REGULATION OF TRANSCRIPTION OF RIBOSOMAL RNA-GENES

DURING AMPHIBIAN OOGENESIS

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1. Introduction

The mechanisms that govern the regulation of the synthetic rate
of a gene product are still poorly understood. In principle, such
regulative mechanisms may act at two different levels of RNA biogene-
sis. The rate of synthesis could be modulated by either (i) direct
coupling of the transcription rate to the requirement of the specific
cell for a certain amount of specific RNA molecules or (ii) post-
transcriptional events, for example degradation, which select the
appropriate number of functional RNA molecules.

The ribosomal RNA (rRNA) synthesis is especially well suited
for elucidating such control mechanisms since under different physi-
ological conditions the ribosome production of a given cell may show
pronounced variations [1-11]. From biochemical experiments alone, how-
ever, an unequivocal differentiation between the alternative regula-
tive mechanisms of ribosome biogenesis at the transcriptional or
posttranscriptional levels is often impossible. A decrease in the
amount of the pre-rRNA species or in the incorporation of nucleotides
into acid-precipitable material does not necessarily mean that the
transcriptional rate of the rRNA-genes is concomitantly reduced but
may likewise indicate a higher instability and turnover rate of the
primary transcription product. To avoid these interpretative
difficulties, we have directly analyzed the transcriptional activity
of rRNA-genes in the electron microscope using the spreading techni-
que of Miller and coworkers [12,13]. The amphibian oocyte has been
chosen for this study because marked variations in the rate of rRNA

synthesis occur during the prolonged diplotene stage of oogenesis [14-18]. Moreover, the spreading technique for visualization the activity of rRNA-genes is particularly successful with this cell system, due to the amplification of rDNA which takes place very early in oogenesis, in the pachytene stage [19,20]. Using this technique we have recently shown that structural alterations of rDNA transcription complexes can be correlated with stages of experimentally induced reduction of transcriptional activity [21]. In this article we report on naturally occurring processes involved in activation and inactivation of rRNA-genes.

2. Results

Alpine newts (Triturus alpestris) were collected during their breeding season. Gel electrophoresis of oocyte RNA labelled in vivo has revealed that radioactivity present in the 28S and 18S rRNA peaks was diminished in mature oocytes and drastically reduced in the previtellogenic oocytes, compared to the lampbrush chromosome stage [22]. The main product synthesized in the previtellogenic oocytes migrated in the 4-5S RNA region. From our biochemical data we calculated that the rate of rRNA synthesis was less than 1% in previtellogenic oocytes and about 30% in mature oocytes relative to that

