Identification of a Novel Class of Tandemly Repeated Genes Transcribed on Lampbrush Chromosomes of *Pleurodeles waltlii*

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ABSTRACT   Electron microscope preparations of lampbrush chromosomes from oocytes of *Pleurodeles waltlii* have revealed a new class of tandemly repeated genes. These genes are highly active, as judged by the close spacing of nascent transcripts. They occur in clusters of >100 copies and are transcribed in units containing roughly 940 base pairs of DNA that are separated by nontranscribed spacers of an estimated DNA content of 2,410 base pairs. The size and the pattern of arrangement of these transcription units can not be correlated with any of the repetitious genes so far described.

Electron microscope analyses of transcriptional arrays on lampbrush chromosomes of amphibian oocytes have shown that most of the lateral loops contain one or a few transcription units (TUs), usually of large sizes (2, 20, 31, 32, 36, 37). Such studies have also shown that in the cases of multiple TUs present on the same loop axis, the TUs are usually dissimilar, as judged from their different contour lengths and opposing transcriptional polarities (36, 37). Although such studies have presented examples showing that even closely linked TUs do not represent repeats of a basic sequence, it is clear from biochemical and electron microscope data that several gene families of amphibians are tandemly repeated. Well-known examples are the ribosomal RNA (rRNA) genes, the genes coding for 5 S rRNA and tRNAs, and the histone genes (e.g., 3-8, 39, 43; for review see reference 26). Moreover, the resistance of certain chromosome loops to digestion with restriction endonuclease HaeIII has been taken as indication that their DNA axes consist exclusively of simple tandem repeats (18). Finally, hybridization *in situ* to nascent RNA of lampbrush loops has indicated that both middle-repetitive and highly repetitive DNA sequences are transcribed from certain loops (27, 42).

This study describes a novel family of homogeneously sized, tandemly arranged TUs, which are different, both by their contour lengths and pattern of arrangement, from repetitious genes so far known.

MATERIALS AND METHODS

Nuclei were manually isolated from midsized oocytes (corresponding to stage IV oocytes of *Xenopus laevis*; 10) of *Pleurodeles waltlii* in 75 mM KCl, 25 mM NaCl, buffered with 10 mM Tris-HCl to pH 7.2. Nuclear contents were dispersed for 10-20 min in 0.1 mM borate buffer (pH 8.5-9.0) and processed for electron microscopy essentially as previously described (31). Grids were rotary shadowed with platinum/palladium (80:20). Micrographs were taken with a Zeiss EM-10 electron microscope operated at 60 kV.

