ULTRASTRUCTURE OF TRANSCRIPTION IN THE NUCLEOLI OF THE GREEN ALGAE ACETABULARIA MAJOR AND A. MEDITERRANEA

M.F. TRENDELENBURG, W.W. FRANKE, H. SPRING and U. SCHEER

Division of Membrane Biology and Biochemistry,
Institute of Experimental Pathology,
German Cancer Research Center,
D-69 Heidelberg, Federal Republic of Germany

It has been demonstrated in a variety of animal cells, with particular clarity in insect and amphibian oocytes and spermatocytes, that transcription of rDNA molecules, that is DNA which contains the genes of precursor molecules of ribosomal RNAs (rRNAs), can be visualized and identified in spread and positively stained electron microscopic preparations of isolated nuclear contents (Miller and Beatty, 1969 a-c; Miller and Bakken, 1972; Miller and Hamkalo, 1972; Derksen et al., 1973; Hamkalo and Miller, 1973; Hamkalo et al., 1973; Scheer et al., 1973; Trendelenburg et al., 1973; Meyer and Hennig, 1974; Trendelenburg, 1974). The transcribed regions appear as clusters of rather regularly spaced intercepts on long, about 70 - 140 Å thick axial fibrils of deoxyribonucleoprotein (DNP) which are mostly uniform in length and are characterized by a series of 120 - 180 Å thick lateral fibrils, the putative pre-rRNA containing ribonucleoprotein (RNP) complexes. These fibril-covered "matrix units" show, at least in the first half, a gradual increase in length of the lateral fibrils, and their insertion knobs on the axis are relatively tightly packed with center-to-center distances of 230 - 340 Å which in the interpretation of Miller and associates (see the references quoted above) represent the spacings of the individual transcriptional complexes with their RNA polymerase A molecules attached to the template. At their free ends they frequently reveal a dense knob up to 300 Å in diameter. The

matrix unit regions are separated from each other by fibril-free intercepts, the so-called "spacer" segments. It has been suggested that these represent the GC-rich regions in rDNA which have been demonstrated by other techniques (e.g. Birnstiel et al., 1968; Dawid et al., 1970; Wensink and Brown, 1971).

It is obvious that, for technical reasons, large nuclei and nuclei with a high relative enrichment of rDNA per total DNA content such as those with amplified rDNA are particularly suitable for such preparations. In the plant kingdom the dasycladacean green algal genus Acetabularia which upon germination of the zygote develops a giant nucleus (up to ca. 100 µm in diameter; Hämmerling, 1931; Schulze, 1939; Schweiger, 1969; Franke et al., 1974) was an attractive first choice for such an analysis. In these nuclei a dramatic increase in nucleolar mass takes place and results in the formation of a giant sausage-shaped or spherical aggregate nucleolar body consisting of distinct nucleolar subunits, which tend to segregate during manual isolation and disruption of nucleus (Fig. 1 a,b; Stich, 1956; Berger and Schweiger, 1974; Spring et al., 1974). Moreover, it has been estimated from the high ribosomal production rate of about 17,000 ribosomes per second that a very high number of rRNA genes must be transcribed in this nucleus (Trendelenburg et al., 1974; Spring et al., 1974; Kloppstech and Schweiger, 1974), suggesting either a high degree of polyploidy or, more likely, amplification of rDNA (Spring et al., 1974; Franke et al., 1974).

In spread and positively stained (with phosphotungstic acid) preparations of nuclear contents and nucleoli isolated from these algae (for details of preparation see Trendelenburg et al., 1974, and Spring et al., 1974) we frequently observed isolated aggregates of a fibrillar substructure, in which at higher magnification typical matrix units were seen. In more favorably spread preparations we noted the existence of three size classes of matrix units (see also Fig. 4). The shortest matrix units observed (mean peak lengths of 1.77 and 1.84 µm, respectively; for the specific ranges see Fig. 4) usually

Fig. 1 a-b. Light micrographs (b, in phase contrast) of a manually isolated giant (primary) nucleus (a, from A. mediterranea; note the large sausage-shaped nucleolar aggregate) and isolated nucleolar subunits (b) with their caverns (vacuolisations). Scales, 10 /um. Fig. 1c presents an electron micrograph survey of spread nucleolar material from A. major, revealing axial fibrils with fibril-covered regions ("matrix units") and fibril free intercepts ("spacers"). Scale, 1 /um.

