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A Novel Type of Chromatin Organization in Lampbrush Chromosomes of *Pleurodeles waltlii:* Visualization of Clusters of Tandemly Repeated, Very Short Transcriptional Units

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A novel chromatin configuration is described in lampbrush chromosomes of *Pleurodeles waltlii* oocytes which is different from transcriptionally inactive chromatin as well as from the various forms of transcribed chromatin hitherto described. This novel type of chromatin is not arranged in Christmas tree-like configurations of densely packed lateral ribonucleoprotein (RNP) fibrils but is characterized by a periodic alternating pattern of thick and thin regions which occur in clusters of some 10,000 repeats. Each thickened unit with an average length of 45 nm contains two closely spaced particles, the putative RNA polymerases, and each thickened unit is separated from the next one by a beaded chromatin spacer with a length of about 80 nm. This chromatin spacer contains on average two particles of approximately 14 nm in diameter, assumed to be nucleosomes. The thickened regions are interpreted to represent short transcriptional units containing approximately 130 base pairs of DNA which are separated from each other by nontranscribed spacers of 240-400 base pairs of DNA. The possibility is discussed that these transcriptional units represent 5S rRNA or tRNA genes.

Key-words: *Lampbrush chromosomes - Amphibian oocytes - Transcription units - Electron microscopy.*

INTRODUCTION

The electron microscopic spreading technique introduced by Miller and Beatty (34) allows the identification of chromatin regions of different functional states. Transcribed chromatin stretches are readily recognized by the occurrence of lateral RNP fibrils, i.e. nascent transcripts attached to the chromatin axis via basal RNA polymerase granules (reviewed in refs. 15, 16, 30, 33, 52). Transcriptionally inactive chromatin, on the other hand, is free of lateral fibrils and usually folded into nucleosomes giving rise to the characteristic "beads-on-a-string" aspect of chromatin filaments (37, 38, 57). A third category of chromatin is also free of transcriptional complexes but exhibits a smooth, non-nucleosomal contour: examples are the spacer regions between transcribed, tandemly arranged ribosomal RNA (rRNA) genes (14, 16, 24, 44, 46), regions corresponding to rRNA genes which are not yet actually transcribed but already activated (11) and minor proportions of non-nucleolar chromatin of the slime mold *Physarum polycephalum* (53) and yeast (41).

As shown previously, a nascent ribonucleoprotein (RNP) molecule has to reach a certain length before it can be detected as a lateral RNP fibril extending from the polymerase granule (13). It has been concluded that transcriptional units coding for RNA molecules of less than 150,000 to 350,000 molecular weight cannot be recognized by their transcriptional products because their nascent transcripts are below the critical size of resolution (13, 25). However, since under appropriate staining conditions RNA polymerase granules are significantly larger and show more contrast than nucleosomal particles (c.f. 14, 24, 25) such small transcription units should, provided that they are transcribed at maximal polymerase packing density, be visible as linear arrays of closely spaced RNA polymerase particles. While a solitary small transcription unit embedded in a nucleosomal chromatin strand and containing only a few RNA polymerases would almost certainly escape detection, highly reiterated and tan-

