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The rare transcripts of interrupted rRNA genes in *Drosophila melanogaster* are processed or degraded during synthesis

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About 50% of the ribosomal transcription units in females of the *bobbed 8* mutant of *Drosophila melanogaster* contain an intervening sequence of 5 kb in the 28S region of the gene. We analysed the transcription of ribosomal genes in this mutant using electron microscopy and found that the majority of the active ribosomal transcription units in larval fat bodies and guts are not long enough to contain the 5-kb intervening sequence; only ~1% of active transcription units have a length consistent with the presence of the 5-kb intervening sequence. Transcription units of this length show an interrupted gradient of nascent RNA fibril lengths indicative of processing or degradation during transcription. The position of the discontinuity in RNA length coincides with the position of the intervening sequence. This observation suggests that even though RNA polymerase may infrequently transcribe an entire interrupted gene, the process does not result in a full-length RNA.

Key words: rRNA genes/RNA processing/transcription

Introduction

Intervening sequences interrupting the coding regions of rRNA genes (intron$^+$ genes) of *Drosophila melanogaster* were discovered in 1977 (Glover and Hogness, 1977; Pellegrini *et al.*, 1977; Wellauer and Dawid, 1977; White and Hogness, 1977). Since then, numerous studies have analysed the influence of these sequences on the transcription of genes that contain them. The majority of studies on transcription of intron$^+$ rRNA genes in insects indicate that most of these genes are transcriptionally inactive (Glätzer, 1979; Long and Dawid, 1979; Jolly and Thomas, 1980; Long *et al.*, 1980, 1981; Kidd and Glover, 1981; Jamrich and Miller, 1982; for review see Beckingham, 1982). On the other hand, Chooi (1979) has presented evidence of vigorous transcription of these genes in *Drosophila* nurse cells. We have analysed the transcription of rRNA genes in fat bodies and guts of the *D. melanogaster* mutant *bobbed 8*. The *bobbed* (*bb*) mutants in *Drosophila* are phenotypically represented by shorter bristles and slower development. The size of the bristles can be directly correlated with the amount of rRNA synthesis (Shermoen and Kiefer, 1975), and *bb* mutants generally represent partial deletions of the nucleolus organizer (for review, see Tartof, 1975; Ritossa, 1976). Since these mutants have reduced levels of rRNA genes, it is reasonable to assume that they are under strong pressure to utilize all or most of their remaining rRNA genes to meet the requirements for rRNA at certain metabolically very active developmental stages.

From our previous study of rRNA synthesis in a similar insect system (Jamrich and Miller, 1982) we concluded that the early larval stages of development require a massive production of rRNA. Taking advantage of this demand, we analysed the transcription of these genes in the first 24 h of *D. melanogaster* larval development. We chose the mutant *bb-8* because of the finding of Tartof and Dawid (1976) that about half of the rRNA genes in females of this mutant contain a uniform 5-kb intervening sequence in the 28S region of the gene. The uniformity and relatively large size of the intron are crucial features for the electron microscopic analysis of transcription of these genes.

Results

Electron microscopic analysis of >1000 rRNA transcription units (TUs) in female larval fat bodies and guts of *D. melanogaster bb 8* showed that ~99% of these TUs must have resulted from the expression of rRNA genes which do not contain the 5-kb intron in the 28S region of the gene. Since ~50% of the ribosomal genes do have the 5-kb intron in the 28S region of the gene, one can infer that most of the intron$^+$ genes are present in a transcriptionally inactive form. Figures 1 and 2 show examples of transcriptionally inactive ribosomal chromatin which are flanked by two active ribosomal genes, and which are longer than typical spacers such as those in Figure 5. Such long stretches of inactive chromatin are not unusual and can frequently accommodate one or more inactive intron$^+$ rRNA genes. Definitive assignment of this chromatin to the intron$^+$ rRNA genes is not yet possible, but the demonstration by Tartof and Dawid (1976), and Hawley and Tartof (1983) that the intron$^+$ and intron$^-$ rRNA genes are interspersed strongly supports our assumption that in most of the cases these long segments contain inactive intron$^+$ rRNA genes.