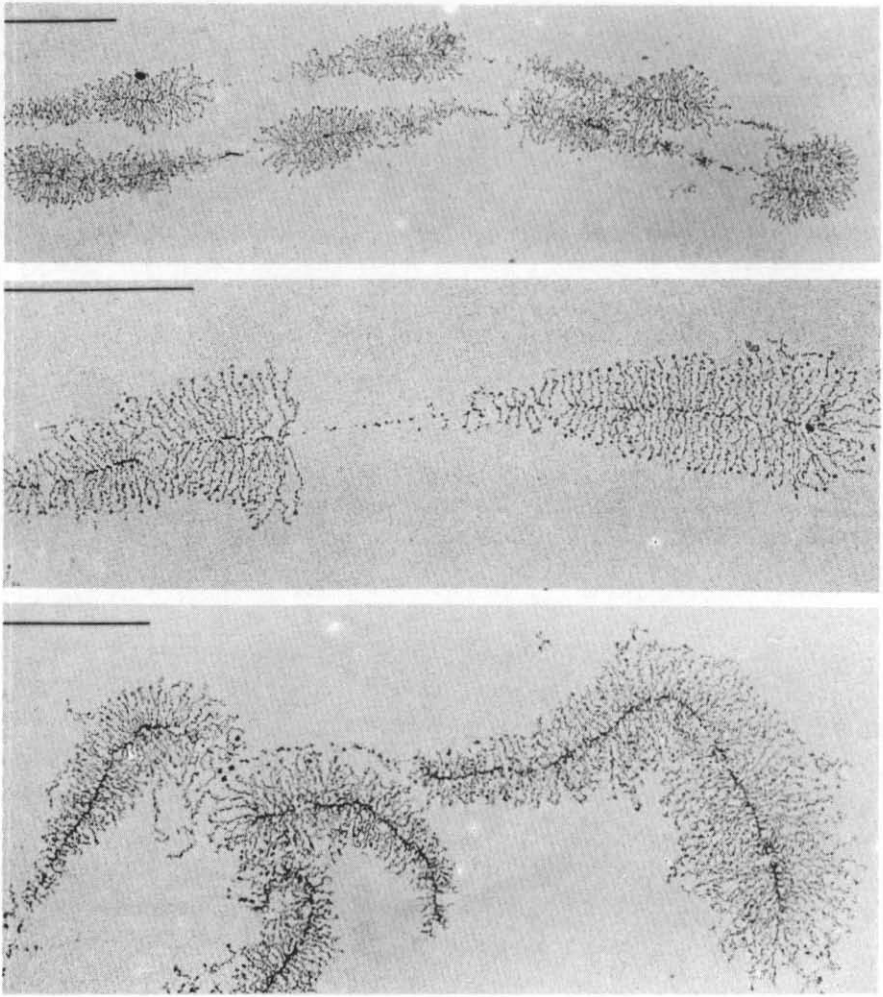


Fig. 2 a,b. Spread material from primary nucleus of A. mediterranea showing the alternating matrix units and spacer intercepts. Note the relatively short spacer regions (a) and the terminal dense knobs at the lateral fibrils. Fig. 2c. Spread preparation of nucleolar material from A. major showing the matrix units of the about 2 /um length class adjacent to a larger matrix unit, probably on the same axis. Scales, 1 /um.