RESULTS

In most spread preparations of chromatin from individual oocyte nuclei of *Pleurodeles*, a special class of transcribed chromatin was found that was clearly different from TUs of rRNA genes and the very large non-rRNA TUs of lampbrush chromosome loops. This novel type of transcriptionally active chromatin consisted of short, tandemly repeated TUs which were separated from each other by nontranscribed spacers. TUs of this gene class were observed to occur either in dense aggregates (Fig. 1 a) or as loosely arranged networks of chromatin (Figs. 1 b, and 2 a). Some of these chromatin aggregates contained >100 such TUs, which were occasionally intermingled with transcriptionally inactive chromatin and/or long featherlike structures of unknown nature (Fig. 1 a). Along a given chromatin strand, the TUs were always arranged with identical polarity (Fig. 2 a-c). Transcribed regions could be clearly distinguished from adjacent nontranscribed spacers by the presence of closely spaced, densely stained granules presumed to contain RNA polymerase molecules. On the average 12 putative RNA polymerase molecules were attached to each repeat. The dark-staining granules were often so densely packed that individual particles could not be resolved but rather formed a uniform thickening of the transcribed chromatin. Lateral fibrils attached to these putative RNA polym-
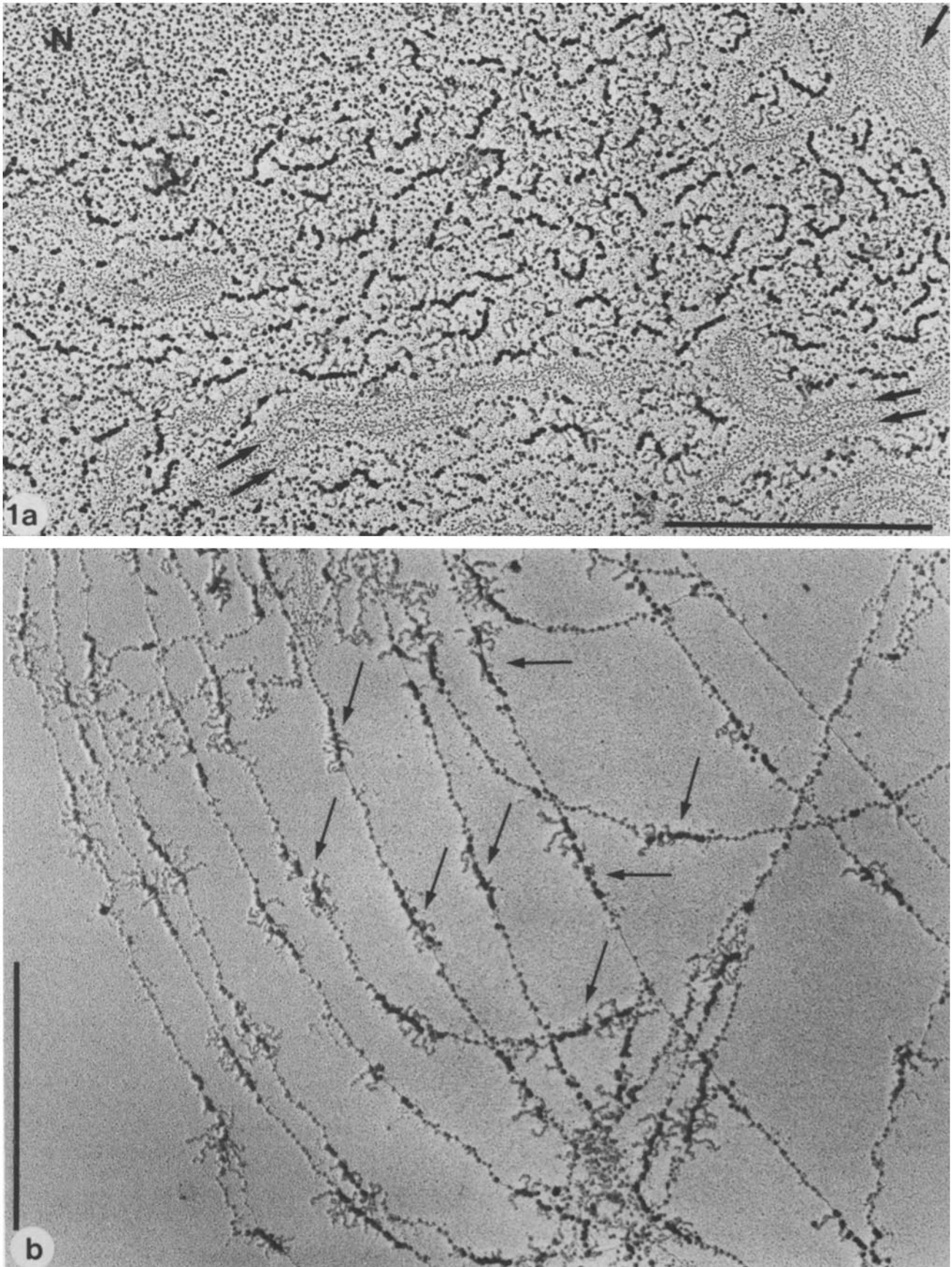
FIGURE 1 Survey micrograph showing clusters of TUs of a defined size class. These TUs occur either in aggregates, often in association with transcriptionally inactive chromatin of beaded morphology and featherlike structures of unknown nature (arrows in *a*), or in the form of a more dispersed chromatin fibril network (*b*). The alternating arrangement of TUs (some are denoted by arrows in *b*) and spacer regions is evident. Note the size difference between nucleosomes (*N*) and RNA polymerase particles. Bars, 1 μm. *a*, × 48,000; *b*, × 48,500.
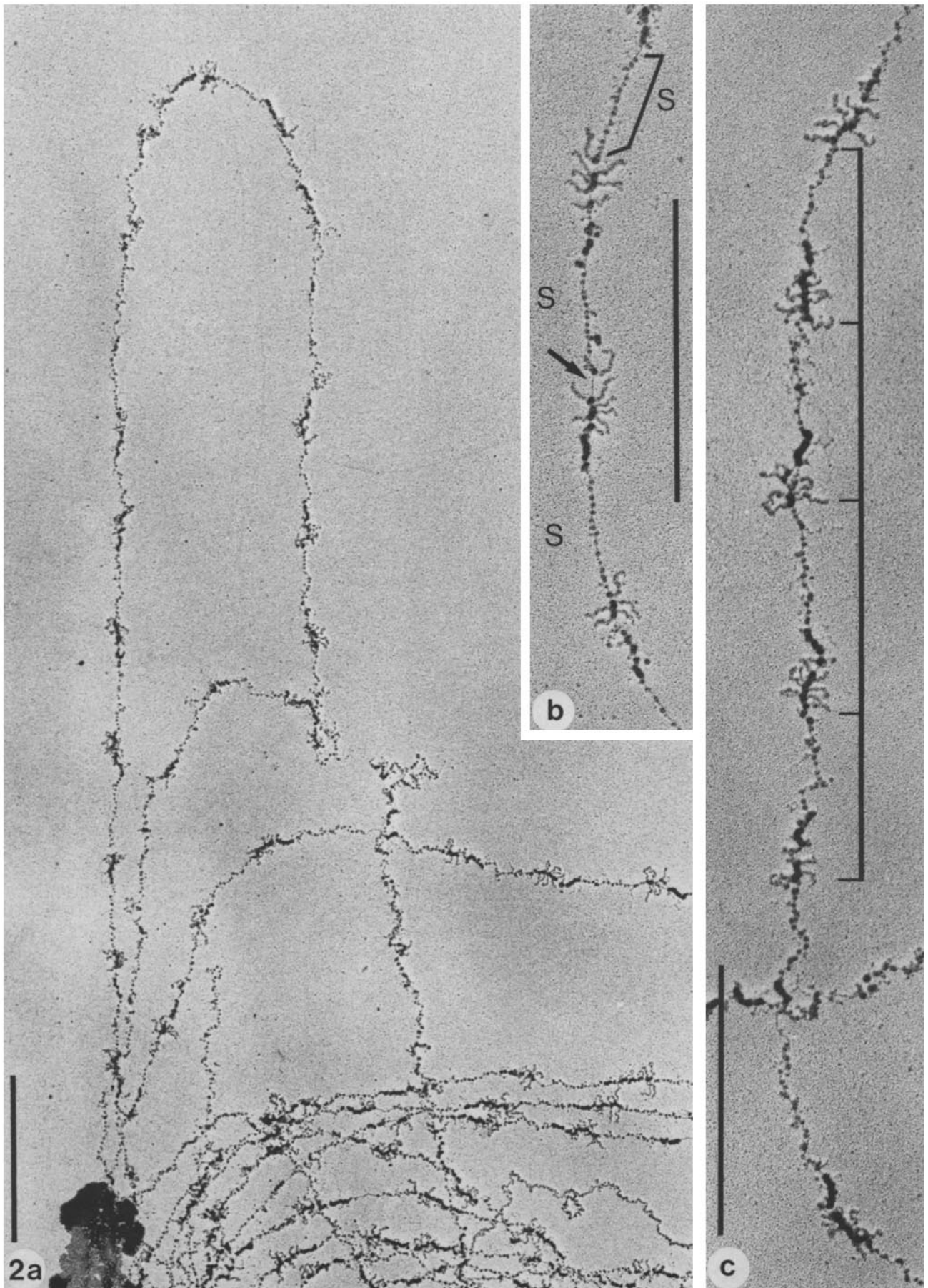
FIGURE 2 Along an individual chromatin strand, the numerous TUs are arranged with identical polarity (a–c). At higher magnification, the beaded appearance of the nontranscribed spacer regions (S) is seen (b and c). Intragenic chromatin stretches between two more distantly spaced RNA polymerase particles, however, are smooth and nonbeaded (arrow in b). Some repeating units (gene + spacer) are denoted by the brackets in c. Bars, 1 μm. a, × 29,000; b, × 50,000; c, × 56,000.