Since it became obvious early in this study that the transcription units representing intron$^+$ rRNA genes are rather rare, special care was taken to recognize all the possible morphological manifestations of the transcription of these genes. Figure 3a shows a schematic representation of an intron$^-$ rRNA gene and the morphology of the resulting transcription unit. Transcription of a rRNA gene without an intron results in a transcription unit of ~8 kb long, whereas, the transcription of an intron$^+$ gene would result in a 13-kb long transcription unit if the 5-kb intron simply increases the length of the gene without disturbing any of the processes involved. This possibility is illustrated in Figure 3b. We were unable to find transcription units of this morphology and therefore we considered other possible morphological manifestations of transcription of intron$^+$ genes. Figure 3c illustrates morphology of the transcription unit if transcription stops at the junction of the 28S portion of the gene and 5′

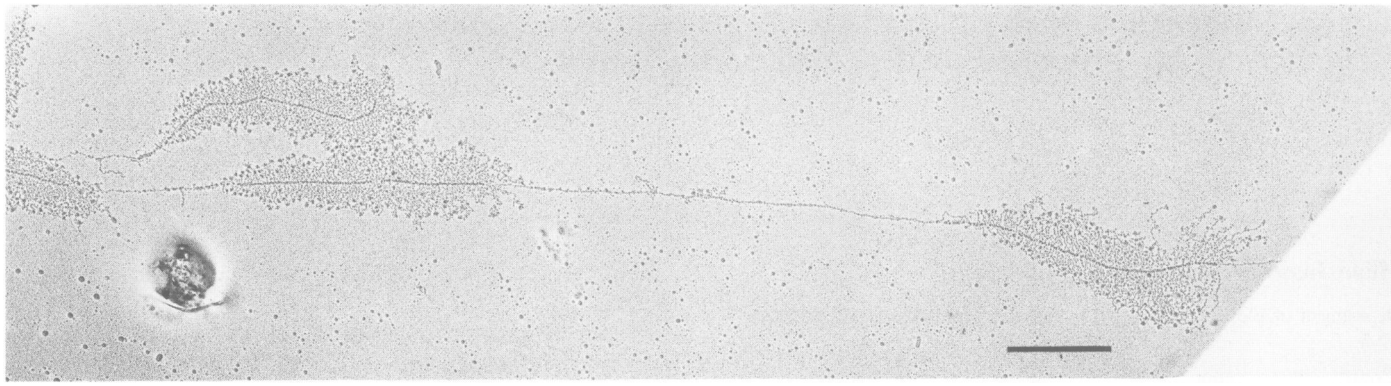

Fig. 1. Electron micrograph of active rRNA genes from *D. melanogaster bb 8* separated by a stretch of inactive chromatin. The stretch of inactive chromatin (4.52 μm) can accommodate at least 13.5 kb of DNA. In our preparations the spacer DNA generally does not have the nucleosomal appearance of the inactive chromatin and therefore the contraction ratio of the DNA in these spacers is likely to be close to B-conformation of DNA which is 1 μm = 3 kb. The preparation was made from the fat body of a 12 h old larva. Scale bar = 1 μm.

