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Morphology of Nucleolar Cistrons in a Plant Cell, *Acetabularia mediterranea*

(ribosomal RNA genes/electron microscopy/spread preparations)

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ABSTRACT **The structural organization of transcriptionally active DNA that contains cistrons for precursor molecules of ribosomal RNA is described in positively stained spread preparations from nuclei and nucleoli isolated from the green alga, *Acetabularia mediterranea* Lmx. These nuclei contain large aggregates of nucleolar subunits in which fibril-covered regions, the putative active cistrons for precursors of ribosomal RNA, alternate with fibril-free intercepts, the "spacers." The length distribution of the different intercepts of this DNA is given, and the pattern is compared with those shown in animal cell systems. The data are discussed in relation to problems of transcription and of amplification of ribosomal RNA genes.**

Transcription of DNA that contains cistrons for precursor molecules of ribosomal RNA (rDNA) has been directly demonstrated by electron microscopy of spread preparations in the amplified extrachromosomal nucleolar genes of amphibia (1–5) and insects (6) and, though with somewhat less clarity, in the chromosomal nucleoli of diverse other cell types (4, 7–10). The observations have shown that the transcriptionally active cistrons of precursors of ribosomal RNA (pre-rRNA) can be identified by their adherent nascent ribonucleoprotein fibrils, occur in clusters, and are separated from each other by rDNA intercepts ("spacer" segments) that are either not transcribed or code only for small RNA molecules that are not covalently linked with the pre-rRNA (1–3, 5, 6, 8, 11). Although the regular pattern of the cistrons and spacers is relatively consistent within a specific group of organisms, a pronounced variability among more distant eukaryotes has been noted (6). The data obtained from such spread preparations are in essential agreement with the corresponding determinations from biochemical studies of pre-rRNA formation and from denaturation mapping analyses of isolated rDNA (5, 10, 12–14; see, however, also ref. 6). In this article we will present the arrangement of transcriptionally active nucleolar genes in a plant cell, the green alga *Acetabularia mediterranea*. *Acetabularia* is of special importance in cytology and cytogenetics because of its mononuclear character in the vegetative phase and its suitability for enucleation and grafting experiments (15–17).

MATERIALS AND METHODS

Primary nuclei (50–80 μm in diameter) were manually isolated from rhizoids of *Acetabularia mediterranea* Lmx. (plant size of 3 cm) in the "5:1" medium of Callan and Lloyd (18) and Gall (19). In some nucleolar isolations we also used the stabilizing, Mg^{2+}-containing medium described by Brändle and Zetsche (20), which, however, was not useful for the spreading procedure. Nuclei were cleaned from adherent cytoplasmic material, and nucleoli were then isolated in the same medium by disrupting the nuclear envelope with microneedles. The isolated nucleoli and nucleolar fragments were rapidly transferred into a drop of water that had previously been adjusted to pH 9 with borate buffer. The further preparation followed the technique of Miller and associates (1–4, 7, 8), with the modification described by Scheer *et al.* (5). For each spread preparation we combined the nucleolar material from 4 to 6 nuclei. Spreadings were evaluated in a Siemens electron microscope 101 or a Zeiss EM 10, both of which were usually