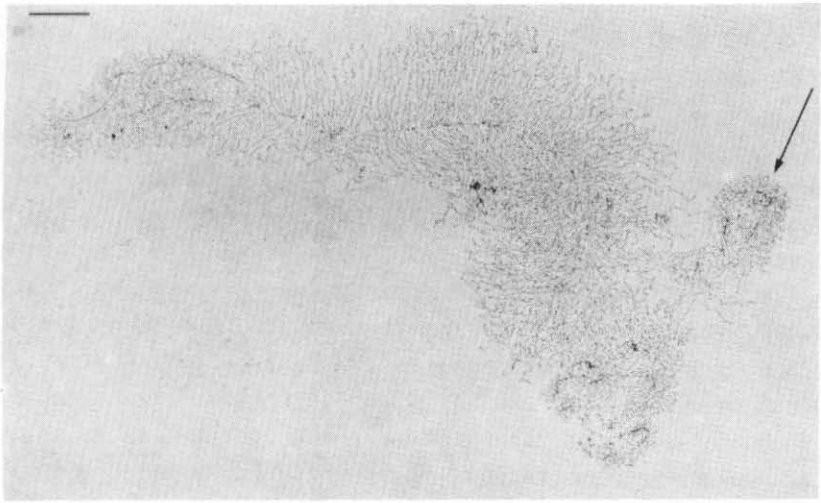
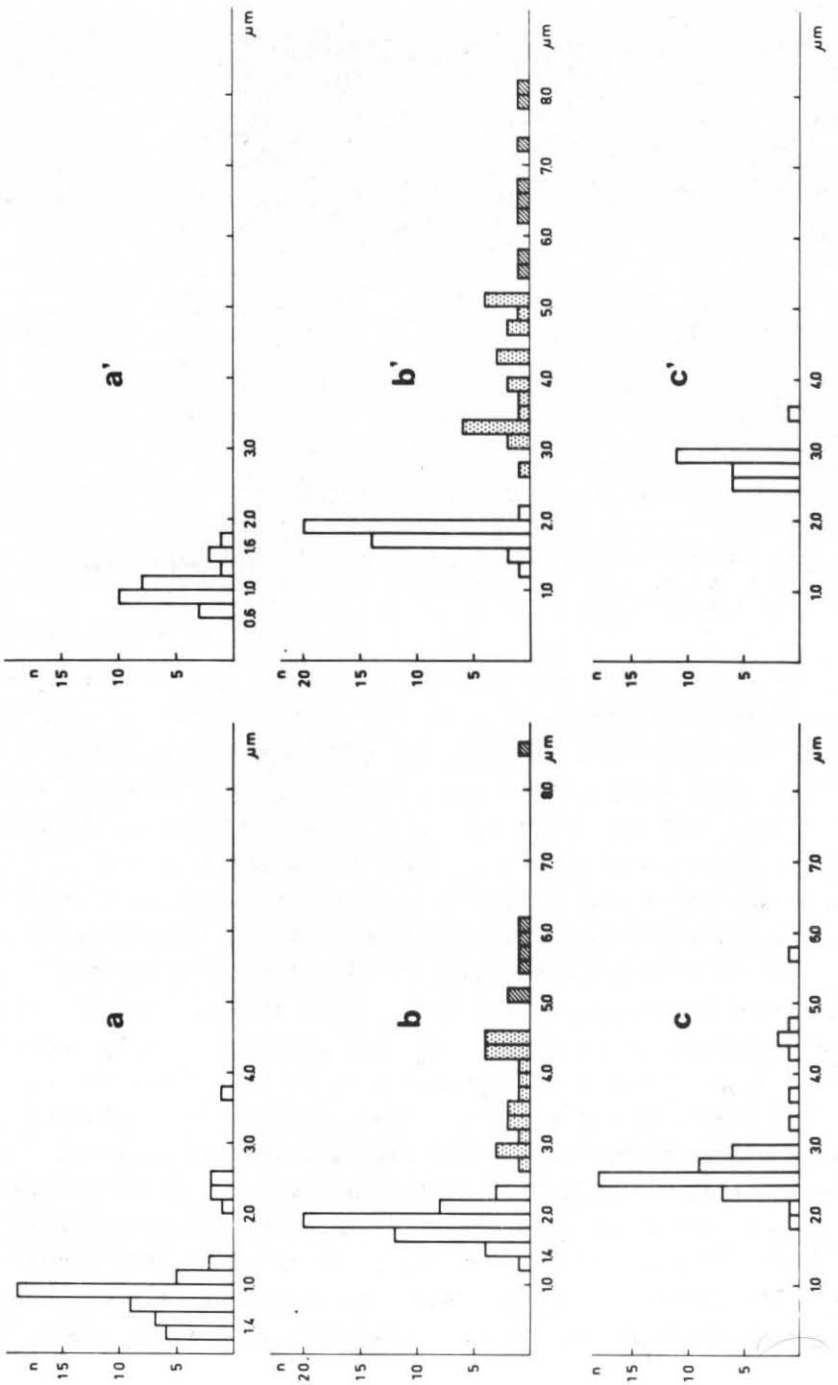


Fig. 3. "Giant" matrix unit as observed in spread contents from an A. major primary nucleus. The arrow denotes an adjacent short type matrix unit. Scale, 1 /um.