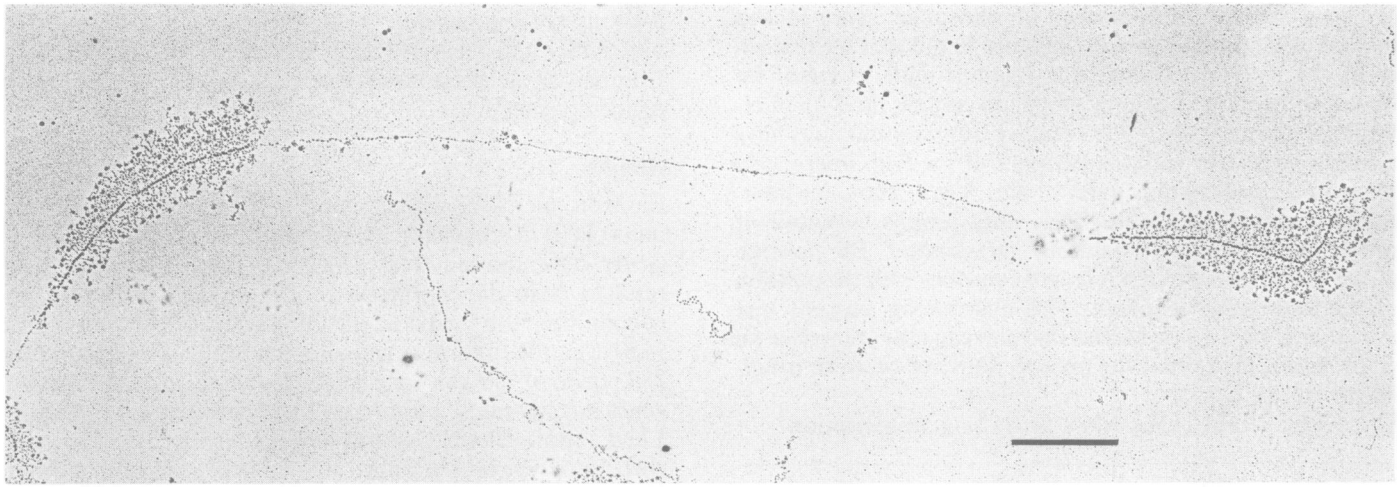

Fig. 2. Electron micrograph of active rRNA genes from *D. melanogaster* larval fat bodies. As in Figure 1, the two active genes are separated by a large stretch of inactive chromatin. The stretch of inactive chromatin (8.4 μm) can accommodate at least 25 kb of DNA. Scale bar = 1 μm.

end of the intron. Such transcription would result in a class of transcription units which are shorter than the intron$^-$ transcription units by 1.3 kb. Since 1 μm of B-DNA is equivalent to ~3 kb, the short transcription units are expected to be 2.26 μm (6.7 kb) long as opposed to the 2.66 μm (8 kb) of the intron$^-$ transcription units. Even if we assume that a difference of 0.4 μm is too small to distinguish accurately between these two classes, the intron$^+$ rRNA genes transcribed in this fashion would be followed by an untranscribed chromatin segment which would have to be long enough to accommodate at least 8 kb of DNA (5 kb intron plus the 1.3 kb second part of the 28S unit plus 2–5 kb of non-transcribed spacer).

Whereas transcription units shorter than 2.66 μm (8 kb) were observed in our preparations (Figure 4), these ribosomal transcription units were heterogeneous in length and most likely represented newly initiating rRNA genes (McKnight and Miller, 1976). The spacer DNA following these genes was typically not long enough to accommodate the rest of the intron$^+$ rRNA gene, in that the sum of the length of the short transcript and its downstream 'spacer' was less than the 16–20 kb that would have been required.

Another possibility is that the genes containing introns are actively transcribed along their length but at a drastically

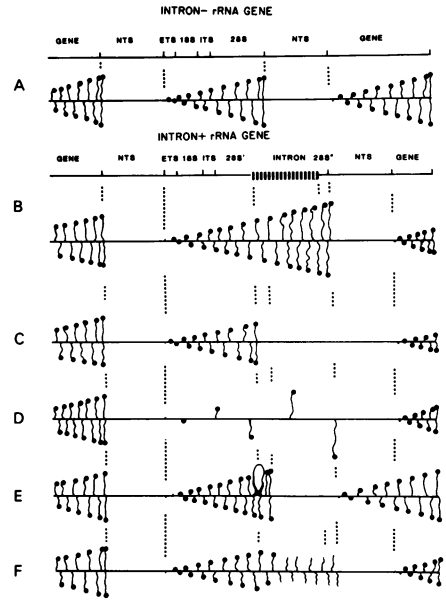

Fig. 3. A schematic representation of possible transcription unit morphologies of intron$^+$ and intron$^-$ rRNA genes. ETS, external transcribed spacer; ITS, internal transcribed spacer; NTS, non-transcribed spacer. For details see text.