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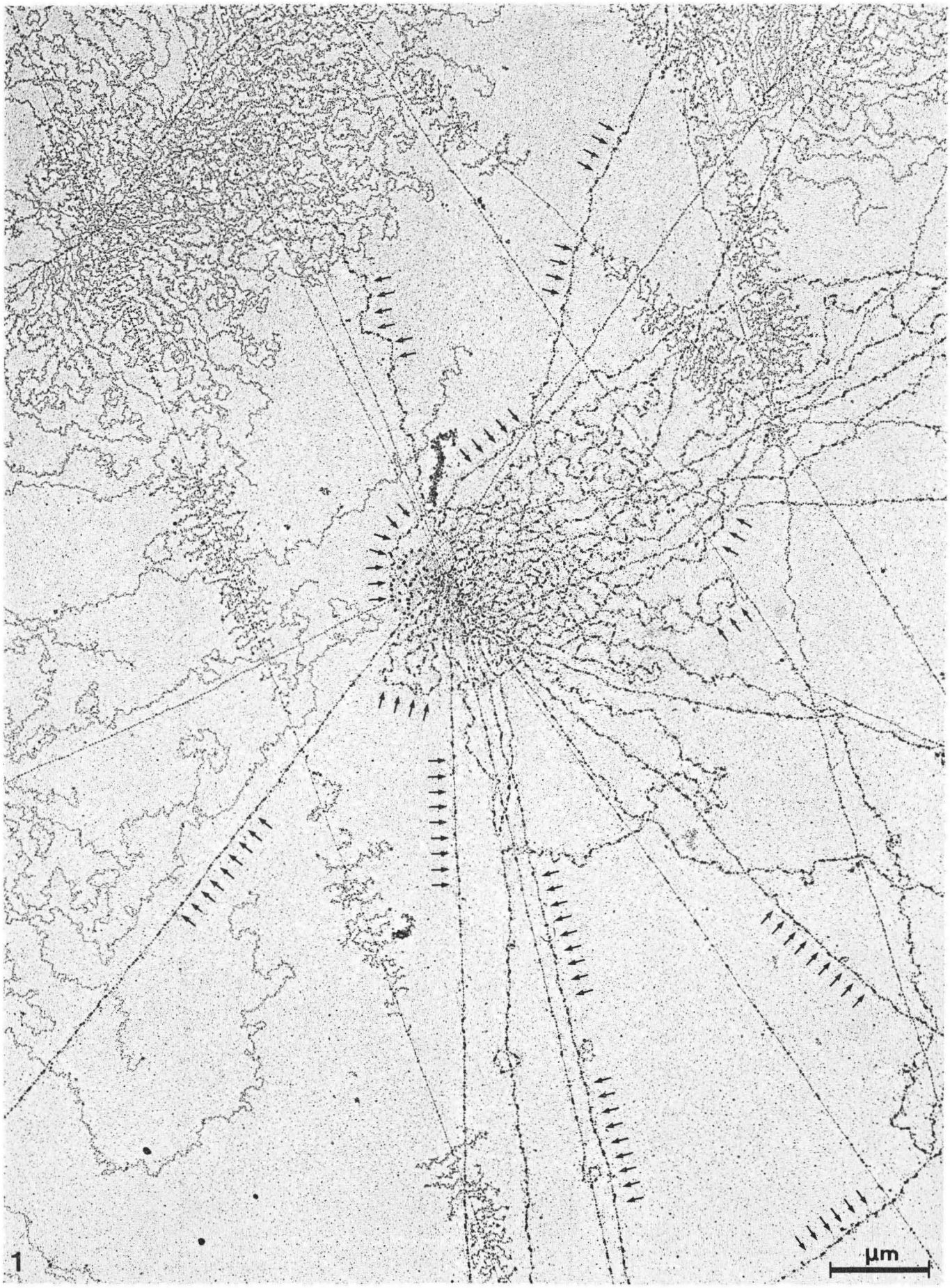
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FIGURE 1. — Survey micrograph of spread chromatin from a growing *Pleurodeles* oocyte showing a part of a transcribed lampbrush chromosome loop (upper left corner), several tandemly arranged transcription units of ribosomal RNA genes and an aggregate of filaments characterized by a periodic thin thick pattern (denoted by the arrows); × 19,000.

demly arranged small genes such as 5S rRNA or tRNA genes should be much easier to identify. In amphibian species the multiplicity of these two gene classes is at least ten thousand and they are arranged in clusters restricted to a few or even a single chromosomal locus (reviewed in ref. 28). In addition it is known that they are transcribed at maximal rate during the growth stage of oocytes (reviewed in ref. 54). In the present communication I describe a novel type of chromatin configuration observed in spread preparations of lampbrush chromosomes from *Pleurodeles waltlii* which is different from typical transcription units so far known as well as from inactive chromatin and is interpreted to represent the transcription of highly reiterated, tandemly arranged short genes.

