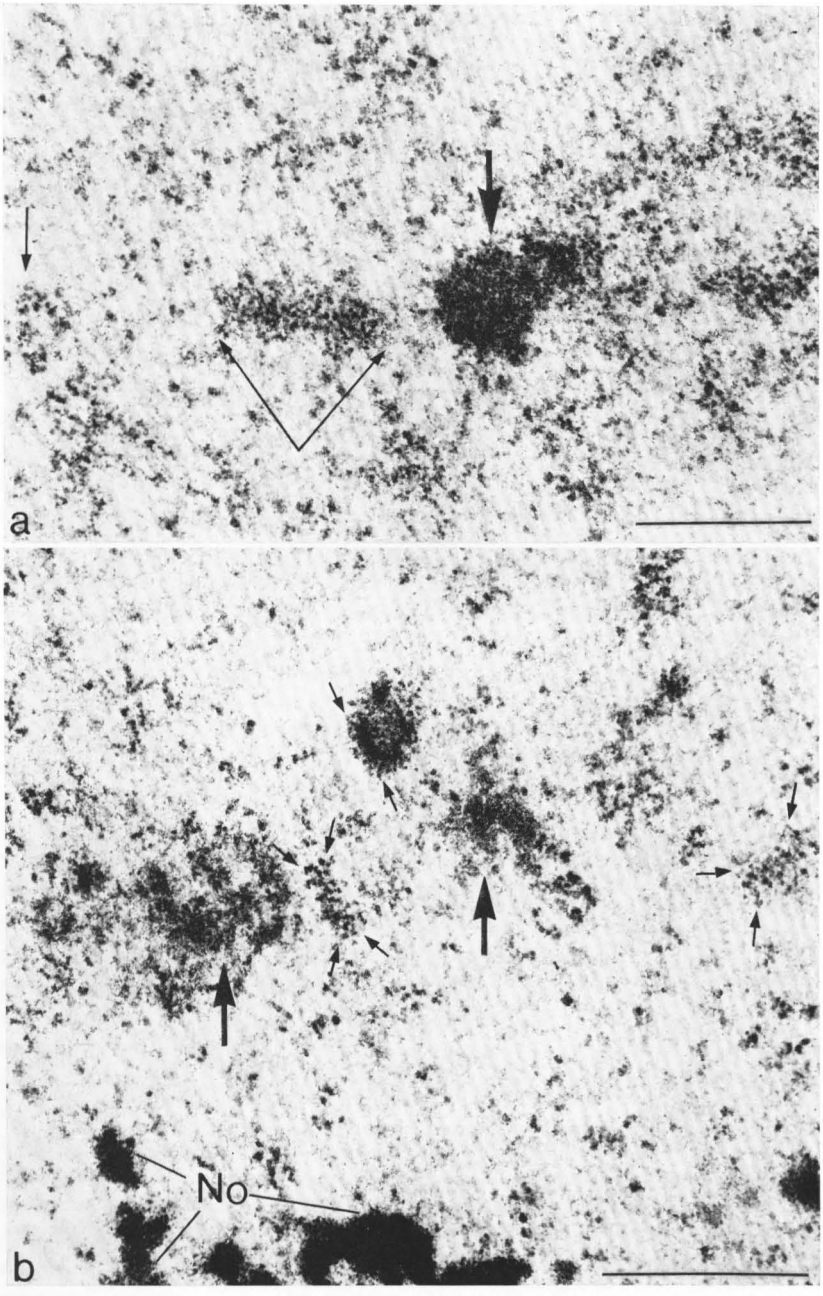

Fig. 5a and b

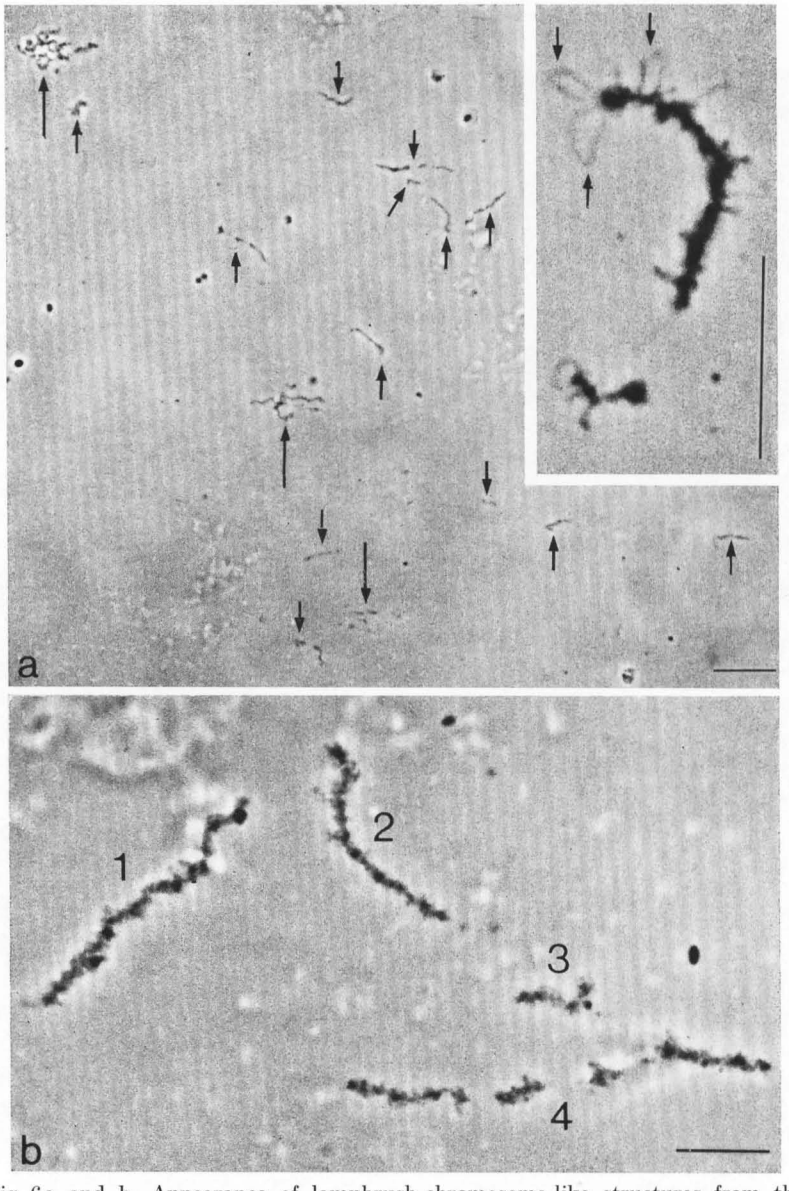

Fig. 6a and b. Appearance of lampbrush-chromosome-like structures from the *Acetabularia* primary nucleus in a chromosome spread preparation as revealed in phase contrast light optics. Fig. 6a presents a survey of such a preparation showing the frequency of these chromosomal structures which occur either single (arrowheads) or in associations of two and more (e.g. at the longer arrows). Fig. b shows a partial magnification of this preparation. Typical chromosome loops are demonstrated in the insert in Fig. a. The lampbrush-chromosomes shown in Fig. b (tentatively labelled *1* to *4*) appear to be in a relatively contracted state. The scale in the insert indicates 5 μm

these loops, although similar in morphology, are generally smaller than those of the chromosomes in, *e.g.*, the amphibian oocytes. They are, however, comparable in size to those described, for example, in the oocytes of the grasshopper, *Decticus* (Kunz, 1967), and of the snail, *Bithynia* (Bottke, 1973). Some chromosomes showed a high density of loop formation (*e.g.* Figs. 7 b and c) whereas others revealed only a fuzzy chromosomal surface and resembled more the morphology described for chromosomes relatively inactive in transcription such as in later stages of oogenesis (Born, 1892; Duryee, 1950; Gall, 1954; Wischnitzer, 1967; Ragghianti *et al.*, 1972; Scheer, 1972). We are as yet unable to clearly correlate loop formation with specific stages of nuclear growth or with transcriptional activities.

Although group formations of the spread isolated chromosomes were not uncommon (*e.g.* Figs. 6–8), including associations of two chromosomes of similar length, typical bivalent formations have not yet been identified. We also repeatedly noted, in squash preparations of whole nuclear contents as well as with isolated nucleolar components, intimate associations of such lampbrush chromosome-like elements, or fragments thereof, with some of the nucleoli (Fig. 9) which perhaps reflects the close associations of lampbrush-like chromosomes with the nucleolar aggregates noted *in situ* (Figs. 1–4).

