TRANSCRIPTIONAL COMPLEXES OF NUCLEOLAR GENES

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The knowledge on the mechanisms of transcription in eukaryotic cell nuclei is mostly advanced for the genes coding for precursors for ribosomal RNAs (pre-rRNA). Both from biochemical (for review see 1) and electron microscopical (2-4) analyses it has been concluded that the DNA regions coding for the high molecular weight (nominal classification: 28S and 18S) rRNA species are transcribed into a common precursor molecule that, in most cell system studied, was found to contain, besides these rRNA sequences, other sequences which are lost during the subsequent processing steps. The pre-rRNA genes are reiterated and tandemly arranged with a characteristic alternating sequence of transcribed ("matrix regions") and virtually untranscribed ("apparent spacer") intercepts (for definitions and nomenclature see 5). However, the relative as well as the absolute lengths of the transcribed and "spacer" portions and, consequently, of the sum of both, the "repeating unit of rDNA", are highly variable among different organisms and may even be heterogeneous in one and the same nucleus (6). For example, when nucleolar material obtained from manually isolated primary nuclei of the green alga *Acetabularia mediterranea* is spread and stained long strands of nucleolar chromatin are seen in the electron microscope which reveal the typical pattern of matrix regions and apparent spacer intercepts (Fig. 1a). The individual transcriptional units which in general are arranged with the same polarity along the axis are the morphological equivalents of active pre-rRNA genes simultaneously transcribed by an average of 110 RNA polymerase molecules which are attached to the axis with lateral fibrils of gradually increasing lengths. These lateral fibrils are ribonucleoproteins and contain the nascent pre-rRNA molecules. The close spacing of the matrix units with extremely short apparent spacer intercepts is evident (Fig. 1a; c.f. also 6-9). Here the mean axial length of the matrix units is about 1.9 μm (for quantitative evaluations see 6,7). Assuming a stretched DNA axis in B-conformation in this intercept (vide infra) one has to expect for the primary transcription product a molecular weight of about 1.9×10^6 daltons. Molecular weight determinations of the pre-rRNA under completely denaturing conditions are in good agreement with this predicted value (Fig. 1b). Thus, we conclude that in *Acetabularia* the pre-rRNA with a molecular weight of 1.9×10^6 D is

only slightly smaller than, if not identical with, the primary transcription product of the pre-rRNA genes, that the processing starts from a pool of accumulated pre-rRNA containing ribonucleoproteins, and that the losses during the processing are minimal (molecular weights of the mature rRNAs: 1.24 + 0.65, i.e. a sum of 1.89×10^6 D).

A similar close correlation between the lengths of matrix units, the estimated molecular weight of the primary transcription product, and the molecular weight of the pre-rRNA as determined by gel electrophoresis is found in the oocyte nucleoli of urodelan (Fig. 1c) and anuran (Fig. 1d) amphibia (c.f. 10). Particularly valuable in evaluation of patterns of rDNA transcription are the circular amplified rDNA molecules as they occur in various amphibia (3, 11-13) and insects (Figs. 1 e,f; c.f. also 14, 15). The lengths of the repeating units and also the relative proportions between transcribed and spacer sections, however, are significantly different from Acetabularia (for a comparison see 16). A quantitative analysis of spread nucleolar preparations from Xenopus laevis oocytes has revealed a mean peak length of the matrix units of 2.4 μm, whereas the corresponding spacer intercepts range from 0.8 to 2.2 μm with a distribution that suggests the occurrence of discrete length classes (major classes ca. 0.8, 1.0, 1.3, 1.8 μm). The resulting heterogeneity in lengths of the repeating units obviously is primarily introduced by the spacer sections (c.f. also 10, 17, 18). The absolute lengths of the repeating units as measured in spread preparations (Fig. 1d) correspond to those calculated from molecular weight determinations of rDNA fragments obtained by restriction endonucleases (17, 18) which indicates that the DNA within the transcribed nucleolar chromatin axes is extended and not arranged in nucleosomes (see also 5).