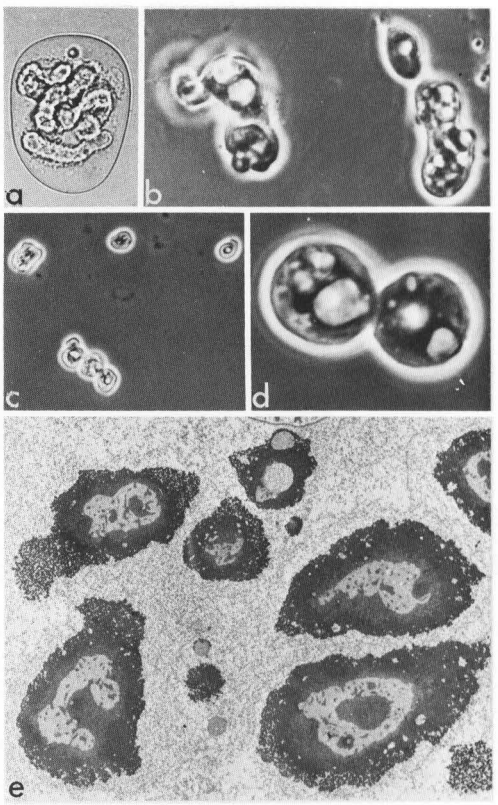

FIG. 1. (a) Phase-contrast micrograph showing a typical isolated primary nucleus of *Acetabularia mediterranea* with its enormous nucleolar aggregate (×413). (b–d) Isolated nucleolar material: (b) relatively large fragments of the nucleolar aggregate, as obtained immediately after rupture of the nuclear membrane (×623); (c) a survey micrograph of the smallest nucleolar subunits, as revealed in a light microscopic squash preparation (×338); (d) at higher magnification, details such as the light central regions are recognized in these nucleolar subunits (×1500). (e) Electron micrographs of sectioned *Acetabularia* primary nuclei fixed *in situ* show the distinct nucleolar subunits, which exhibit a light core surrounded by a fibrillar and a granular zone (×1800).

Abbreviations: rDNA, stretches of DNA that contain the cistrons for the precursor molecules of ribosomal RNA; pre-rRNA, precursors of ribosomal RNA.