Miller and his associates (Miller and Beatty, 1969; Miller and Hamkalo, 1972; Hamkalo and Miller, 1973; Miller and Bakken, 1973) have demonstrated, in amphibian oocyte nuclei, the electron microscopical appearance of spread and positively stained chromosomal structures which they interpreted as images of the transcriptional units contained in lampbrush chromosome loops. These are characterized by very long matrix units, correspondingly very long lateral fibrils in the terminal intercepts of these matrix units, a high package density of the points of attachment of the lateral fibrils to the deoxyribonucleoprotein axis, *i.e.*, of the putative RNA-polymerase B containing transcriptional complexes. In the primary nuclei of *Acetabularia mediterranea* and *A. major*, we noted the occurrence of similar matrix units in spread preparations of whole nuclear contents (see also Spring *et al.*, 1974) as well as of suspensions of virtually nucleolus-free nuclear material (Fig. 10). The matrix units observed were usually very long (5–14 μm) and also showed a very close spacing of the associated lateral fibrils. The largest lateral fibrils encountered in such matrix units were about 3 μm. Miller and associates described lateral fibrils longer than 10 μm in lampbrush chromosome loops of *Triturus*. Interestingly, clear spacer intercepts adjacent to such matrix units were hardly detected. These structures seem to correspond to the cistron classes D and E according to the classification given in our previous article (Spring *et al.*, 1974).