In other organisms such as in some insects, spread preparations of active nucleolar material (from oocytes) reveal very long matrix units (mean length in Acheta: 5.6 μm, in Dytiscus: 3.7 μm; Figs. 1 e-h; c.f. also 14, 19) suggestive of the existence of very large primary transcription products. In gel electrophoretic analyses of the pre-rRNAs obtained from the same material, however, only a pre-rRNA of about 2.8×10^6 could be found (for Acheta see also 19). This might indicate that here processing starts while the precursor molecule is still growing and that a complete read out product of this transcriptional unit does not exist. The "pre-rRNA" peaks found in gel electrophoretic analyses represent the first stable processing product and not necessarily the primary transcription product (see also 5). Cleavage in the growing RNA molecules may well escape detection in the electron microscope, due to the high packing density of the RNA within the lateral RNP fibrils. Only under conditions which favour the unravelling of the lateral RNP fibrils one notes specific discontinuities in the fibril length gradient (Fig. 2a) and short additional matrix units such

as the "prelude regions" (10) and other forms of "spacer transcripts"(5).

In some cells which show a natural enrichment and amplification of pre-rRNA genes such as various amphibian and insect oocytes, it is possible to directly demonstrate differences in the transcriptional activity of rRNA genes. In cell stages characterized by a relatively low level of rRNA synthesis the structural arrangement of transcriptional complexes is significantly different, as compared to stages of high synthetic activity (Figs. 2 c-h; c.f. also 11). In many units the average number of lateral fibrils per matrix unit is drastically reduced (compare Figs. 2 c-e with Fig. 1c) which results either in forms of nearly homogeneous reduction in the lateral fibril density and, consequently, to larger distances between the (active) RNA polymerases (Figs. 2 c,d), or in gaps between fibril covered portions of a matrix unit (Fig. 2e). A typical appearance of a group of partially active rRNA genes is shown in Fig. 2g: here all adjacent genes seem to be affected to the same extent. In contrast to this situation, other nucleolar chromatin strands of the same preparation exhibit another basically different structural change (Fig. 2 f,h). Some genes appear fully active whereas adjacent genes are completely or nearly completely inactive. This clearly shows that the transcriptional activity in each gene is regulated individually per gene, most probably by the frequency of initiation events of the RNA polymerases.

The presence of "dormant" rRNA genes interspersed between fully active ones (Fig. 2h) allows another examination of the question whether or not the transcription process leads to a foreshortening of the transcribed DNA intercept (c.f. 4). In this case the lengths of the transcriptionally active intercepts (denoted by arrows in Fig. 2h) should be significantly longer than the sum of two "normal" spacer units plus one matrix unit (2 su + 1 mu). A statistical evaluation of length measurements from a great many preparations of mature amphibian oocytes, however,did not reveal a significant difference between the measured and expected 2 su + 1 mu lengths. This leads to the conclusion that even the simultaneous transcription of one rRNA gene by about 100 polymerases does not result in a measurable change of the length of the rDNA containing chromatin axis.

Fibril-free nucleolar chromatin axes (both untranscribed spacer intercepts and inactive gene sections) sometimes appear studded with 80 to 150 Å large particles which presumably represent RNA polymerase molecules bound to the rDNA (11, 20, 21). The alternative interpretation, namely that these particles represent "nucleosomes" (22, 23) seems to be excluded (for detailed discussion see 5) since (i) these particles are not consistently observed and are distributed rather irregularly along the chromatin strand; (ii) they are still present after treatments

that lead to the disappearance of nucleosomes in, for example, hen erythrocyte chromatin (e.g. after incubation in 0.3 to 0.5% of the anionic detergent Sarkosyl NL-30; Fig. 2f); and (iii) there is no inverse correlation between the specific length and the number of particles attached as this would be expected from the five to sixfold shortening of DNA per nucleosome (c.f. 23).