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Fig. 1 a,b. Electron micrographs of spread and positively stained nucleolar material from previtellogenic oocytes of Triturus alpestris. The typical appearance of the amplified nucleoli of this oogenic stage is demonstrated in an ultrathin section (inset of Fig. 1b), showing the firm attachment of the nucleolus to the nuclear envelope and its rather uniform composition of densely packed fibrils. When this nucleolar material is spread it becomes evident that the whole nucleolar body (diameter of about 10 /um) consists of aggregated "naked" fibrillar material. In the preparation shown in Fig. 1a no matrix units are seen. Only occasionally are individual, isolated lateral fibrils noted (inset) which probably contain transcription products. Note, however, that dense 80-120 A particles, similar to those interpreted as polymerase molecules in matrix units, are rather evenly distributed along the axes (Fig. 1a). Fig. 1b shows a large aggregate of "naked" axial fibrils with one identifiable matrix unit (arrow). Note that this single matrix unit already reveals the maximal packing density of lateral fibrils, thus suggesting full gene activity. Note also the many small aggregates of densely stained fibrillar material which perhaps represent components from chromosomes or the nuclear sap. Scales indicate 2 /um (Fig. 1 a,b); 0.2 /um (inset of Fig. 1a); 1 /um (inset of Fig. 1b).
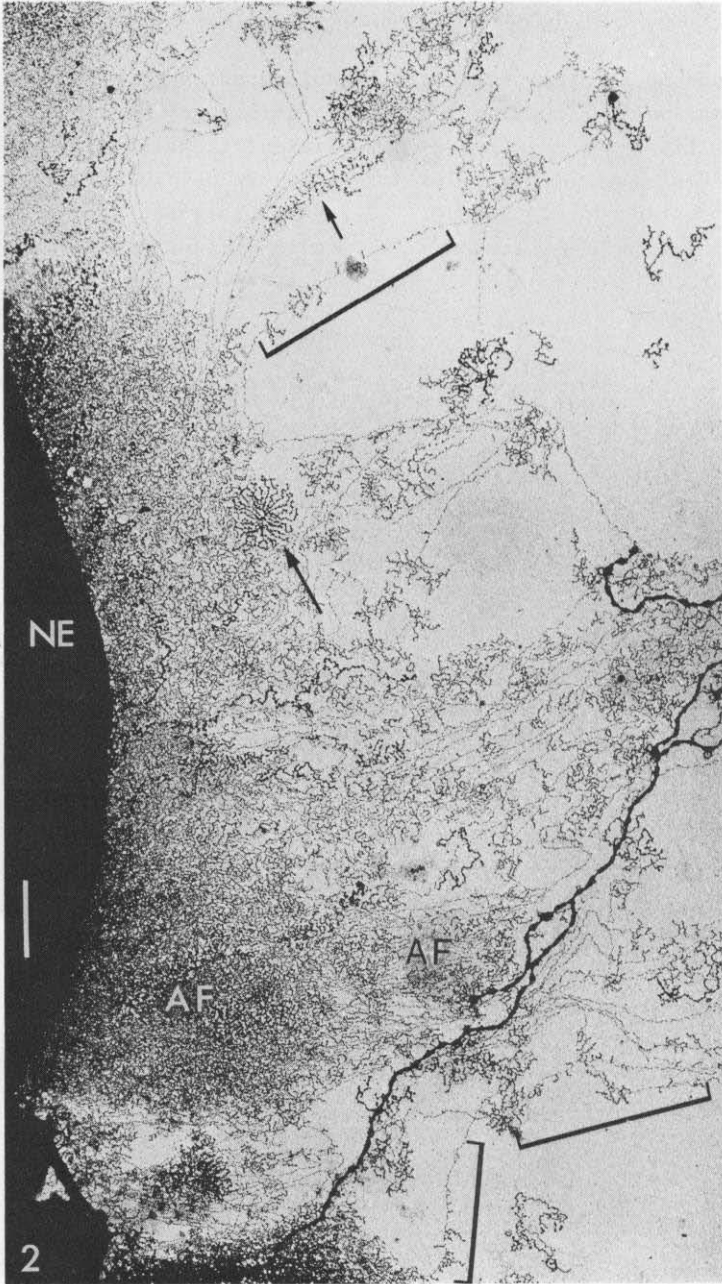
1a

