Ultrastructure of Gene Transcription in Spermatocytes of *Trichosia pubescens* Morgante, 1969 (Diptera: Sciaridae) *

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In spread preparations of whole spermatocytes of *Trichosia pubescens* the ribosomal transcription units could be identified by virtue of their "Christmas-tree-like" morphology. The ultrastructural features of these matrices are similar to those described in other eucaryotes. However, in contrast to the previously described systems the "spacer" unit between these matrix units is very small or non-existent at all. In addition, axial fibers displaying much longer lateral fibrils, irregularly spaced, and not as closely packed as in the ribosomal cistrons, were found. These are likely to represent active nonribosomal transcription. In a few instances these lateral fibrils show a gradual increase in their length.

Introduction

The spreading technique developed by Miller and Beatty [1, 2] for visualization of active gene in the extrachromosomal nucleoli of amphibian oocytes provided considerable information on the morphology of the ribosomal RNA cistrons. This approach has been extended to other eucaryotes [3—8]. In all these cases one can distinguish axial fibers covered with fibrils of gradually increasing length alternating, in a regular pattern, with axial fiber segments free of lateral fibrils. The fibril covered segment ("matrix unit") probably represents the transcription active ribosomal cistrons, while the fibril free segment ("spacer") is most likely an intergene sequence. This latter segment, in some instances has some fibrils attached to it, but at a much lower density than the matrix units [5, 7, 9, 10].

In some cases it has been possible to spread whole cells, rather than isolated nuclei [3, 6, 11, 12]. The ribosomal cistrons can then be identified in the basis of their close morphological similarity to that described in nucleoli of amphibian oocytes. Furthermore, in such whole cell spread preparations it has also been possible to visualize other types of transcription units, presumably of non-nucleolar origin [12, 13].

In the present work we have studied the ultrastructural features of transcriptionally active chromatin, interpreted as ribosomal cistrons, in spread preparations of spermatocytes of *Trichosia pubescens*. In addition, an attempt has been made to describe nonribosomal transcription units.

Material and Methods

T. pubescens were reared as described previously [14]. Although the detailed cytology of the spermatocytes of this species is not well documented, preliminary observations show that the spermatocytes begin to mature in the fourth instar larva just before the appearance of the eye spots and that meiosis takes place just before and during pupation. Within each larva the maturation of the spermatocytes is synchronous as evidenced by the uniform size and similar morphology of the spermatocytes at any stages in the maturation process.