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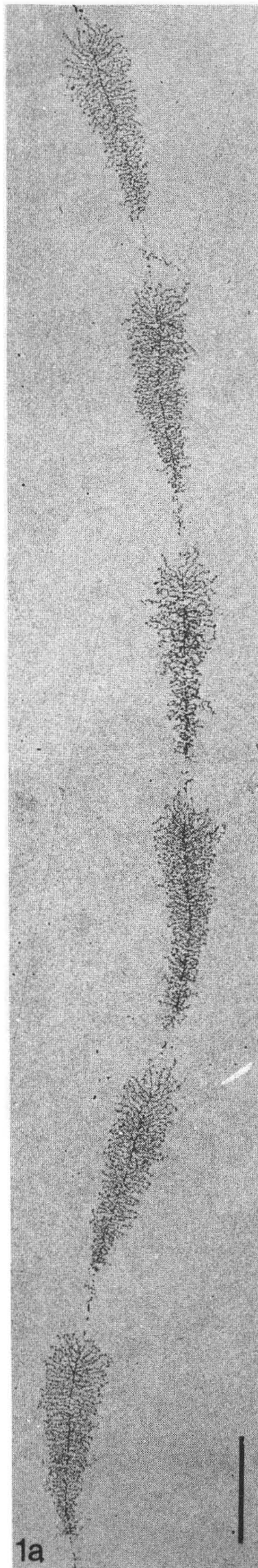
Fig. 1. Electron microscopic spread preparations of active nucleolar chromatin isolated from primary nuclei of Acetabularia mediterranea (1a), from oocytes of Triturus cristatus (1c), Xenopus laevis (1d), Acheta domesticus (1 e-g), and Dytiscus marginalis (1h). All preparations were positively stained, some were with additional metal shadowing (1a). Note the differences in absolute and relative lengths of matrix units, spacer intercepts and repeating units among the different organisms. In the two insect species the amplified rDNA is often arranged in circles containing one (1e), two (1f), or more pre-rRNA genes plus the adjacent spacer portions. A length gradient of lateral fibrils is visible only in initial parts of the matrix units of Acheta (1g) and Dytiscus (1h) whereas fibrils attached to the middle and terminal regions have a nearly equal length but frequently show a "terminal knob" (1 e-h). Fig. 1b presents a gel electrophoretic analysis under completely denaturing conditions (3-7% exponential acrylamide gel containing 98% formamide at $45^{o}C$; c.f. ref. 24) of labeled nuclear RNA of Acetabularia mediterranea. The plants were incubated for 26 hrs in sea water containing 100 μCi/ml ^{3}H-uridine (45 Ci/mM); then 25 primary nuclei were manually isolated and processed as described (6, 7). The UV-absorption pattern of co-electrophoresed marker RNAs is shown by the dotted line (TMV, tobacco mosaic virus; Escherichia coli 23S and 16S rRNAs). The estimated molecular weights of the three distinct radioactivity peaks are given in million Daltons.