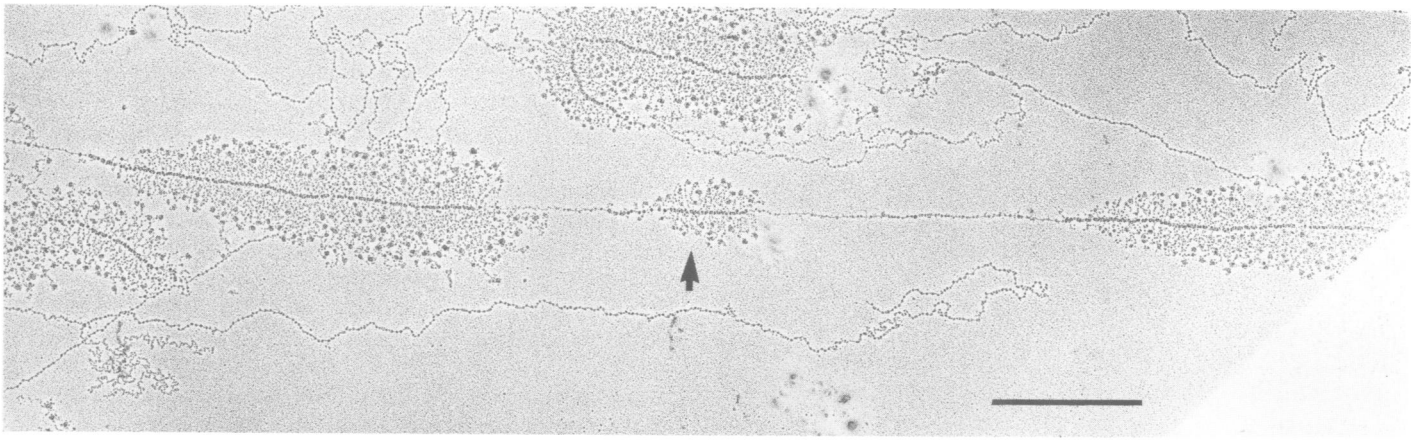

Fig. 4. Electron micrograph of rRNA genes from the gut of a 24 h old *D. melanogaster bb 8* larva. The short transcription unit (2.94 kb) (arrow) probably represents a newly initiating gene since the downstream 'spacer' (2.2 μm) is not likely to have much more than 6.6 kb of DNA.