occured in highly repeated arrangements. The maximal number of individual matrix units of this size class counted in such aggregates was 120 in A. major and 130 in A. mediterranea. As many as 23 matrix units could be counted and traced on one individual well extended axis. The typical morphology of these transcriptional structures is presented in Figs. 1 and 2. The quantitative evaluation for both species is illustrated in Fig. 4. The ultrastructural organization is in principle the same as in the animal cells studied (table; see also Berger and Schweiger, 1974). These short transcriptional units of Acetabularia are different in some properties as is described in detail elsewhere (Trendelenburg et al., 1974; Spring et al., 1974). They are much shorter than the corresponding matrix units in chromosomal and extrachromosomal nucleoli of all animal cells studied (table) but contain about the same number of lateral fibrils (ca. 110) than the larger pre-rRNA cistrons of, e.g., various amphibia and the fruit fly Drosophila (for refs. see table). When one compares the molecular weight of the RNA that can be transcribed from a DNA piece


a
b
c
a'
b'
c'
n
µm

of this length with the determined molecular weights of the cytoplasmic rRNA of Acetabularia (Woodcock and Bogorad, 1970; Spring et al., 1974; Kloppstech and Schweiger, 1974) it is obvious that little, if any, RNA is lost during the processing of the pre-rRNA. From the number of individual nucleolar subunit bodies and the number of matrix units per subunit aggregate one can estimate that about 17,000 pre-rRNA cistrons occur in an A. mediterranea primary nucleus and 21,000 in A. major. This high number of rRNA genes, the dramatic increase in relative nucleolar mass, concomitant with the development of a large aggregate nucleolar body cytochemically positive for DNA (Spring et al., 1974), and the apparently isolated state of the nucleolar units might serve as an indication of rDNA amplification after zygote germination, similar to what has been described in other cases of giant nuclear growth such as in oogenesis of amphibia and insects (for review see Birnstiel et al., 1971).

In addition to these short matrix units, one finds in nuclear contents as well as in isolated nucleoli from both species of Acetabularia longer matrix units, not infrequently on the same axis and interspersed with the short ones (e.g. Fig. 2c; Trendelenburg et al., 1974). Among these larger matrix units, one might distinguish one class of lengths ranging from 2.7 to about 5 /um and the "giant" matrix units which can even exceed 10 /um in length (Spring et al., 1974) and in their terminal regions reveal lateral fibrils as long as 2 - 3 /um (Figs. 3 and 4). It remains to be clarified whether these longer units are related to rDNA or represent the transcription of other genes (for a more detailed study on these giant matrix units see Spring et al., 1974).

Fig. 4. Length distribution of the matrix units (b and b'), spacer intercepts (a and a'), and total repeating units (c and c') as observed in spread material from primary nuclei of A. mediterranea (a,b,c) and A. major (a',b',c'). As to the measurements of spacer intercepts and repeating units only those associated with (or containing) the short matrix units have been considered. Note the three size classes of matrix units, the short ones (white blocks), those of intermediate size (dotted blocks), and the extremely long (giant) ones (hatched blocks). N, number of measurements.

Table

Structural data of arrangement of ribosomal RNA cistrons in nucleolar DNA

Object	Topological state	Method	Matrix unit length (/um)	Spacer unit length (/um)	Repeat unit length (/um)	Ratio spacer: matrix unit	%nucleolar DNA (double strand) homologous to rRNA	References
Acetabularia mediterranea	?	spread	1.84§	0.96	2.80	0.51	74	1
Acetabularia major	?	spread	1.77§	1.05	2.82	0.59	73	2
Acheta domesticus	extrachrom.	spread	5.58	5.49	11.07	0.98	21	3
Dytiscus marginalis	extrachrom.	spread	3.71	4.39	8.1	1.18	29	4
Chironomus thummi	chrom.	spread	2.2	0.45	2.65	0.20	87	5
Drosophila melanogaster	chrom.	spread	2.65	ca.0.4	ca.3.1	ca.0.15	67	6
Drosophila hydei	chrom.	spread	2.5	0.9	3.4	0.36	61	7
Triturus viridescens	extrachrom.	spread	2.2-2.5	0.73-0.83+	2.9-3.3	ca.0.33	71	8-11
Triturus alpestris	extrachrom.	spread	2.9	2.2	5.1	0.76	43	12
Triturus cristatus	extrachrom.	spread	2.4	2.2	4.6	0.92	48	12
Triturus helveticus	extrachrom.	spread	2.6	2.2	4.8	0.85	46	12
Xenopus laevis	extrachrom.	spread	2.0-2.5	0.67-0.83+	2.7-3.3	ca.0.33	77	9
Xenopus laevis	extrachrom.	spread	2.6	2.1	4.7	0.81	49	12
Xenopus laevis	extrachrom. + chrom.	Denat. rDNA	3.1	2.3	5.4	0.74	43	13
Xenopus muelleri	extrachrom. + chrom.	Denat. rDNA	3.1	1.9	5.0	0.61	46	14
HeLa Cells	chrom.	spread	3.5	ca.3.5	7.0	1.0	36	6,11

§ only the short matrix unit type has been considered.