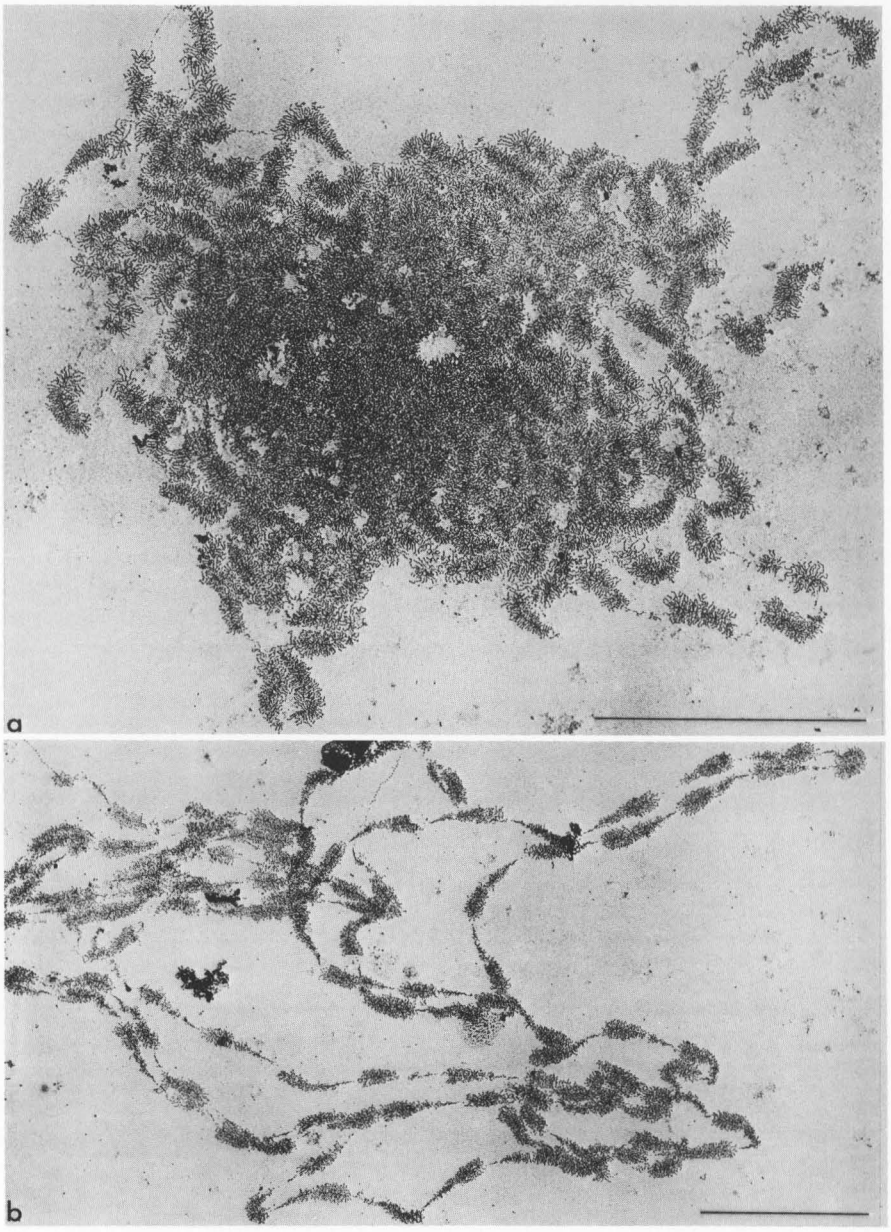

FIG. 2. Survey electron micrographs of positively stained and spread nucleolar subunits isolated from *Acetabularia* nuclei after moderate (a) or extensive (b) spreading. The composition of fibrillar elements, the deoxyribonucleoprotein-axes, and the smaller lateral fibrils, is clearly recognized. Note the sequence of matrix-covered and matrix-free intercepts. Scales indicate 5 μm.

operated at 60 kV. Instrument magnification was controlled with a series of grating replicas. For total screening of grids, use was made of the quick specimen stage transport system of the Zeiss EM 10 at very low magnifications. Ultrathin section preparations of nuclei fixed *in situ* were prepared and examined as described elsewhere (21, 22).

RESULTS

After germination of the *Acetabularia* zygote the primary nucleus enlarges, concomitant with total cellular growth, within 2–3 months, from 3 to 100 μm in diameter. During this nuclear growth a dramatic increase of total nucleolar mass occurs, so that in the maturing giant nucleus up to 50% of the nuclear interior is occupied by the usually sausage-shaped, dense nucleolar aggregate (Fig. 1*a;* see also refs. 16, 23–26), which corresponds to the morphometrically calculated total nucleolar volume of 70×10^3 to 150×10^3 μm^3 (refs. 24 and 25; Spring, Trendelenburg, Scheer, Franke, and Herth, *Cytobiologie*, in press). This nucleolar body, however, does not represent one extended ordinary nucleolus (see refs. 23 and 25) but rather appears to be an irregular aggregate of numerous subunits. Each of these nucleolar subunits is characterized by the existence of a relatively electron-transparent central region surrounded by a shell that structurally resembles the *pars fibrosa* of other nucleoli and an outer region with the granular substructure typical for *pars granulosa* (Fig. 1*e;* for review see ref. 27). Such a composition by defined subunits is also suggested from the observation that, upon isolation, the large nucleolar mass tends to fragment into well-defined smaller bodies (Fig. 1*b–d*). We believe that the smallest of such fragment bodies (Fig. 1*d*) represent the individual nucleolar units. These fragments show a striking similarity in size (diameter of about 10 μm) and appearance to