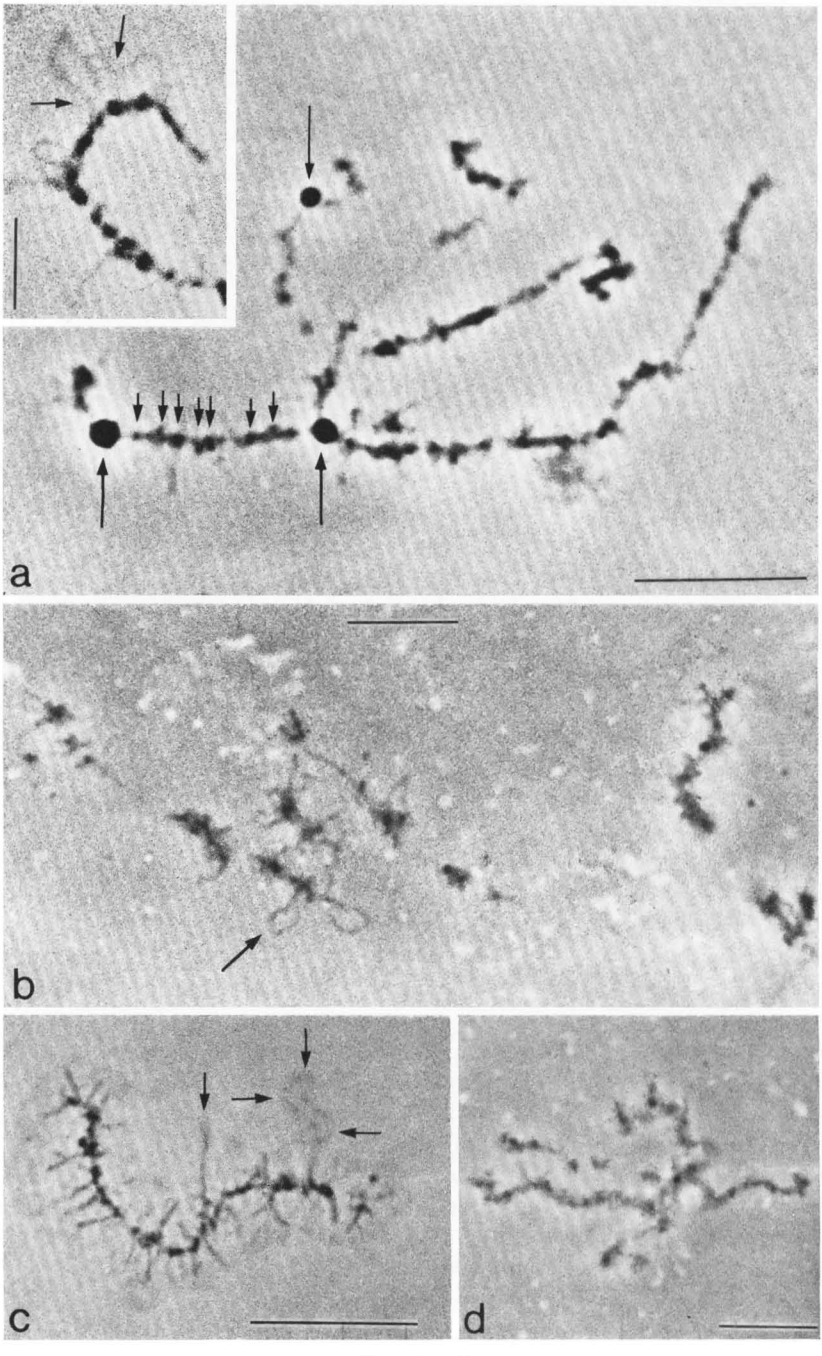

Fig. 7a—d

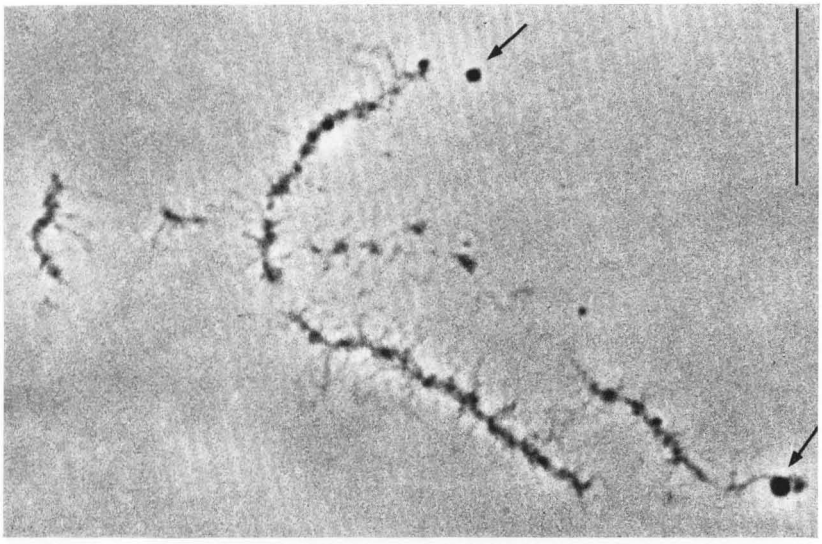

Fig. 8. One of the frequent "pair-associations" of lampbrush-type chromosomes each of which here reveals a (pre)terminal large globule (arrows). Such chromosome associations, however, cannot be identified as bivalents

Concluding Remarks

Our observation of lampbrush-type chromosomes in *Acetabularia* presents the first significant demonstration of this chromosome type in a plant cell. (The loop structures described by Nagl, 1970, in the polytene chromosomes of *Phaseolus* suspensor cells were not clearly defined with respect to their chromosomal relation and would anyway rather be homologous to a "puff" formation.) At the moment one cannot decide as to whether the *Acetabularia* lampbrush chromosomes also represent a stage of meiotic prophase as is the case in all the animal examples of lampbrush chromosomes so far described. While it has been assumed

Fig. 7a—d. Details of spread lampbrush-chromosome-like structures isolated from *Acetabularia mediterranea* as revealed with phase contrast optics. Fig. 7 a shows, on the chromosome axis, chromomere—like small knobs (0.7–1.2 μm in diameter; some are denoted by arrowheads) and larger dense globules (diameters from 2 to 4.5 μm, indicated by arrows), together with the finely extended lateral loops which are illustrated with particular clarity in the insert of (a) (arrows). (b) shows some less contracted appearance of such chromosomes with rather long lateral loops (*e.g.* at the arrow). An example of a very loop-rich (perhaps transcriptionally very active) chromosome is shown in (c) (some long loops are accentuated by arrows). Associations of chromosomes occur frequently, *e.g.* (d), but true bivalents were not yet identified with clarity. The scale in the insert of (a) represents 5 μm