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erase granules were not detected in the regions proximal to the site of transcription initiation. This observation supports previous conclusions from studies of rRNA gene transcription that a growing RNP fibril must reach a certain length before it is detectable as a nascent fibril (15, 25). The lateral fibrils associated with more distal regions of these TUs were of uniform thickness (~14 nm) and did not show the terminal knobs typical of nascent ribosomal ribonucleoprotein fibrils.

Occasionally, putative RNA polymerase granules were spaced more distantly. In such situations, the chromatin fiber visible between them revealed a thin, nonbeaded configuration (Fig. 2b). By contrast, the nontranscribed spacer regions showed the typical beaded conformation of nucleosomal chromatin (Figs. 1b and 2b–c). The average frequency of nucleosomes in these spacer regions was 33/μm, a value similar to that reported for inactive chromatin of a variety of species, including Pleurodeles (28, 38).

The distributions of contour lengths of these TUs, nontranscribed spacers and the resulting repeating units are shown in Fig. 3. The histograms indicate that the TUs represent a homogeneous size class (mean value 0.32 ± 0.05 μm) much shorter than TUs of rRNA genes (cf. 2, 16). The TUs are separated by spacer regions showing a slightly heterogeneous length distribution (mean value 0.41 ± 0.05 μm). The mean value of the entire repeating unit is 0.74 ± 0.07 μm. These chromatin contour lengths were converted into DNA lengths by assuming a fully extended B-conformation of the transcribed DNA (13, 14, 34, 41) and a nucleosomal compaction of the spacer DNA between these genes. Thus, the TUs were estimated to contain an average of 940 base pairs of DNA. This suggests that the molecular weight of the primary RNA products of these genes is ~0.3 million or 11 S. The DNA content of the spacer regions was estimated, assuming an average DNA compaction ratio of 178 base pairs/nucleosomal unit (38; for Xenopus cf. reference 34), to be on the average 0.82 μm or 2,410 base pairs. This results in a mean value of 3,350 base pairs for the entire repeating unit.