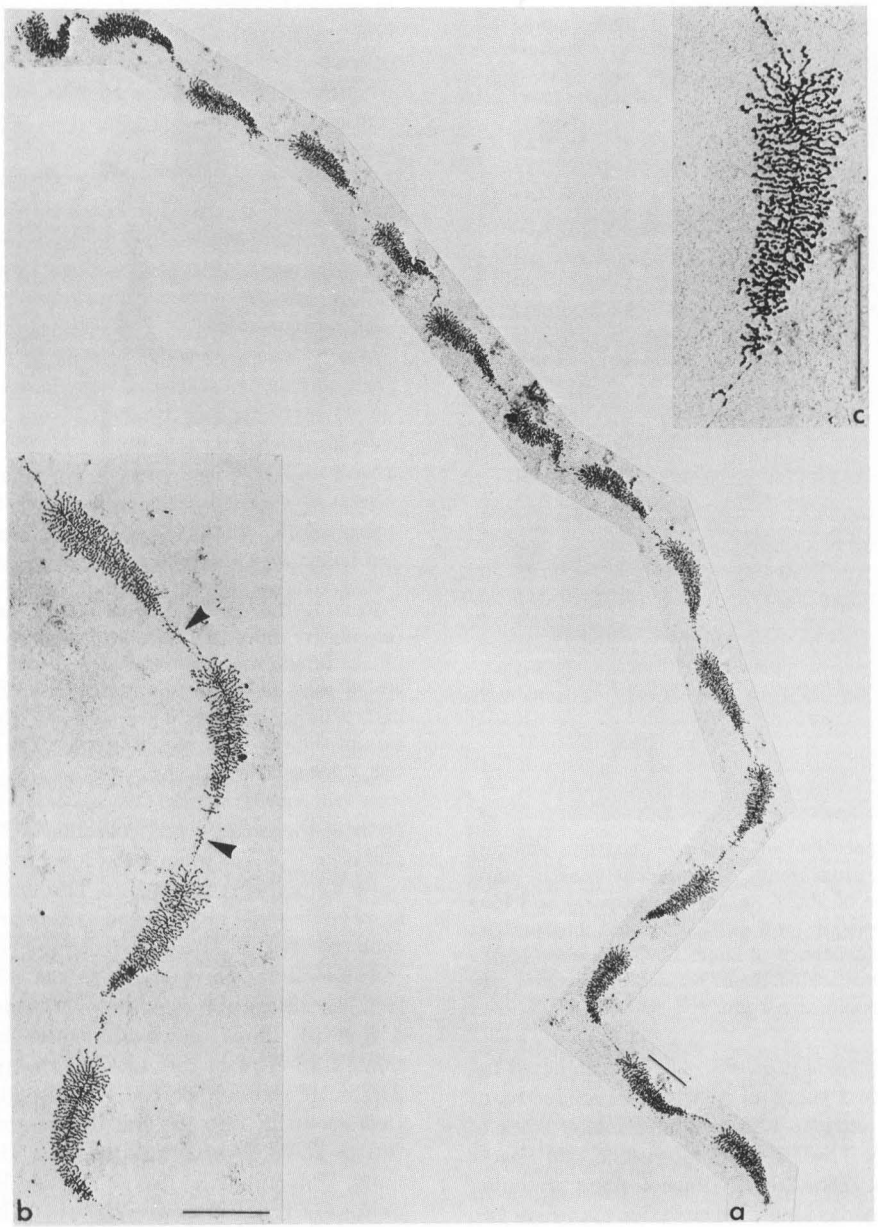

FIG. 3. (a) Well-spread nucleolar material showing the regular pattern of matrix units and "spacer" segments. Note the length gradient of lateral fibrils within each matrix unit. (b) Note also occasional groups of small fibrils associated with some of the spacer regions (e.g., at the arrowheads). (c) At higher magnification, the dense packing of lateral fibrils within the matrix units is shown. Scales indicate 1 μm.

the extrachromosomal nucleoli described in amphibian oocytes (e.g., refs. 28–33).

In the spread and positively stained electron microscopic preparations, distinct morphological units (Fig. 2), measuring from 10 to 20 μm in diameter, were observed. These were composed of fibrillar substructures, which were either aggregated (Fig. 2*a*) or more extended (Fig. 2*b*). They consisted of long axial and shorter lateral fibrils (Fig. 2*b*) and closely resembled the structures described for amphibian and insect oocytes (1–3, 5, 6). These fibrillar aggregate units probably represent the spread nucleolar subunits described above. Under more favorable spreading conditions one can recognize, along the axial (putatively rDNA-containing) fibrils, sequences of repeating units, each consisting of virtually naked ("spacer") and fibril-covered (cistronic) intercepts (Figs. 2*b* and 3). Each series of lateral fibrils is characterized by a marked length gradient and a very close packaging of fibril insertion points of about 150–250 Å. Only rarely were prominent "terminal knobs" (see refs. 1–6) observed on these fibrils. The whole situation closely resembled that described in nucleolar material from various animal cell types (1–10) and is interpreted as a demonstration of the transcription of the cistrons coding for the precursor molecules of ribosomal RNA (1–3, 5), which are separated by spacer segments. Over most of its length, the axial fibrils containing the rDNA were in an transcriptionally active state, suggesting that the majority of genes are switched on. Within the individual nucleolar subunits we could count as many as 130 cistrons.