1b
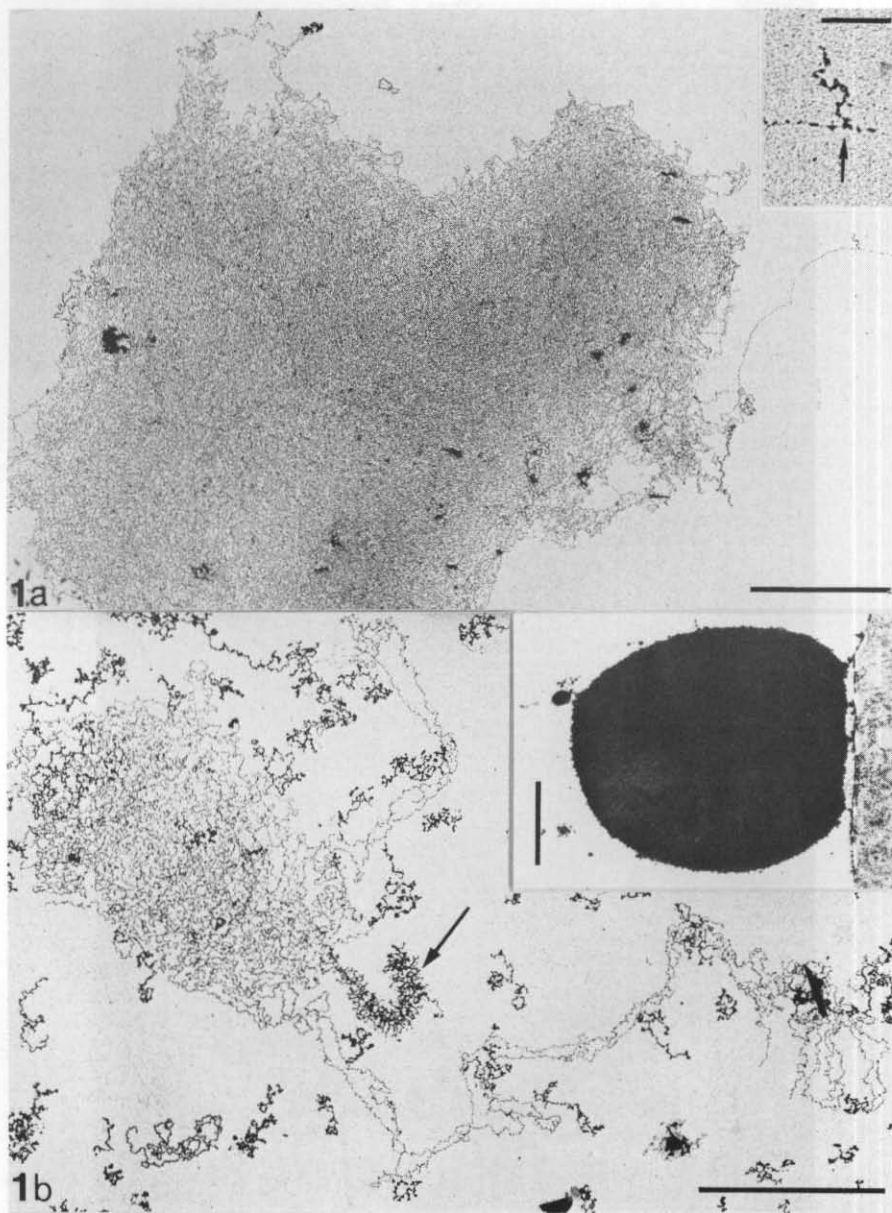
Fig. 2. Spread preparation of a nuclear envelope (NE) together with the attached nucleolar material, isolated from previtellogenic oocytes. Most of the nucleolar fibrils occur in aggregated "naked" fibrils (AF) but some groups of lateral fibrils, either densely clustered in typical matrix units (arrows) or with larger intervals (e.g. as indicated by the brackets) are seen. Scale indicates 1 µm.
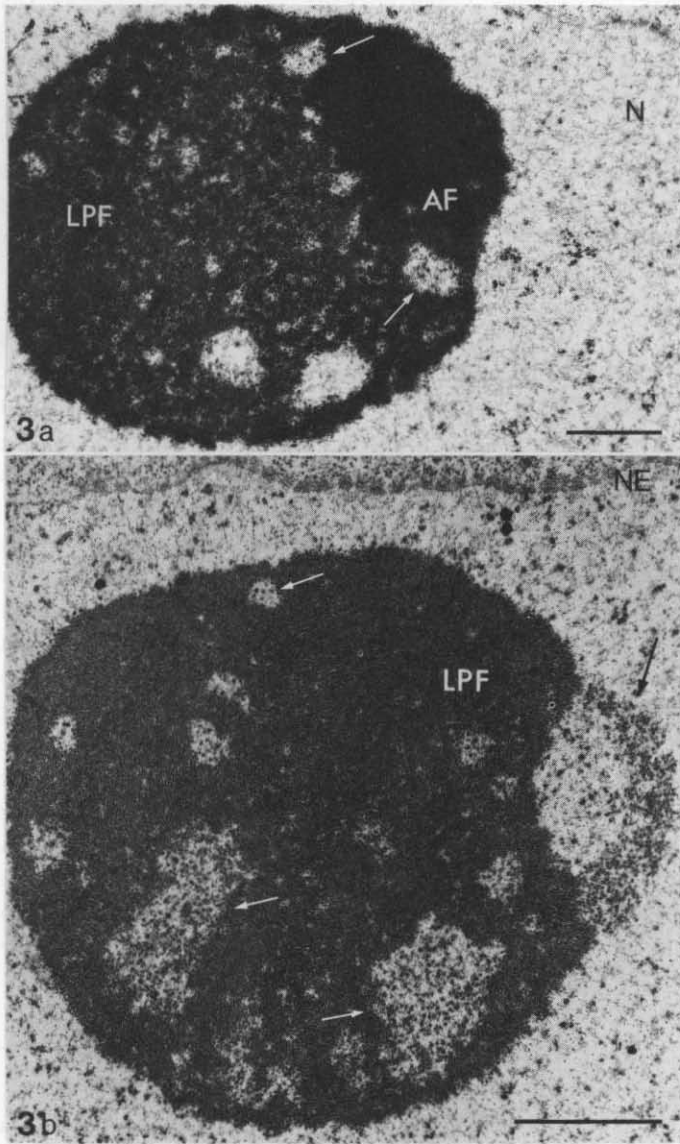
Fig. 3 a,b. Typical ultrastructure of peripheral nucleoli as revealed
in ultrathin sections at early lampbrush oocyte stages of T. alpes-
tris. Note the separation of zones with densely packed fibrils (AF
in Fig. 3a) from zones with loosely packed fibrils (LPF), and vacuo-
lized regions in which some granular particles are identified
(arrows). Fig. 3b represents the characteristic appearance of a more
advanced state of nucleolar transcriptional activity in which most of
the fibrils are relatively dispersed. N, nucleus; NE, nuclear
envelope; scales indicate 1 µm.