+ these values were reported to be variable: "1/3 matrix length up to 20 /um". The values listed here refer to the most frequently given spacer length of 1/3 matrix unit.

References: 1, Trendelenburg et al. (1974); 2, Spring et al. (1974); 3, Trendelenburg et al. (1973); 4, Trendelenburg (1974); 5, Derksen et al. (1973); 6, Hamkalo et al. (1973); 7, Meyer and Hennig (1974); 8, Miller and Beatty (1969a); 9, Miller and Beatty (1969b); 10, Miller and Beatty (1969c); 11, Miller and Bakken (1972); 12, Scheer et al.(1973); 13, Wensink and Brown (1971); 14, Brown et al. (1972).

REFERENCES

Berger, S. and Schweiger, H.G. (1974). Protoplasma (in press).

Birnstiel, M.L., Speirs, J., Purdom, I., Jones, K., and Loening, U.E. (1968). Nature 219, 454.

Birnstiel, M.L., Chipchase, M., and Speirs, J. (1971). Progr. Nucl. Acid Res. 11, 351.

Brown, D.D., Wensink, P.C., and Jordan, E. (1972). J. Mol. Biol. 63, 57.

Dawid, I.B., Brown, D.D., and Reeder, R.H. (1970). J. Mol. Biol. 51, 341.

Derksen, J., Trendelenburg, M.F., Scheer, U., and Franke, W.W. (1973). Exp. Cell Res. 80, 476.

Franke, W.W., Berger, S., Falk, H., Spring, H., Scheer, U., Herth, W., Trendelenburg, M.F., and Schweiger, H.G. (1974). Protoplasma (in press).

Hämmerling, J. (1931). Biol. Zentralblatt 51, 633.

Hamkalo, B.A., and Miller, O.L. (1973) Ann. Rev. Biochem. 42, 379.

Hamkalo, B.A., Miller, O.L., and Bakken, A.H. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 915.

Kloppstech, K., and Schweiger, H.G. (1974) Protoplasma (in press).

Meyer, G.F., and Hennig, W. (1974) Chromosoma 46, 121.

Miller, O.L., and Bakken, A.H. (1972) Acta Endocrinol. Suppl. 168, 155.

Miller, O.L., and Beatty, B.R. (1969a) Science 164, 955.

Miller, O.L., and Beatty, B.R. (1969b) Genetics Suppl. 61, 134.

Miller, O.L., and Beatty, B.R. (1969c) J. Cell. Physiol. 74 Suppl. 1, 225.

Miller, O.L., and Hamkalo, B.A. (1972) Int. Rev. Cytol. 33, 1.

Scheer, U., Trendelenburg, M.F., and Franke, W.W. (1973) Exp. Cell Res. 80, 175.

Schulze, K.L. (1939) Archiv Protistenkunde 92, 179.

Schweiger, H.G. (1969) Curr. Top. Microbiol. Immunol. 50, 1.

Spring, H., Trendelenburg, M.F., Scheer, U., Franke, W.W., and Herth, W. (1974) Cytobiologie (in press).

Stich, H. (1956) Chromosoma 7, 693.

Trendelenburg, M.F. (1974) Chromosoma (in press).
Trendelenburg, M.F., Scheer, U., and Franke, W.W. (1973) Nature New Biology 245, 167.
Trendelenburg, M.F., Spring, H., Scheer, U., and Franke, W.W. (1974) Proc. Nat. Acad. Sci. US 71 (in press).
Wensink, P.C., and Brown, D.D. (1971) J. Mol. Biol. 60, 235.
Woodcock, C.L.F., and Bogorad, L. (1970) Biochim. Biophys. Acta 224, 639.

ACKNOWLEDGEMENTS

The authors thank Drs. S. Berger and H.G. Schweiger (Max-Planck-Institut für Zellbiologie, Wilhelmshaven, Federal Republik of Germany) for valuable discussions and cooperation. The work has been supported by the Deutsche Forschungsgemeinschaft.