Although within most nucleolar fragments the relative length of the specific cistron and spacer intercepts was rather uniform (e.g., Figs. 3 and 5), we noted the existence of different types of arrangement between different units. There were, for example, some rDNA axes on which the spacers were extremely small, if existent at all (e.g., Fig. 2*b*). There were also

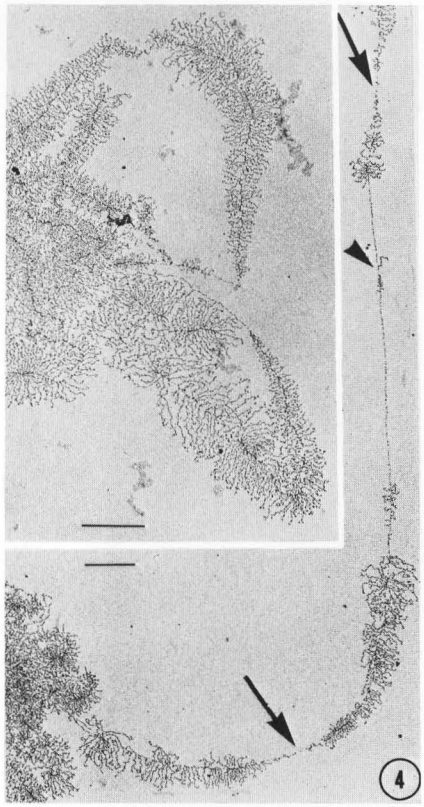

FIG. 4. Spread preparation of nucleolar cistrons showing typical "tail-to-tail" arrangements of adjacent matrix units (arrows), the occurrence of short (in the upper part) and long (in the lower part) cistrons, as well as of short and long matrix-free intercepts, and the existence of short fibrils in some of the spacer regions (arrowhead). The insert shows a typical "very long" matrix unit. Scales indicate 1 μm.

others with very long spacers (e.g., Fig. 4). We also noted between different axes two types of cistrons, a predominant short category (mean length, 1.85 μm) and longer ones of about 4–6 μm (Fig. 4). This diversity of cistron and spacer lengths is also apparent from the distribution plots presented in Fig. 5. We also repeatedly identified situations in which two adjacent cistronic units showed series of lateral fibrils increasing in length in opposite directions beginning at a common starting point (representing Fig. 4), a situation interpreted as "tail-to-tail" arrangements, corresponding to the "head-to-head" arrangements described by Miller and Beatty (3) in the amplified nucleoli of *Triturus viridescens*. In addition, tiny clusters of short fibrils were observed within some of the spacer intercepts (Fig. 3), similar to those recently described in spread amphibian nucleoli (5).

DISCUSSION

This study has shown that the arrangement of cistrons for precursor rRNA molecules within the nucleolar DNA is similar in principle in diverse plant and animal cells. One can identify, in transcriptionally active nucleoli, rather regularly repeated cistronic units covered with a set of lateral fibrils gradually increasing in length and spaced by intercepts that either bear no fibrils or show small clusters of fibrils separated from the cistronic fibrillar gradients. In this connection it is also noteworthy that, in *Acetabularia*, such structural arrays are directly demonstrated in *isolated nucleolar* material and, thus,