The present experiments were performed on testes isolated from larvae immediately before the appearance of the eye spots and two days thereafter. The testes were dissected in 5:1 medium [7, 15] and the adhering fat body was removed. The testes were then transferred to a few drops of bi-distilled water buffered with 0.01 M borate buffer (Merck) pH 9.22 (20 ml of bi-distilled water plus five drops of borate buffer; final concentration about 0.05 mM). The spermatocytes were freed in this medium by disrupting the testes with a forceps. The cells were then collected with a micropipette and transferred to a few drops of the spreading medium in a siliconized cavity slide. Since the comercially available
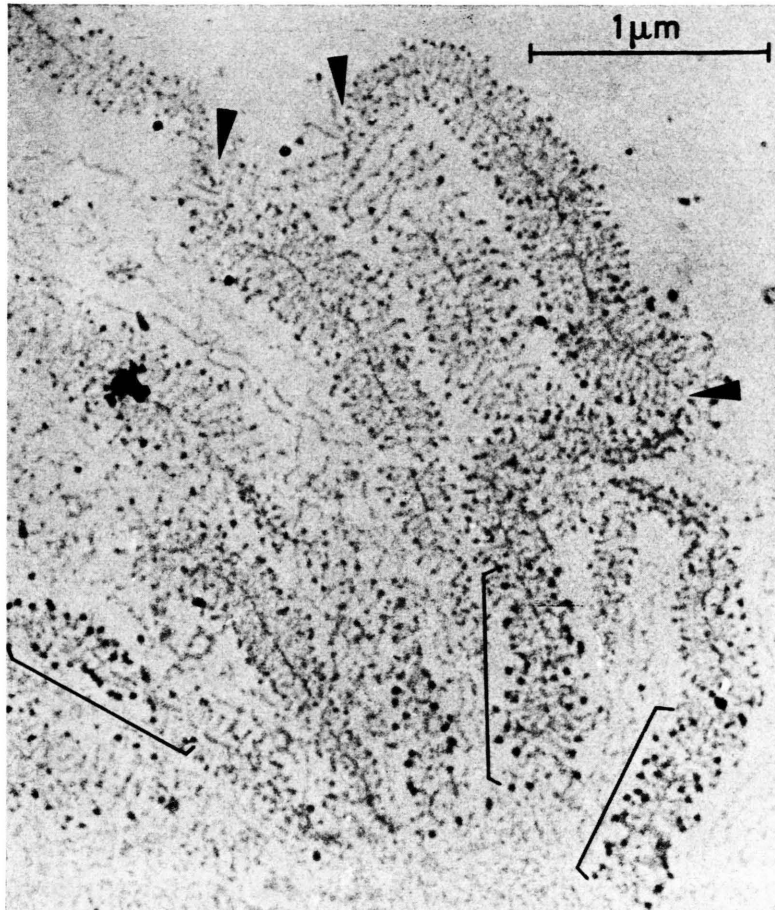
Fig. 1. Transcriptionally active (pre)rRNA cistrons as they appear in spreads
of *Trichosia pubescens* spermatocytes. Dispersed amidst the matrix units one can