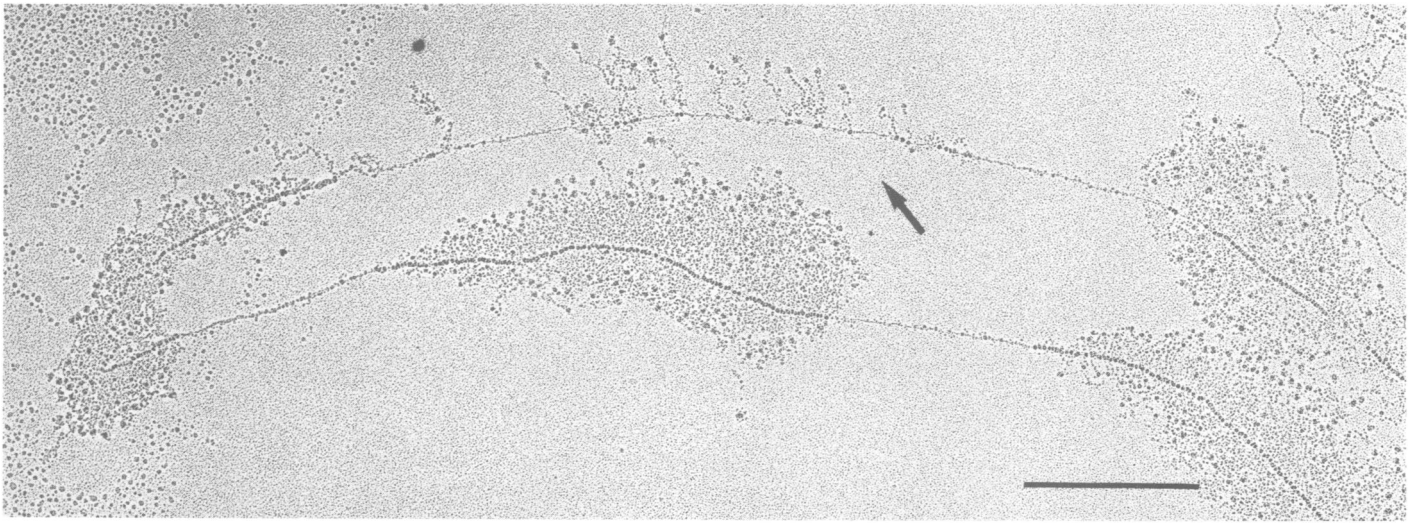

Fig. 5. Electron micrograph of rRNA genes from *D. melanogaster bb 8* larva. Note the gene with the drastically reduced RNA polymerase density (arrow). Scale bar = 1 μm.

lower RNA polymerase density (Figure 3d). Transcription in such a way could explain the relatively low abundance of transcripts from interrupted genes found by biochemical methods and still leave open the possibility that these genes are transcribed. We observed rRNA genes with drastically lower RNA polymerase density than that of neighbouring genes (Figure 5), but they are extremely rare (three genes out of >1000) in this physiologically very active stage, indicating that during this period the rRNA genes are turned on by an all or none phenomenon. Nevertheless, the observed genes with low RNA polymerase density were not long enough to be intron$^+$ genes and they may reflect promoter mutations or local inaccessibility of the gene to RNA polymerase molecules.

The fourth possibility we considered was that transcription of an interrupted gene pauses at the beginning of the intron, the intron DNA loops out and transcription resumes at the junction of the 3′ end of the intron and the 28S′ region (Figure 3e). This possibility is rather difficult to exclude with absolute certainty since the looped out DNA might resemble the RNA or be entangled in the mass of transcripts. However, from a careful analysis of a large number of transcription units we concluded that this is not the general mechanism of transcription of intron$^+$ rRNA genes. Nevertheless, if this is a very rare event it is possible that it escaped our attention.

Although our search for transcription units of unusual length or morphology was largely unsuccessful, we did find transcription units in larval fat bodies and guts which have a length corresponding to ~13 kb of DNA. These represented ~1% of the total rRNA transcription units. Figure 6 shows five rRNA genes in tandem. In this slightly overstretched preparation, genes 1, 3 and 4 (as well as the majority of the genes in the preparation) have a mean length of 3.01 μm. Since the expected size of intron$^-$ rRNA genes is 8 kb (for review see Beckingham, 1982), it follows that in this preparation 1 μm of DNA represents ~2.65 kb. Gene 5 has a length