in lampbrush chromosome stage oocytes [22]. This suggested that ribosome biogenesis is not a continuous process throughout oogenesis but that at the onset of yolk platelet deposition the synthesis of the high molecular weight rRNAs is dramatically increased until the oocyte has reached its final size, whereupon rRNA production is again reduced (for similar data obtained in Xenopus laevis oocytes see [14-18]). It should be emphasized, however, that rRNA synthesis is never completely suppressed but that in all oocyte stages examined a significant amount of synthesis takes place (see also [23]).

The amplified nucleoli of previtellogenic oocytes of Triturus alpestris are closely associated with the inner nuclear membrane and consist of a dense fibrillar mass without a typical pars granulosa (Fig. 1b, inset). In spread and positively stained preparations (for methodology see [13,24]) these fibrillar aggregates became partially unravelled and were shown to consist of long strands of nucleolar chromatin (Fig. 1a). Typical "matrix units", i.e. transcriptional units characterized by the attachment of ribonucleoprotein fibrils that contain the growing precursor molecules for the rRNAs, could be found at a few sites within these aggregates, most of which were composed of "naked", i.e. transcriptionally inactive, rDNA-containing axes (for nomenclature see [12,13,24]). Most of the lateral fibrils, however, were separated from each other by relatively large distances compared to the maximal possible packing (see below), resulting in incomplete, "diluted" matrix units which contained 10 to 80 lateral fibrils per 2.8 /um of axial length, i.e. the average length of a "normal" matrix unit [12,24]. The relatively low number of lateral fibrils per matrix unit resulted either in homogeneously diluted matrix units or in the formation of local groups of nascent fibrils alternating with fibril-free, intramatrical intervals (Fig. 2).