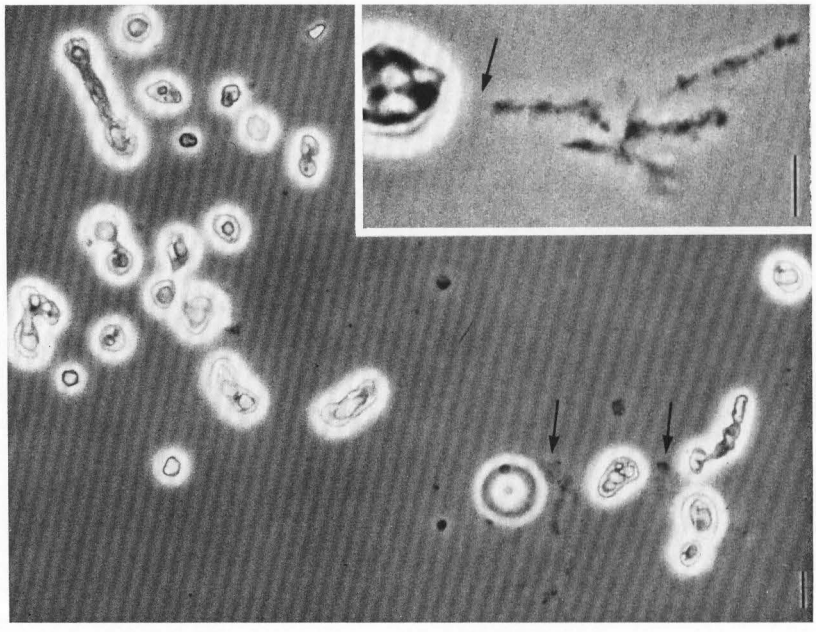

Fig. 9. Isolated nucleolar aggregates and subunits as revealed by phase contrast light microscopy. Note the instances (arrows) of an intimate relationship (perhaps due to connection or adherence) of such globular nucleolar components with lampbrush chromosome-like strands. The insert shows this association at higher magnification

for a long time that in *Acetabularia* meiosis occurs at or after cyst formation (Schulze, 1939; Puiseux-Dao, 1966; Woodcock and Miller, 1973) some recent observations (Green, 1973; Franke *et al.*, 1974; Trendelenburg *et al.*, 1974; Spring *et al.*, 1974; Berger *et al.*, 1975) including our present demonstration of lampbrush chromosomes emphasize the need for a reexamination of the life cycle of this alga and would be in accord with the alternative hypothesis that in this organism, like in many other green algae, meiosis begins immediately after germination of the zygote but is characterized by an extended meiotic prophase which lasts for most of the vegetative phase.

Fig. 10. Electron micrograph of a spread and positively stained preparation of nuclear content material which was virtually free from nucleolar structures but did contain some lampbrush-like chromosomes. One notes typical matrix units (the beginning and the terminus are denoted by arrows) of the long type (type D or E of the classification given by Spring *et al.*, 1974) with very small if existent at all, adjacent spacer intercepts. Note also the occasional occurrence of unusually extended, lateral fibrils (arrowhead). The scale indicates 1 μm

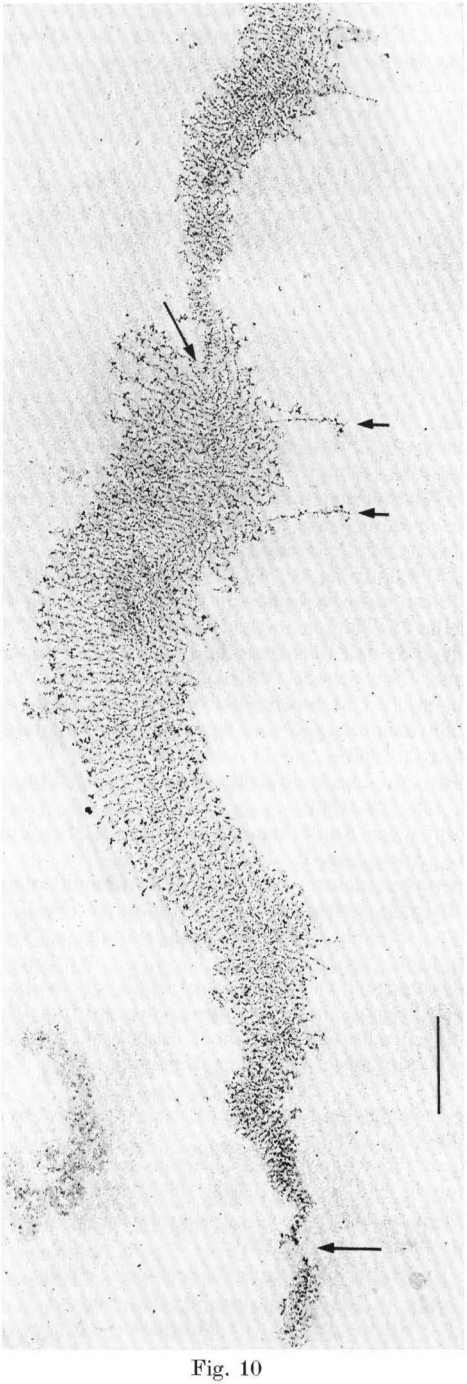

Fig. 10

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