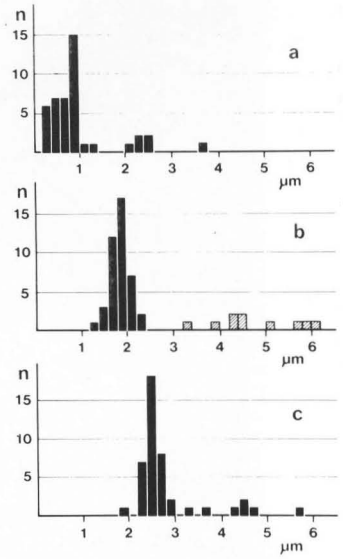

FIG. 5. Diagram of length distributions of spacer regions (a), matrix units (b), and total repeated units (c). The hatched blocks in (b) represent the category of very long matrix units, which were less frequently observed. The values of these long matrix units, however, have not been considered in the presentation of the repeated unit diagram. (The ordinate gives the number of measurements, *n*.)

confirm the earlier interpretations of Miller and Beatty (1–3) and others (4–11) who used whole nuclei in their preparations.

In amphibian oocytes, in *Drosophila* spermatocytes and embryonic cells, in *Chironomus* salivary glands, and in HeLa cells (but not in *Acheta* oocytes; see ref. 6), the length of the cistronic units corresponds to the codon lengths calculated from the molecular weights of the identified pre-rRNAs (2, 4, 5, 8–10). Thus, one would expect mean lengths of the primary transcript in the *Acetabularia* nucleus of about 1.8 to 2.0 $\times$ 10^6 daltons for the more frequent short matrix units (left group in Fig. 5*b*) and of considerably larger molecules (up to about 6 $\times$ 10^6 daltons) for the extraordinarily long units. Unfortunately, due to preparative difficulties, determinations of the molecular weight distribution of pre-rRNAs in the primary nucleus of *Acetabularia* have not been made. One must therefore compare these figures calculated from electron microscopy with the somewhat higher values determined biochemically for various algae, yeasts, and higher plants (34–45). It is, however, a clear and inevitable consequence from the present findings and from the determinations of cytoplasmic rRNA molecular weights of *Acetabularia* (1.3 and 0.7 $\times$ 10^6 see refs. 46–48) that only a very small portion, if any at all, is lost during the processing steps. The occurrence of exceedingly long matrix units has been documented as the only class in the house cricket oocyte (6) and, in smaller subpopulations, also in amphibian oocytes (ref. 5; see also ref. 49), and in this article for *Acetabularia* as well. It cannot be decided whether this reflects the existence of different length classes of rRNA cistrons, such as have been discussed in various biochemical studies (34, 50, 51), or whether it reflects the "read-through" transcription of adjacent cistrons (see also refs. 5, 42, and 52). An alternative explanation would be that in this organism a class of primary transcripts exists that does not contain the sequences of both large rRNAs. The observed heterogeneity of repeated unit lengths, as well as the

existence of the exceedingly long cistrons (hatched blocks in Fig. 5*b*) are further in contrast to the hypothesis of Perry and associates (35) of a uniform eukaryotic repeating unit (see also our ref. 6). Assuming about 130 cistrons per nucleolar subunit and a total number of 100 such subunits per fully grown nucleus (roughly determined in light microscopical squash preparations), one can approximate the total number of pre-rRNA cistrons as about 13000. This figure is by far in excess of the cistronic number reported in other Chloro- and Chromophyta (e.g. refs. 53 and 54) and, together with the observations of the dramatic increase of the nucleolar mass relative to the total nuclear mass during germination and development (23–25), might be indicative of an rDNA amplification process. (For indications of ribosomal gene amplification during plant developmental processes, see also refs. 53 and 55; for contrasting statements, see ref. 56). It should also be mentioned that very high rDNA contents have been reported in various higher plant tissues (56–59). Studies must be done to clarify whether this nucleolar enlargement during *Acetabularia* vegetative phase growth is concomitant with, or preceded by, the production of extrachromosomal rDNA copies.

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