DISCUSSION

The transcriptional arrays described here demonstrate the presence of clusters of certain nonribosomal, spacer-separated genes that are intensely transcribed on lampbrush chromosomes. The nature of these genes, however, remains unknown. Possible candidates to be discussed are the genes coding for 5 S rRNA, tRNAs, and histones. Genes coding for tRNAs and 5 S rRNAs are much shorter in all eukaryotes studied so far. Precursor molecules, interpreted to represent primary transcripts of individual tRNA genes, are ~110 ribonucleotides long (e.g., 17, 30) and the corresponding TUs (~0.04 μm contour length) could accommodate only one or two RNA polymerase particles. 5 S rRNA genes have similar dimensions as tRNA genes, and it is generally assumed that, in amphibia, the 5 S rRNA molecules (~120 residues) represent the primary gene transcript (e.g., 19, 24). Even if one allows for the possible existence of slightly larger primary transcripts (135 residues [9]), the 5 S rRNA genes of amphibian oocytes would have space only for a maximum of two RNA polymerase particles. Thus, it seems highly unlikely that the TUs described here represent tRNA or 5 S rRNA genes.

Whereas the reiteration number of histone genes is only 20–50 in Xenopus laevis (23), amphibia with higher contents of genomic DNA seem to have much higher numbers of histone genes. In Triturus cristatus, for example, a histone gene fre-
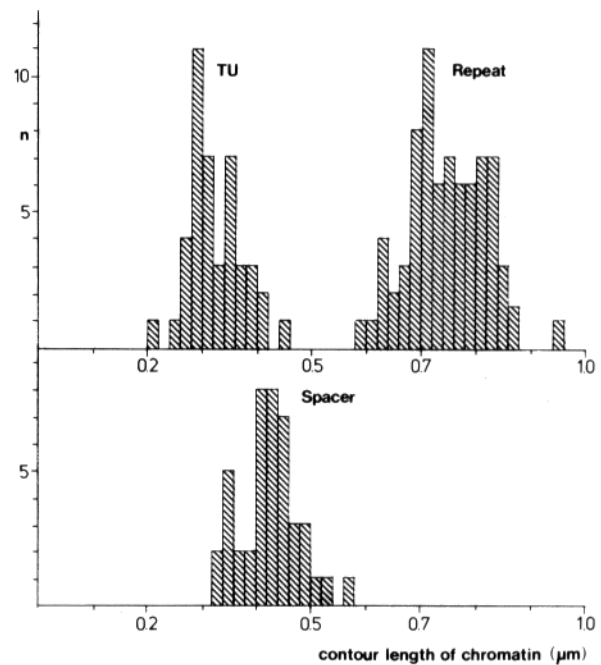

FIGURE 3 Histogram of the size distribution of TUs, spacer regions, and repeating units.

quency of 300–600 has been determined (39), and a comparably high number of histone genes may be present in the genome of Pleurodeles. Furthermore, amphibian histone genes seem to be clustered at a few loci, as shown by in situ hybridization (33). Recently, the histone repeat unit containing five of the histone genes of the newt species Notophthalmus viridescens has been cloned and analyzed (J. Gall, personal communication). The size of this repeat unit (9,000 base pairs) seems to exclude the possibility that the TUs described here represent the synthesis of a common precursor to four or five different histone mRNAs as reported for HeLa cells and sea urchin (29, 40) as well as Triturus (39). It also indicates that the TUs discussed here do not code for precursors of histone mRNAs separately initiated on the individual histone genes (21).

The genes described here could code for so-called "low molecular weight nuclear RNAs" ("small nuclear RNAs" or snRNAs [35, 44]). It has been estimated that mammals contain between 100 and 2,000 copies for the different snRNA genes (12, 22), and recently it has been shown that at least certain subclasses of snRNA genes occur in clusters (1). Although the size of the snRNA species usually ranges between 100 and 300 nucleotides, it is conceivable that their primary transcripts are considerably larger (11) and of sizes compatible to that of the TUs described here.

In summary, the repetitive TUs described cannot, at the moment, be correlated with genes of defined content. However, because they are easily detectable in electron microscope preparations, it should be feasible to identify their nature by in situ hybridization techniques at the electron microscope level and to study the regulation of their transcription in different developmental stages.

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