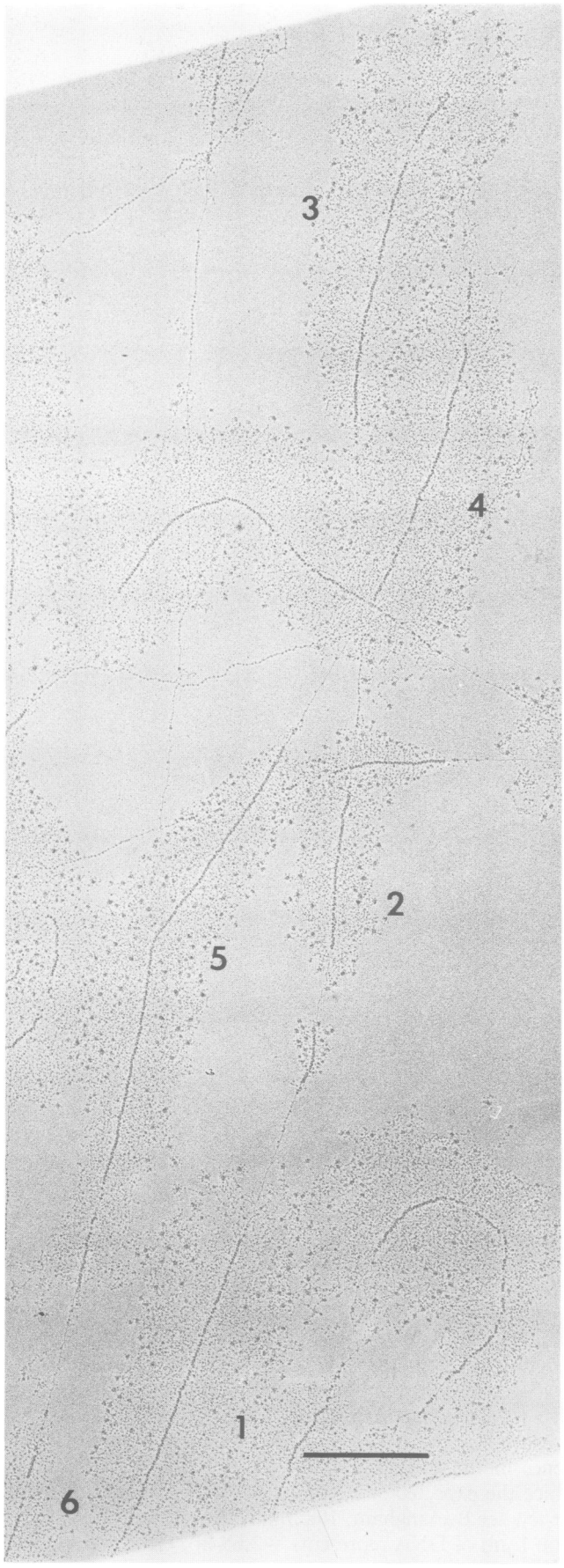

Fig. 6. Electron micrograph of rRNA genes from the fat body of a 24 h old *D. melanogaster bb 8* larva. Gene 5 has a length corresponding to 13 kb of DNA. Scale bar = 1 μm.

of 4.87 μm, corresponding to a transcription unit of ~13 kb. A schematic diagram of this transcription unit is depicted in Figure 3f. This gene shows some unusual features aside from its length: whereas all normal rRNA genes have the typical 'Christmas tree' appearance, with 'knob' like structures at the end of progressively longer RNP fibrils, gene 5 (seen at higher magnification in Figure 7) does not show a continuous lengthening of RNP fibrils. At ~6.5 kb from the beginning of the ribosomal transcription unit, a substantial part of each nascent RNP fibril appears to be clipped off and the 'knob' like structures disappear. As indicated in Figure 6, the apparent processing or degradative step takes place at or near the boundary of 28S′ region and the 5′ end of the intervening sequence. It appears that the RNA transcripts are not cleaved in the immediate proximity of the RNA polymerase molecules, suggesting that a certain secondary or tertiary structure is required for the cleavage. The fact that the RNP fibrils do not seem to increase appreciably in length in the putative intron region indicates that either the RNA polymerase and associated RNP move along the axis without further transcription, or, more likely, that sequences from the intervening sequence are degraded at about the same rate as they are transcribed. To our knowledge this is only the second time that processing or degradation simultaneous with rRNA transcription has been described in a eukaryotic system. The first report, by Grainger and Maizels (1980), demonstrated processing during the trancription of rRNA genes in the cellular slime mold *Dictyostelium discoideum*.