distinguish long fibers free of lateral fibrils, probably inactive chromosomal fibers.
The beginning and the end of some cistrons are indicated by arrows. Some
lateral fibrils displaying unusually large knobs are indicated by brackets..
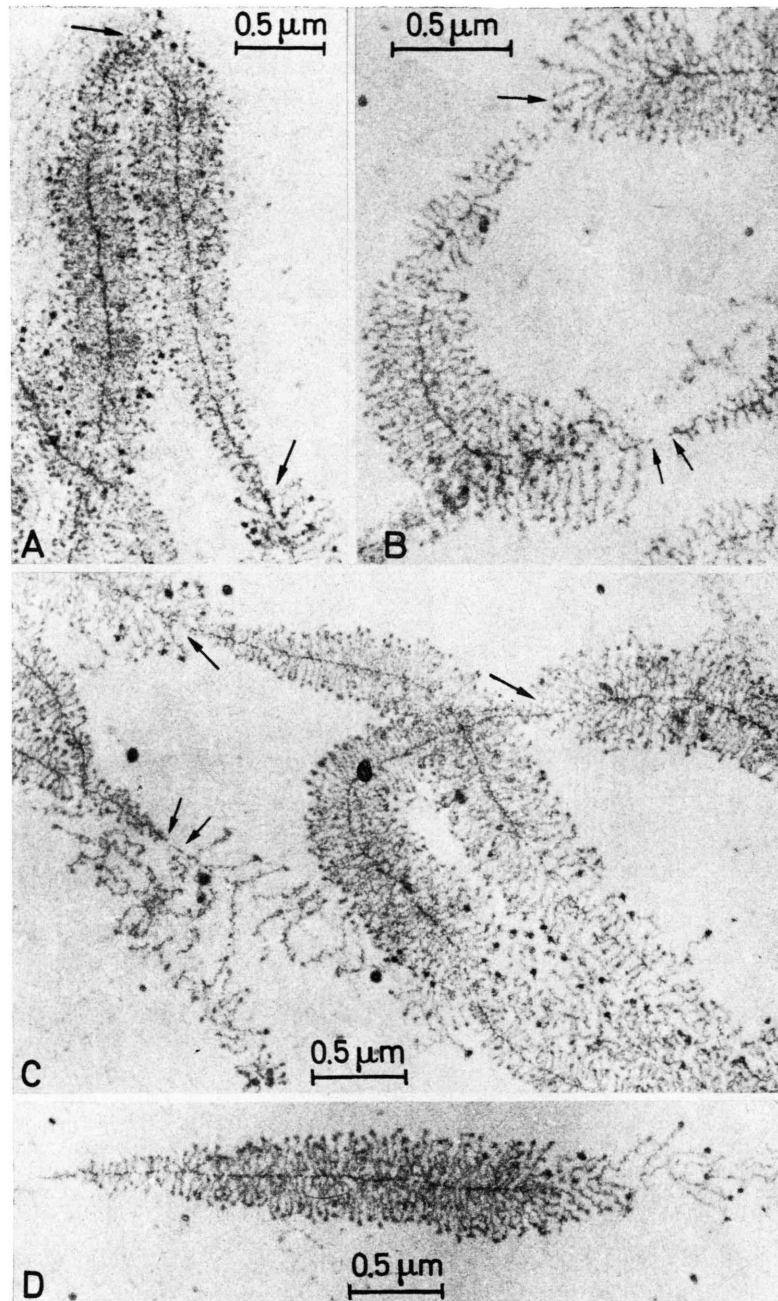
Fig. 2. Details of the ultrastructure organization of the matrix units at high magnification. The tandemly arranged matrix units do not show any intercalated segment (big arrows in A, B and C). In stretched matrices a very short "spacer" can be observed between the end of one matrix and the beginning of the next one (indicated by the small arrows). The matrix shown in D is strongly stretched in the more distal region.
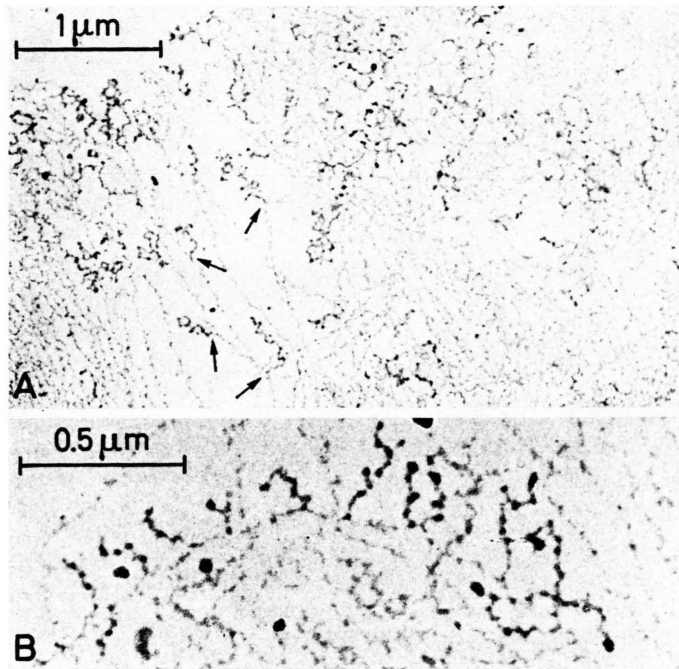