Fig. 4 a,b. Electron micrographs showing the appearance of a fully transcribed nucleolus isolated from a lampbrush stage T. alpestris oocyte as revealed after spreading and staining. The abundance of more than 100 complete matrix units in this nucleolar body is demonstrated in the survey of Fig. 4a. Almost all genes seem to be transcribed. Fig. 4b presents details of fully transcribed rRNA-genes of nucleolar material from the same stage. Note the close packing of the lateral fibrils within the matrix units and the occurrence of "knobs" at the free ends of the lateral fibrils attached to the more terminal regions of matrix units. Scales indicate 5 /um (Fig. 4a) and 1 /um (Fig. 4b).
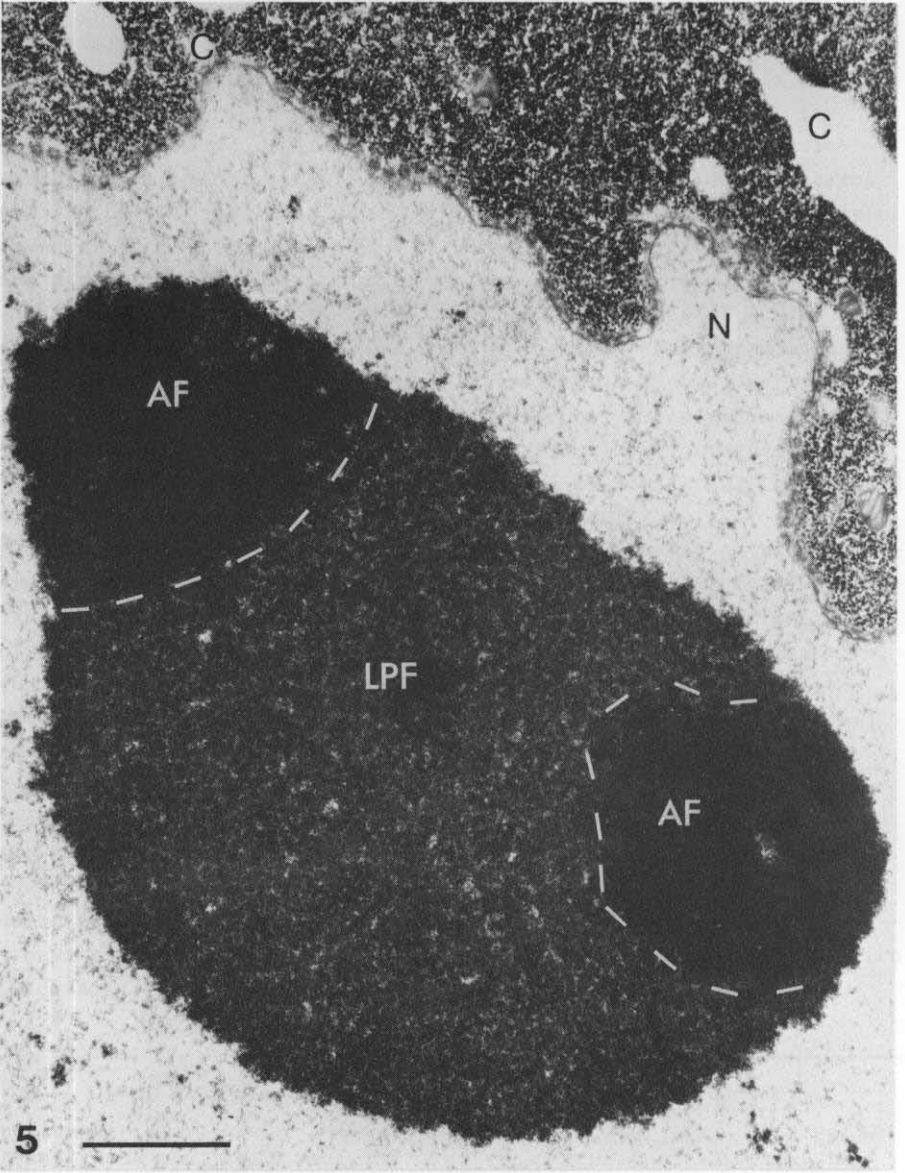
4 a