In summary, our results suggest that the majority of intron$^+$ rRNA genes are not used even under a high requirement for rRNA synthesis. This is so despite the reduced number of available rRNA genes in the *bb 8* genome, and our data confirm the similar conclusions of Long *et al.* (1981). Our results also indicate that the occasional expression of intron$^+$ genes does not result in full length transcripts, even though the RNA polymerase might transcribe the entire length of the interrupted gene. Such processing or degardation of nascent transcripts would produce RNA molecules of heterogeneous length which could confuse the biochemical analyses of the transcription of interrupted genes, especially those using 'Northern' blotting and S1 protection as analytical tools. Finally, it is conceivable that the long transcription units result from the insertion of a 5 kb long transposable element into an intron$^-$ rRNA gene. We presently cannot unequivocally identify the nature of the additional 5-kb sequence seen in these transcription units. In either case, the presence of this sequence seems to be detrimental to the resulting transcript.

Materials and methods

Isolation of *Drosophila* tissues and the preparation of chromatin for electron microscopy was conducted as described in Jamrich and Miller (1982). A stock of *D. melanogaster bb 8* was kindly provided by Dr. Kenneth Tartof.

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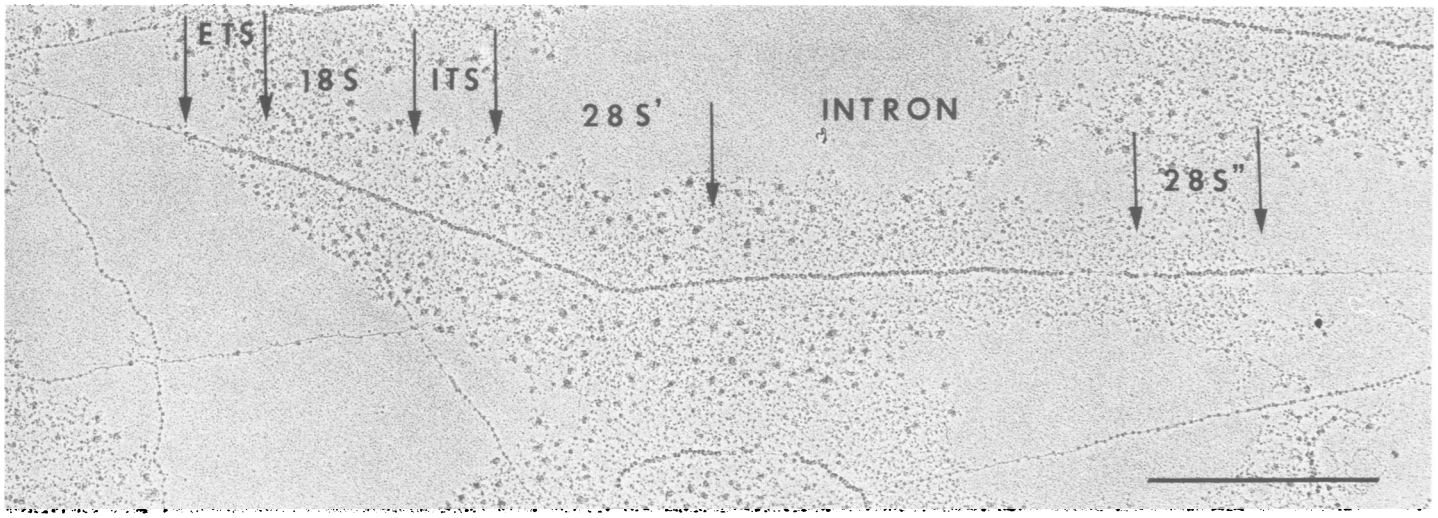

Fig. 7. A higher magnification of gene 5 from Figure 6. The relative position of 18S, 28S, external transcribed spacer (ETS) and internal transcribed spacer (ITS) regions, as well as the 5 kb intron, are indicated. Note the shortening of RNP fibrils near the boundary of the 28S′ region and the intervening sequence. Scale bar = 1 μm.

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