Fig. 2. Fig. 2a presents a matrix unit of amplified nucleoli from Acheta oocytes after prolonged incubation in very low salt concentrations in which the lateral fibrils tend to unravel, thereby revealing discontinuities in their length increment. Fig. 2b shows nucleolar material from a Xenopus laevis lampbrush stage oocyte where a separate small "spacer transcript" (pair of arrows) precedes a "normal" pre-rRNA matrix unit. Figs. 2 c-h represent examples of partially active rRNA genes isolated from mature Triturus oocytes. The general decrease in lateral fibril density leads either to larger spacings of the lateral fibrils (Figs. 2 c,d) or to the group-wise disappearance of fibrils within a matrix unit (Fig. 2e, arrows). Fig. 2f shows fully covered, i.e. fully active genes adjacent to nearly inactive ones. The arrow denotes two lateral fibrils attached to an otherwise "dormant" gene region. The dispersion of the nucleolar material of this preparation has been done in a solution containing 0.3% Sarkosyl NL-30 (for details see 5) but particles are still attached to the fibril-free chromatin axes. Partial inhibition of transcription (Fig. 2 g,h) results either in a homogeneous dilution of lateral fibrils in adjacent genes (g) or in the complete removal of lateral fibrils in some (totally inactive) gene regions (arrows in h). All bars represent 1 μm.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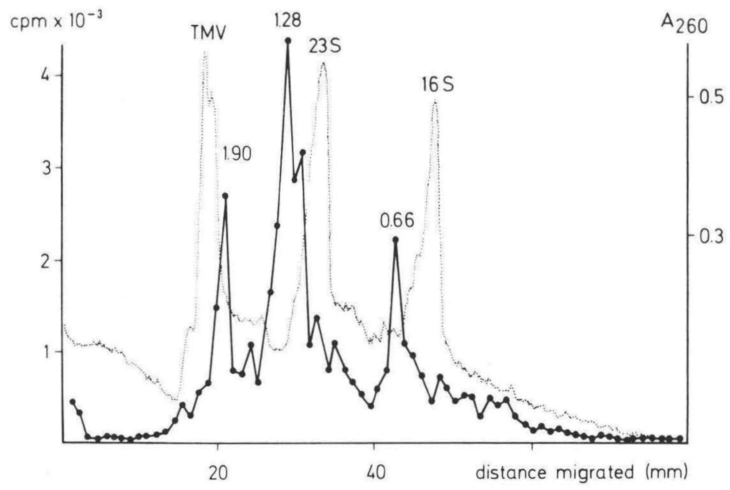
cpm x 10⁻³
TMV
1.28
23S
A260
16S
1.90
0.66
4
3
2
1
0.5
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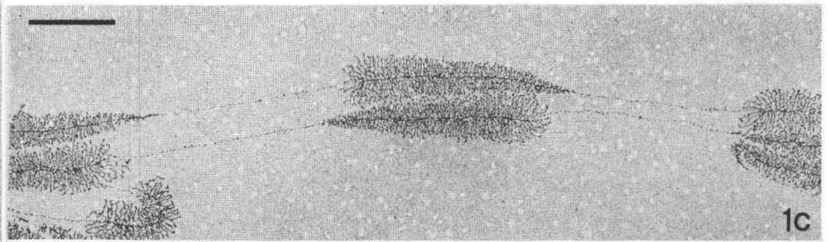
20
40
distance migrated (mm)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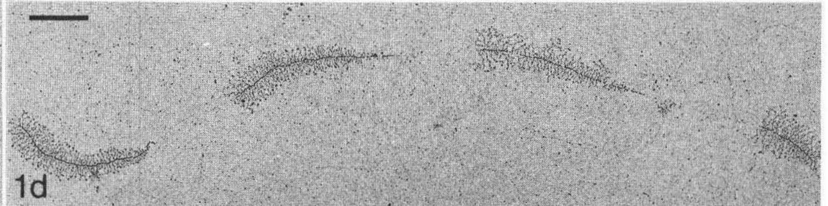
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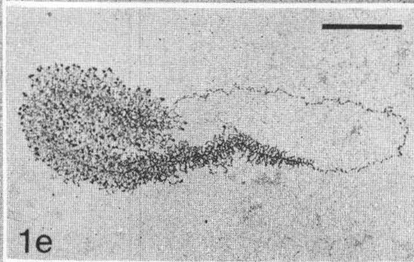
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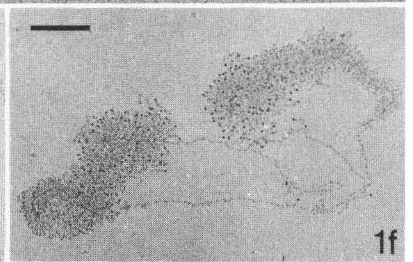
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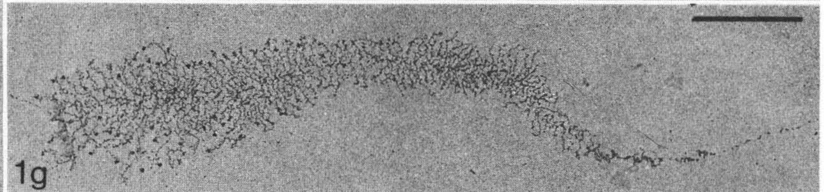
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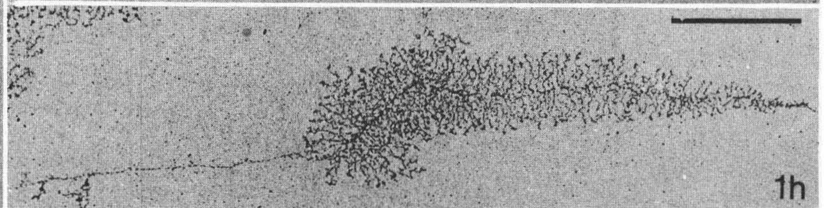
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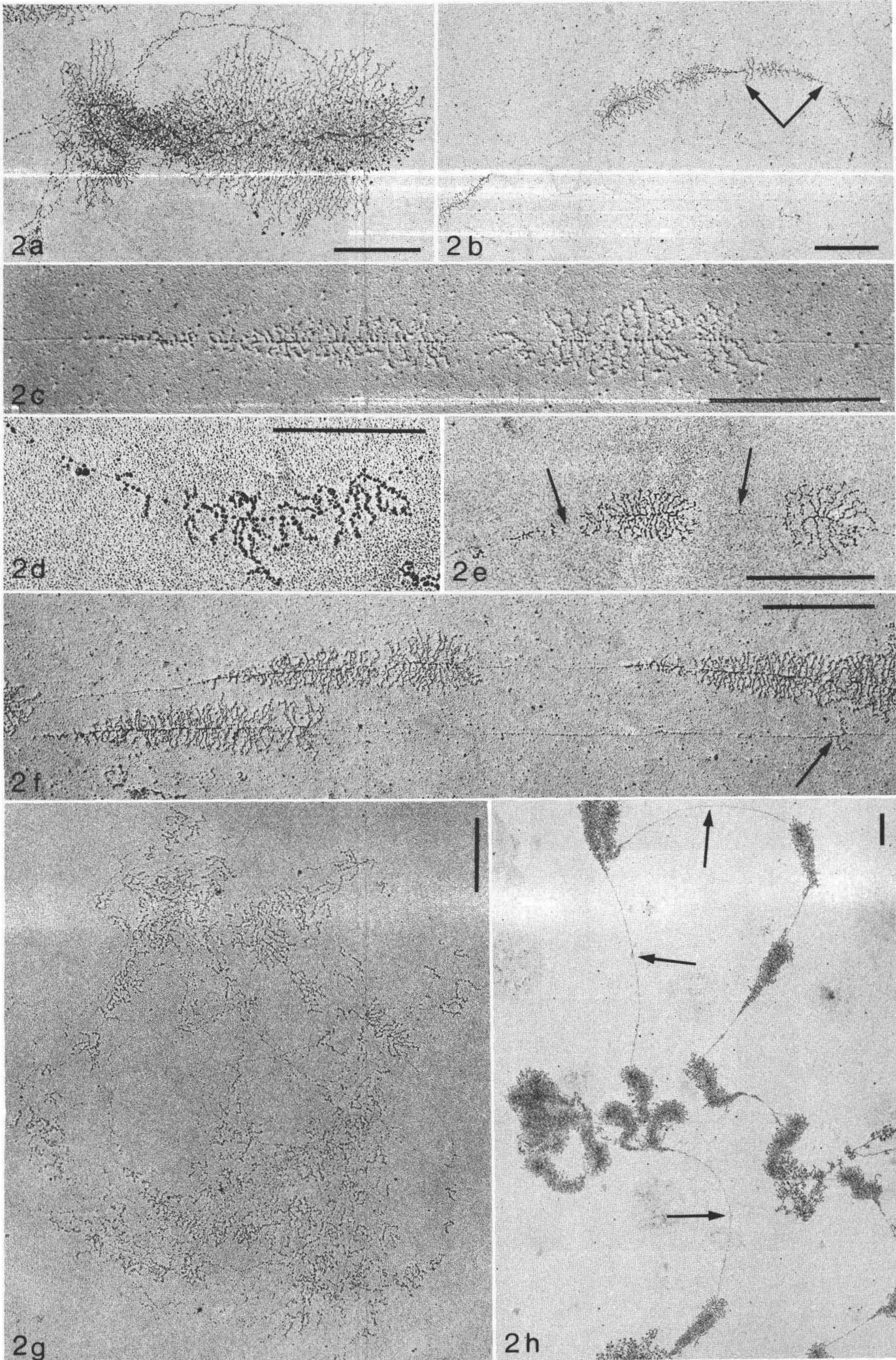
2a
2b
2c
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