4 b
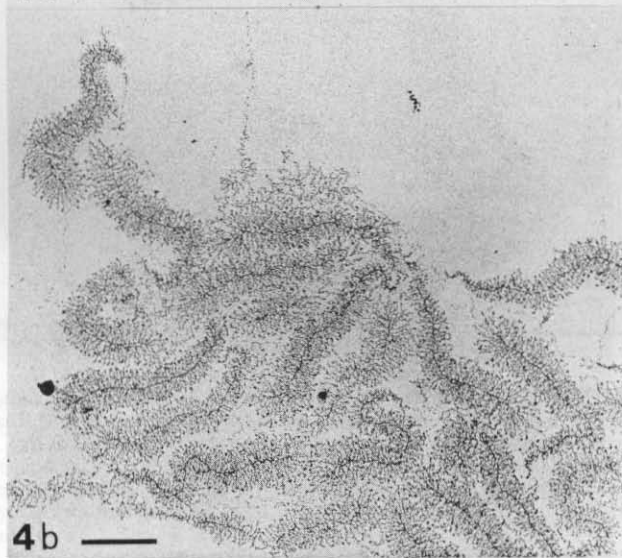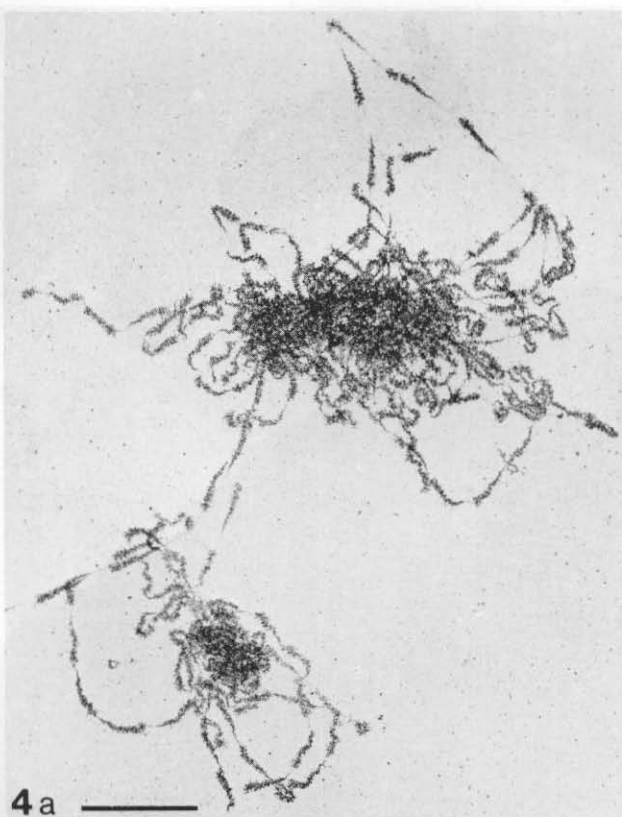
Fig. 5. Electron micrograph of an ultrathin section through the nuclear (N) periphery of a mature T. alpestris oocyte. The nucleoli show a "segregation" into zones with densely packed fibrillar aggregates (AF), usually somewhat excentrically located, and a central zone with looser packed fibrils (LPF). Note the high ribosome density in the cytoplasm (C). Scale indicates 1 /um.

Fig. 6. Spread preparation of a nucleolus from a mature oocyte of
T. alpestris. A large portion of the rDNA is not at all transcribed
and appears in the form of large aggregates of "naked" axial fibrils
(lower right). Fully transcribed rRNA-genes are found adjacent to
this transcriptionally inactive nucleolar chromatin and are connec-
ted to it by extended axial fibrils without visible transcription
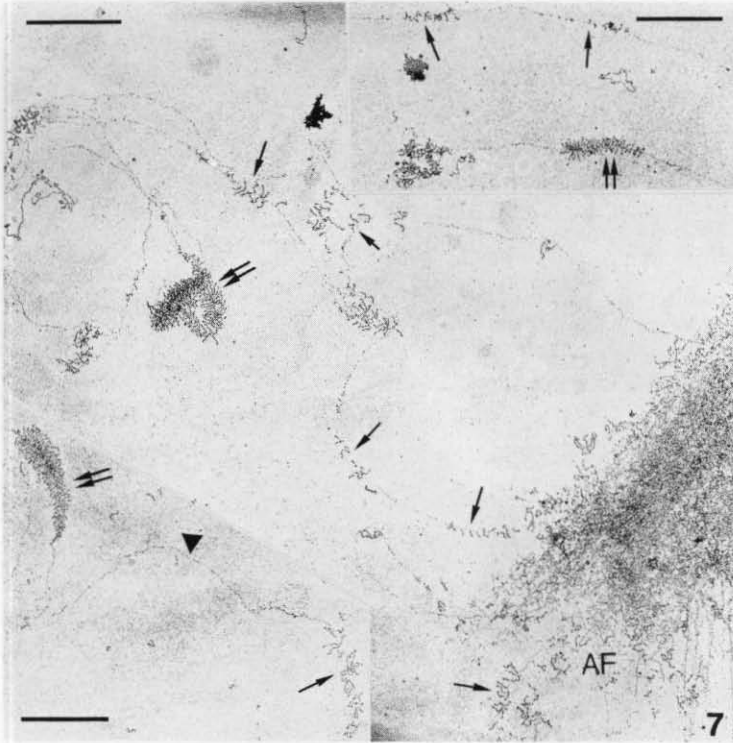complexes (arrows). Scale indicates 2 µm.