MATERIALS AND METHODS

An ovary piece was removed from an anaesthetized *Pleurodeles waltlii* female and placed in modified Barth medium (12). A mid-sized oocyte (1.0-1.2 mm diameter) was selected, transferred into buffered "3:1-medium" (75 mM KCl, 25 mM NaCl, 10 mM Tris-HCl, pH 7.2) and the nucleus was manually isolated under a dissecting microscope. The nuclear content was dispersed for about 30 min in a droplet of low salt buffer (0.1 mM Na-borate, pH 8.5-9.0) placed on a siliconized glass slide and then centrifuged through 1% formaldehyde (prepared from paraformaldehyde), 0.1 M sucrose, 0.1 mM borate buffer (pH 8.5-9.0) onto a freshly glow-discharged, carbon-coated grid essentially as described (34, 35). After centrifugation the electron microscopic grid was briefly immersed in 0.4% Kodak Photo-flo, air dried, stained in ethanolic 1 % phosphotungstic acid, dehydrated in 100% ethanol and air dried. Finally the preparation was rotary-shadowed with platinum-palladium (80:20) at an angle of ca. 7°. Micrographs were taken with a Zeiss EM 10 electron microscope operated at 60 kV.

RESULTS

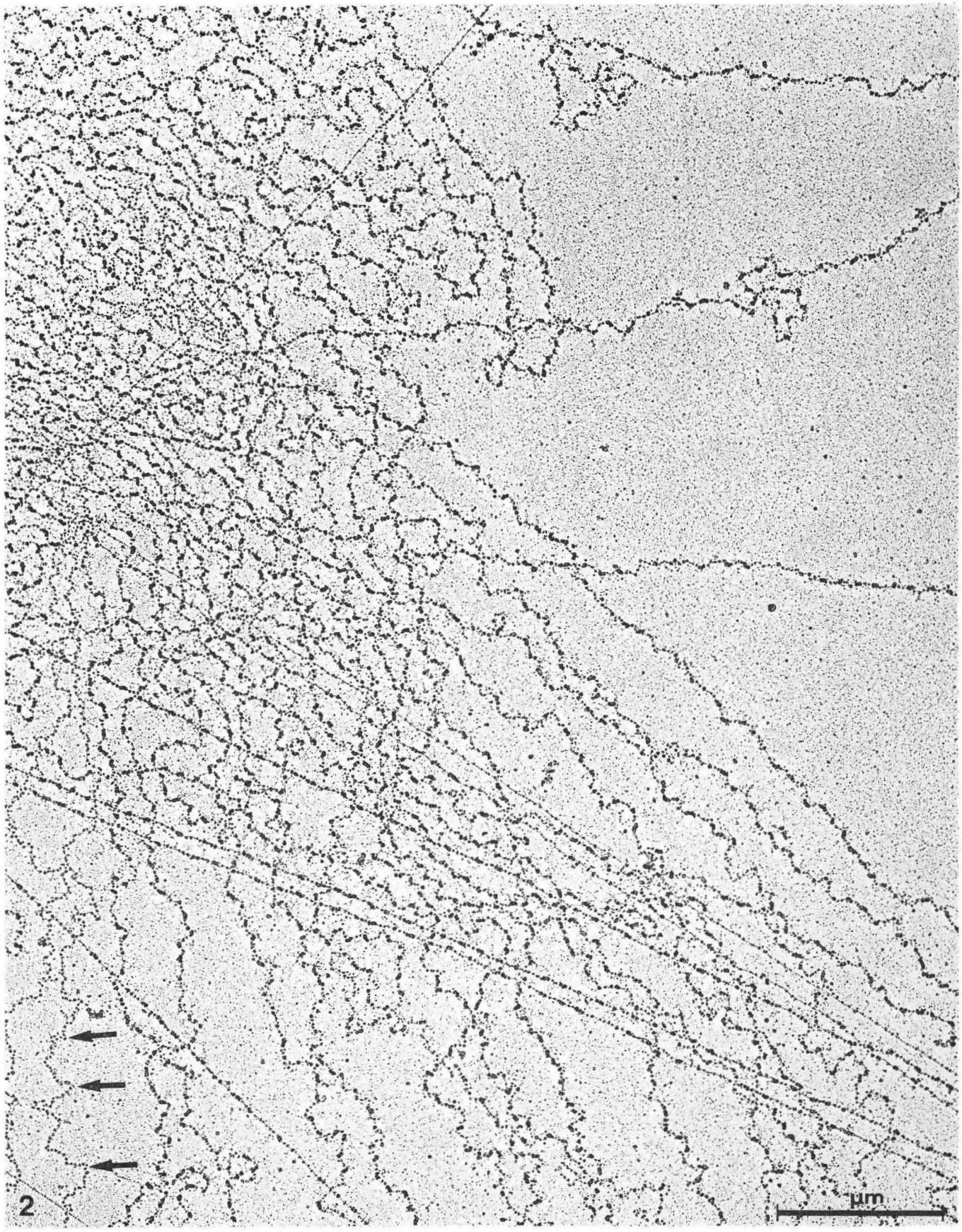
In spread preparations of chromatin from growing *Pleurodeles* oocytes transcribed lampbrush chromosome loops and rRNA genes predominate (Fig. 1; for a detailed morphological description see refs. 16 and 52). Occasionally, foci of fibrillar aggregates were found from which long filaments extend (Figs. 1 and 2). Unlike nucleolar chromatin and the chromatin of lampbrush chromosome loops, these filaments were not associated with visible lateral RNP fibrils. However, they were also clearly distinguished from the non-transcribed, uniformly beaded nucleosomal chromatin strands which were often intermingled with or closely adjacent to them by the occurrence of a periodic alternating pattern of rather short thick and thin regions (Figs. 2 and 3). In a preparation made from a single oocyte nucleus usually only one or two such filamentous aggregates could be found, often containing at least 10,000 of these tandemly arranged, thickened regions. The longest individual filament traced had a length of 67 μm and contained about 530 serially arranged repeat units. At higher magnification, it became apparent that each thickened unit with an average length of 45 nm usually contained two closely spaced granular particles, which in metal shadow cast preparations, appeared with a diameter of 20-22 nm (Figs. 3*a*-3*d*). The regions interspersed between the thickened units with an average length of 80 nm revealed a beaded organization similar to that of inactive chromatin. These interspersed regions ("spacers") usually contained two beads of nucleosomal size (diameter of about 14 nm) but not infrequently three or only one were also found (Figs. 3*a* and 3*b*). The length variability of the beaded spacer regions was not distributed in an apparent pattern along a given filament but seemed to occur at random.