Fig. 4. Transcriptionally active chromatin fibers interpreted as sites of nonribosomal RNA synthesis. Arrows indicate the attachement of some lateral fibrils (growing RNA molecules) to the axial fibers (chromosomal fibers). In B a higher magnification of an unusually regular distribution of the lateral fibrils along the axial fiber.
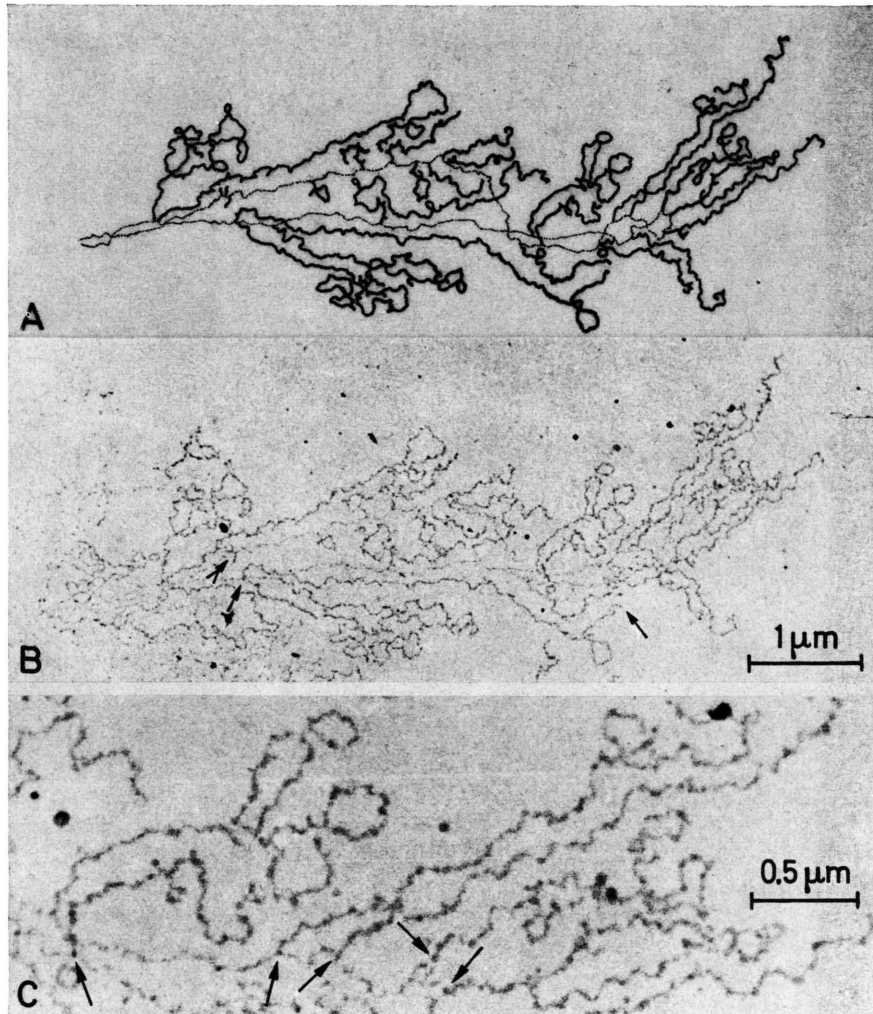
Fig. 5. A very long nonribosomal transcriptional unit displaying a short-to-long gradient of lateral fibrils. The axial fiber and the lateral fibrils observed in the electron micrograph shown in B are represented, respectivelly, by thin and thick lines in the drawing shown in A. The initiation site of the transcription unit is indicated (double arrow). The single arrows in B indicate a very long lateral fibril. Fig. 5 C is a high magnification of one section of the transcription unit shown in B. The arrows indicate the attachement points of the lateral fibrils to the axial fiber.