Fig. 7. Spread rDNA-containing axes of nucleoli from mature oocytes of T. alpestris, showing the coincident occurrence of different degrees and forms of reduction in lateral fibril density in adjacent matrix units. Fully transcribed or almost fully transcribed matrix units (double arrows) alternate with intercepts that show a much larger spacing (arrows) or a complete absence of lateral fibrils (e.g. at the triangle in the lower inset). Note that non-transcribed regions are much less readily spread and tend to appear as fibrillar aggregates (AF). Scales indicate 2 /um.

Fig. 8 a,b. Sequence of 15 successive rRNA-genes, revealing the typical appearance of reduced nucleolar transcription activity in the mature T. alpestris oocyte. The individual cistrons are numbered in sequence. Note the wide range of morphological forms, from almost complete matrix units (e.g. No. 15; see also the lower left of Fig. 8a) to totally untranscribed intercepts (e.g. Nos. 1,13,14) and the whole spectrum of cistrons with intermediate forms of coverage with lateral fibrils. The arrows in the upper part of Fig. 8a and the bottom part of Fig. 8b denote the same marker structure and allow the identification of this axis. Scale indicates 2 /um.

8a **8b**
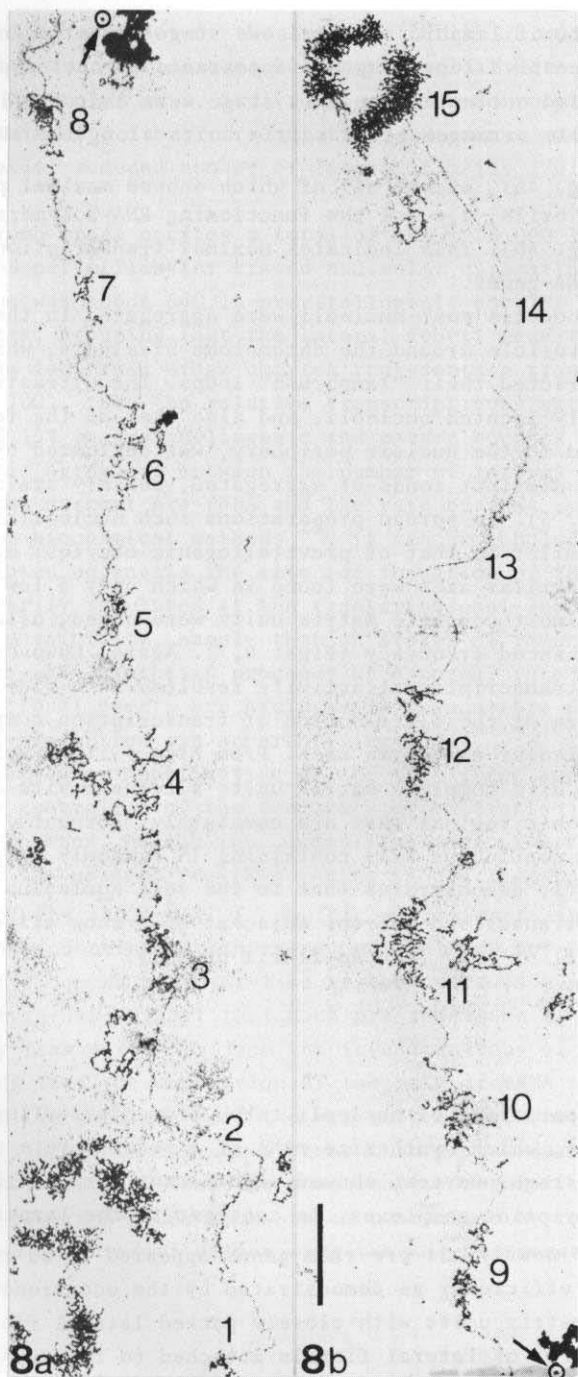
The nucleoli of lampbrush chromosome stage (vitellogenic) oocytes showed a progressive loosening and appearance of nucleolar granules (Fig. 3). Spread nucleoli from this stage were characterized by the closest possible arrangement of matrix units along the rDNA-containing axes (Fig. 4a), almost all of which showed maximal packing of the lateral fibrils, i.e. of the functioning RNA-polymerase A molecules (Fig. 4b). This indicates maximal transcriptional activity of the pre-rRNA-genes.