DISCUSSION

The filaments described in the present study are characterized by periodically arranged thick and thin regions and are therefore clearly different from chromatin fibers displaying the typical beaded nucleosomal organization as well as from transcriptional units of rRNA genes and the large transcriptional units of lampbrush chromosome loops (16, 52). They are also different from the tandemly reiterated non-ribosomal transcription units recently described in lampbrush chromosomes of *Pleurodeles* (45) by a much smaller repeat size and the apparent absence of lateral RNP fibrils. Furthermore, the regular pattern of thick and thin regions distinguishes them also clearly from non-

FIGURE 2. — Same preparation as in Fig. 1 showing a cluster of filaments with numerous tandemly repeated thickenings. Note that beaded nucleosomal chromatin fibers adjacent to these filaments (e.g. in the lower left, arrows) are morphologically clearly distinguished; × 31,000.

FIGURE 3. — At higher magnification the tandem arrangement of thick regions with interspersed beaded "spacers" is clearly seen (a-d). The structural appearance of adjacent nucleosomal chromatin strands (arrows in b) is different from these filaments. Each thickened region usually contains two closely spaced 20-22 nm thick particles and is separated from the next one by a beaded chromatin spacer containing on average two particles of nucleosomal size (arrows in c and d). a, × 78,000; b, × 118,000; c, × 244,000; d, × 160,000.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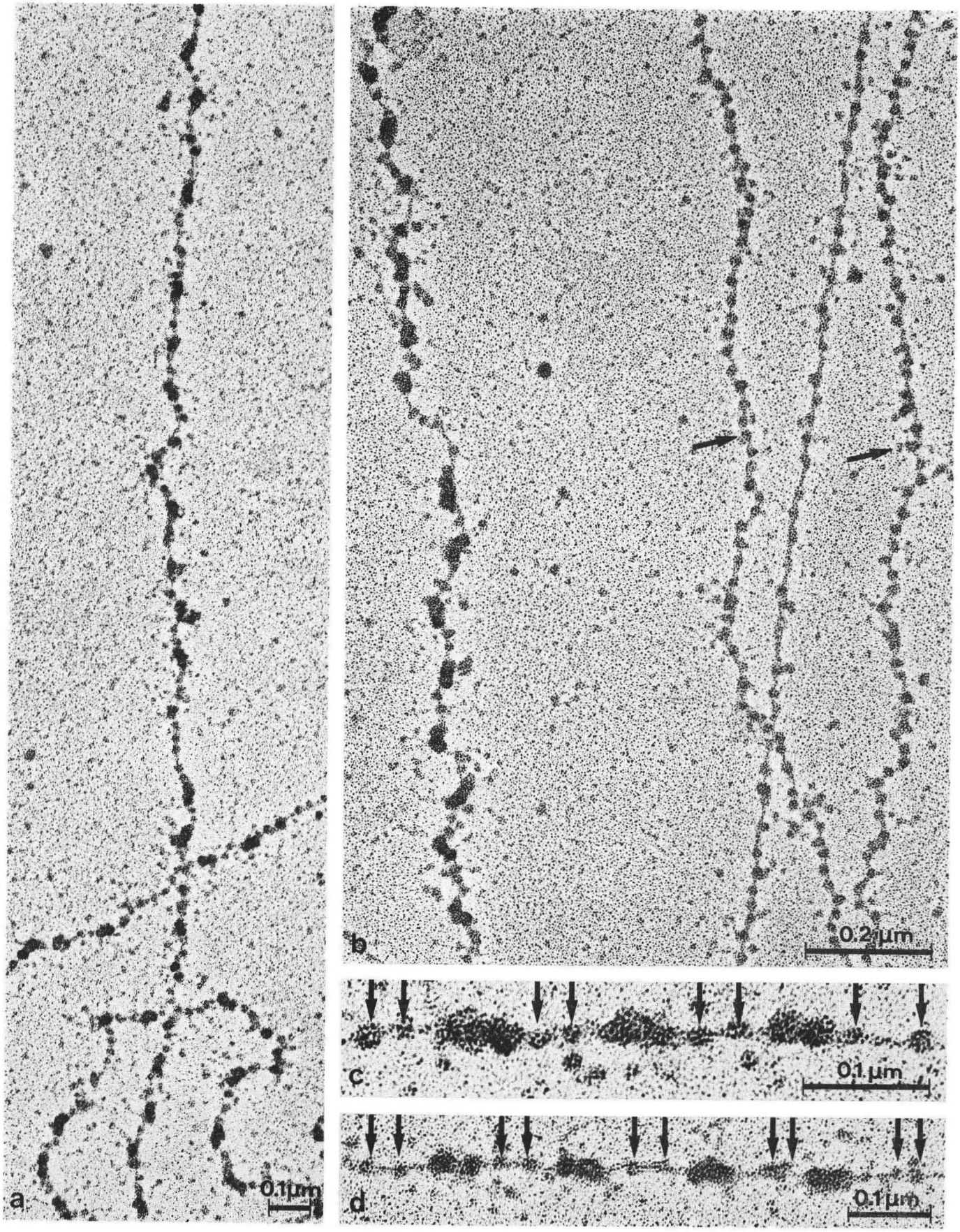
a
0.1 µm
b
0.2 µm
c
0.1 µm
d
0.1 µm

chromatin skeletal filaments present in amphibian oocytes (17, 36).