detergent Joy (Procter and Gamble) facilitates spreading of whole cells (see ref. 3) the spreading medium was prepared by mixing five drops of borate buffer with 20 ml of freshly bidistilled water containing four μl of a 1% solution of Joy. The spermatocytes were allowed to spread in this medium for 15 – 20 min at 20 – 22 °C. The spread material was collected on carbon coated parlodion grids according to Miller and Beatty [1, 2] as modified by Scheer et al. [10]. The grids were stained with phosphotungstic acid prepared according to Miller and Beatty [1, 2] and examined in a Philips E. M. 201 at 60 kV. The magnifications were corrected using grating replicas.

The lengths of the matrix units were measured from electromicrographs with a curvo-meter and the actual length in μm computed from micrographs of grating replicas. Each matrix unit was measured five times and averaged. A total of 43 matrices were measured to calculate the mean length of a matrix unit.

Results

In spread preparations of *T. pubescens* spermatocytes, the chromatin material appears as large aggregates of positively stained fibers, densely packed in the central region and more loosely spread in the periphery. In the well spread regions, dispersed amidst the chromatin material, it was possible to distinguish transcriptional units (matrix units) characterized by a "Christmas-tree-like" morphology. In most cases, only a few tandemly repeated matrix units spread out of the chromatin aggregates; therefore, the number of active rRNA cistrons cannot be estimated. In one preparation a single nucleolus displaying 83 matrix units could be observed. This value probably represents a minimum value for the number of active rRNA genes.

As in other systems investigated so far, the matrix unit is covered by lateral fibrils of gradually increasing length (Figs 1, 2 *). Most matrix units contain 110 – 150 very close packed lateral fibrils but sometimes units with only a few fibrils are observed. In the latter case, the fibrils were probably detached during the spreading procedure. In well spread matrices, the length of the more distal lateral fibrils ranges from 0.23 to 0.39 μm, displaying only about 1/8 of the matrix length. The length of the matrix units ranges from 2.0 to 2.7 μm (Fig. 3), with a mean length of 2.3 μm. Since the length distribution appears to be a normal one, the

matrix units are homogeneous with respect to length. The lateral fibrils, like in some other organisms bear small terminal knobs at their free end [1, 4, 5, 8]. However, in some matrices these knobs become very dense and large in the distal third of the matrix;
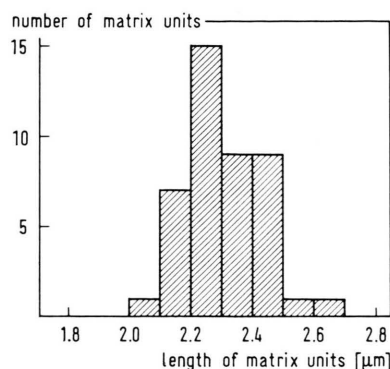

Fig. 3. Length distribution of the matrix units in spread preparations of *T. pubescens* spermatocytes.

attaining a maximum diameter of about 350 Å (Fig. 1). Within a single nucleolus, the length of the lateral fibrils with such knobs is only about 2/3 of that of the corresponding fibrils of matrices without large knobs. Similar large knobs were observed in some nucleolar matrices of *Drosophila hydei* spermatocytes [6]. In stretched matrices small strongly stained granules could be distinguished on the insertion points of the lateral fibrils to the axial fiber. These granules have been observed in other organisms and have been interpreted as RNA polymerase molecules involved in the transcription of the axial DNA fiber.

In adjacent matrix units, the termination and the next initiation sites appear to overlap, suggesting that "spacers" between the matrices are very small or non-existent at all. Only in stretched matrices one could distinguish an intercalated segment of the fiber axis free of lateral fibrils, between adjacents matrix units (Fig. 2). Even in these cases the intercalated segments are not longer than 0.07 μm. This extremely short "spacer" unit is not the result of the spreading conditions since even in fully extended cistrons the intercalated segment is hardly distinguishable. This apparent absence of "spacer" units resembles the situation observed in some giant matrix units of *Acetabularia* [7, 16].

* Figs 1 and 2 see Plate on pages 186 a and 186 b.

In addition to the matrix units described above, axial fibers diplaying only a few lateral fibrils were found dispersed amidst the transcriptionally inactive chromatin (Figs 4, 5)**. Repetitivity in these transcriptional units as well lateral knobs on these fibrils have never been observed. As compared to the rRNA cistrons, in these cases the number of lateral fibrils on a given length of the axial fiber is much smaller, the lateral fibrils are, usually, much longer and they are irregularly spaced along the axial fiber (Figs 4, 5). In some instances, a gradual increase in length was shown by the lateral fibrils attached along a given axial fiber segment. An example of this situation is shown in Fig. 5. There one can follow an axial fiber about 9.5 μm long displaying a short-to-long gradient of lateral fibrils. In this case, the more distal lateral fibril are about 4.5 μm long suggesting that, like the rRNA fibrils, these fibrils are foreshortened during the transcription process. However the possibility that these fibrils have been broken during the transcription process or during the spreading procedure cannot be excluded.