In mature oocytes most nucleoli were aggregated in the center of the germinal vesicle around the chromosome bivalents, which at this stage had retracted their "lampbrush" loops. The ultrastructure of these centrally located nucleoli, and also that of the few nucleoli which remained in the nuclear periphery, was dominated by the appearance of distinct zones of aggregated, densely stained fibrillar material (Fig. 5). In spread preparations such nucleoli showed an appearance similar to that of previtellogenic oocytes: aggregates of "naked" fibrillar axes were found in which only a low number of complete or almost complete matrix units were noted, often in a regionally enhanced frequency (Figs. 6,7). Again, however, the reduction of transcriptional activity resulted in a widely hetero-geneous pattern of the distribution of transcription complexes along the nucleolar chromatin axes. From Fig. 7 it is evident that along a given axis complete matrix units alternate with dilute units or even cistronic regions that are completely "dormant". Fig. 8, which shows a continuous axis containing 15 tandemly arranged repeating units, demonstrates that in the same nucleolus some genes can be fully transcribed whereas adjacent ones show either no activity at all or only reduced fibril density.

3. Discussion

Spread preparations of nucleoli taken from previtellogenic or mature oocytes, which synthesize rRNA at a reduced rate as compared to lampbrush stage oocytes, showed significant morphological changes of the transcription complexes. In contrast to the lampbrush stage oocytes where nearly all pre-rRNA-genes appeared to be transcribed with maximal efficiency as demonstrated by the occurrence of tandem-ly arranged matrix units with closely packed lateral fibrils [12,24], the total number of lateral fibrils attached to the rDNA-containing axes was significantly lower in previtellogenic and mature oocytes.

The resulting arrangement of the lateral fibrils in nucleolar chromatin strands revealed a marked morphological heterogeneity. On the same nucleolar axis, individual or grouped complete matrix units alternate with fibril-free cistronic segments and matrix units with a variable, reduced number of lateral fibrils ("diluted matrix units").

In lampbrush stage oocytes a total of about 23,000 lateral fibrils were attached per millimeter traced nucleolar chromatin axis, whereas this value was about 640 in previtellogenic oocytes and 3,350 in mature oocytes. Assuming that the lateral fibril density present in nucleoli from lampbrush stage oocytes represents a transcriptional activity of 100%, then the relative transcriptional activity is about 3% and 15% in previtellogenic and mature oocytes respectively. From the basic agreement between the number of lateral fibrils containing the nascent pre-rRNA and the rate of rRNA synthesis as determined by biochemical methods [22], it can be concluded that during amphibian oogenesis the rate of formation of 28S and 18S rRNA is primarily regulated at the transcriptional level. The alternative possibility, namely that in stages of reduced ribosome formation pre-rRNA synthesis proceeds at a normal rate and posttranscriptional events are exclusively responsible for the reduced rRNA production, is apparently excluded.

The activation or inactivation of the rRNA-genes apparently is individually controlled by the frequency of initiation events by the RNA-polymerases. Mechanisms controlling exclusively the elongation rate of the nascent pre-rRNA chains should lead to the appearance of the whole set of complete matrix units in stages of reduced rRNA production. Furthermore, our data do not support the concept that a common promotor-like region controls a whole set of genes [25] but rather suggest that each pre-rRNA-gene is regulated individually (see also [26]). From the investigations of Roeder [27], it is obvious that the modulation of the rate of rRNA synthesis is not due to different levels of the activity of the nucleolar RNA-polymerase, since this enzyme is present in excess during all stages of amphibian oogenesis. Thus the quantitative regulation of transcription of pre-rRNA-genes seems to be controlled by regulatory factors [28] which interact either with the RNA-polymerases or with the template.

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