The two granular particles occurring in each thickened region of a repeat unit resemble in size and staining the RNA polymerase granules which serve as attachment sites of nascent transcripts to nucleolar and nonnucleolar chromatin of *Pleurodeles* oocytes (1, 14-16, 44-46, 49, 52). They are morphologically indistinguishable from the first two RNA polymerases of typical transcription units where the transcripts are still hidden in the relatively large enzyme complexes (13, 25, 45). In view of observations indicating that only transcribing RNA polymerases are stably attached to amphibian oocyte chromatin *in vivo* (e.g. 5, 47, 48, 51) these thickened, tandemly reiterated regions are interpreted as transcriptional units containing two RNA polymerases each. Assuming a fully extended state of the DNA covered by RNA polymerases in these regions, as it has also been shown for other kinds of chromatin (14, 16, 30, 43, 50, 55, 56), an average transcription unit covered by two 20-22 nm particles is estimated to contain 130 base pairs of DNA.

Although the interspersed ("spacer") regions exhibit a beaded organization it is not known whether these beads represent true nucleosomes, i.e. whether the spacer DNA is foreshortened from its B-length (for the occurrence of nucleosome-like beads without concomitant DNA compaction see 44). Therefore, the spacer DNA length is either 240 base pairs (assuming extended spacer conformation) or about 400 base pairs of DNA (assuming two nucleosomes per spacer). The total repeat unit therefore may contain 370 base pairs of DNA, if in extended form, or 530 base pairs, if packed into nucleosomes.

Which kinds of genes can be correlated with the size and pattern of arrangement of these small transcriptional units? The most likely candidates are the 5S rRNA and tRNA genes. The 5S rRNA multigene family is especially well characterized in amphibian species. The 5S gene is encoded by 120 base pairs of DNA while the spacer regions have different lengths in different amphibian species (see refs. 23 and 28 for reviews). For instance, the "oocyte-type" 5S repeating unit of *Xenopus laevis* oocytes comprises 660-870 base pairs of DNA (10, 31, 32) while that of the newt *Notophthalmus viridescens* is much shorter and consists of 231 or 269 base pairs of DNA (22). The multiplicity of the 5S rRNA genes has been reported in *Xenopus laevis* as 9,000 (4), 15,000 (20) and 24,000 (6) and it has been shown by *in situ* hybridization that the genes occur in clusters at the ends of most or all the chromosomes (39). In urodelan species the number of 5S genes might be even higher in proportion to the increase in genomic DNA content (40) and they can be clustered at a few or even a single chromosomal location (2, 3, 21, 26, 27, 40).

Primary transcripts of tRNA species are slightly smaller than 5S rRNA. For instance, in *Xenopus laevis* the primary transcripts for $tRNA^{met}$ and $tRNA^{leu}$ are 88 and 98 nucleotides long, respectively (18, 19). The overall organization and transcription of the tRNA genes resemble that of the 5S genes. The haploid genome of *Xenopus laevis* contains at least 7,800 genes coding for tRNAs with an average of about 200 genes for each tRNA species (8). The location and arrangement of eight tRNA genes has been determined within a cloned 3.18 kilobase DNA unit from *Xenopus laevis* which is clustered about 100 times at one chromosomal site (7). In two salamander species studied, all tRNA genes seem to be clustered at a single chromosomal locus (26).

Although the overall organization of DNA containing 5S rRNA and tRNA genes correlates well with the dimension and the arrangement of the transcriptional units described here, alternative interpretations cannot be excluded. For instance, they might represent transcription units of other repetitive small genes such as those coding for small nuclear RNAs (snRNAs; 9, 29, 42).

In summary, the present study describes a type of very short transcription unit without visible attached RNP fibrils. This indicates that it is possible to visualize small genes which accomodate no more than two RNA polymerases in electron microscopic spread preparations. Therefore, it should also be possible to study the transcription of cloned 5S rRNA, tRNA or snRNA genes *in vitro* or after microinjection into amphibian oocyte nuclei by electron microscopic methods.

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