Discussion

Results obtained in the present study demonstrate that at least two kinds of gene transcription can be visualized in spread preparation of *T. pubescens* spermatocytes. As far as transcriptional units which show a "Christmas-tree-like" morphology are concerned, we assume that they represent transcriptionally active rRNA cistrons. Some circumstancial evidences lead to this conclusion: the organization of these matrices is very similar to those coding for rRNA in the extrachromosomal nucleoli of amphibian oocytes [1, 2, 10]; like the rRNA cistrons of other eucaryotes, these matrix units are tandemly repeated, and the length of the matrix unit is close to that expected for a double stranded DNA (in the B conformation) coding for RNA molecule with a molecular weight similar to that reported for (pre) rRNA molecule of several insect species. Although the exact M.W. of the (pre) rRNA in *T. pubescens* is not known it is unlikely to be much different from the M.W. reported for (pre) rRNA in other diptera; namely 2.6×10^6 D [17] and 3.3×10^6 D [18] for *Chironomus tentans*; 3×10^6 D for *Chironomus thummi* [19]; and 2.6×10^6 D for *D. hydei* [4]. The

length of DNA (in B conformation) coding for such a molecule should be $2.6 - 3.3$ μm which is close to the matrix length found here: $2.0 - 2.7$ μm.

In some matrices, lateral fibrils displaying unusual very large knobs have been observed. These large knobs are present only in the distal third of the matrix units and do not show any visible gradual increase in size in the more distal regions. The length of the fibrils displaying such knobs is reduced to about 2/3 of that of the corresponding fibrils without large knobs, suggesting that the lateral fibrils are folded up in such cases. In a few instances it was possible to observe that the first lateral fibrils displaying these very large knobs are a bit shorter than the immediately preceding one that do not have such knobs. These observations suggest that a minimal length of lateral fibrils is required for folding and/or association with proteins, resulting in such (very) large knobs. The fact that those large knobs are only observed in some cistrons is probably due to different spreading conditions in different regions of the grids [10].

In all eucaryotes so far examined a distint "spacer" unit alternates with the rRNA matrix units. The length of this intercalating segment varies in different species and the dipterous insects have the shortest one, ranging from 0.15 μm in *Chironomus thummi* [4] to 0.17 μm in *D. hydei* [6]. We report here an extremely short "spacer", if any at all, between the rRNA cistrons of *T. pubescens*. The function of such a spacer is not known, but our finding suggest that a long one is not necessarily required.

The morphology of the transcriptionally active chromatin here interpreted as nonribosomal units is quite similar to that observed in some other eucaryotes cells ([12, 13]; Glätzer, personal communication). For example: the length of the lateral fibrils is often longer than those of the rRNA cistrons and these genes are less active than the rRNA cistrons as shown by the small number of lateral fibrils attached to a given length of axial fiber. The finding of transcriptional unit of about 9.5 μm in length displaying a short-to-long lateral gradient suggests that very long RNA molecules with a molecular weight of about 9.5×10^6 D are transcribed during the maturation process of *T. pubescens* spermatocytes. Similarly, Hennig et al. [13] have observed that in *D. hydei* spermatocytes very long RNA molecules, more than 10.0 μm longer, are transcribed during the maturation process.

** Figs 4 and 5 see Plate on page 186 c and 186 d.

Note added during revision: An apparent lack of spacer between the rRNA cistrons of a green alga was described by S. Berger and H. G. Schweiger, Molec. Gen. Genet. **139**, 249 – 275 [1975].

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