Localization of 5S Gene Loci on Polytene Chromosomes of *Glyptotendipes barbipes* (Staeger) and Transcription of RNA from These Sites

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Ribosomal 5S RNA (5S RNA) is transcribed from tandemly repeated sequences of the eucaryotic genome (Tartof and Perry 1970). Repetition of the genes for 5S RNA has permitted localization of these DNA sequences on the chromosomes of *Drosophila melanogaster* (Wimber and Steffensen 1970), *Xenopus laevis* (Pardue *et al.* 1973), Hela cells (Steffensen *et al.* 1974, Johnson *et al.* 1974), *Glyptotendipes barbipes* (Wen *et al.* 1974), *Zea mays* (Wimber *et al.* 1974), *Taricha granulosa* (Leon 1976), *Chironomus tentans* (Weislander *et al.* 1975) and *Notophthalmus viridescens* (Pukkila 1975) by *in situ* hybridization (Gall and Pardue 1969) with purified 5S RNA. An interesting result of these studies is the finding that the 5S genes are often clustered in several different regions of the genome. For example, the 24,000 repeats of the genes in *X. laevis* are distributed on the telomeres of almost all of the 36 chromosomes of this organism (Pardue *et al.* 1973). The 5S genes in Hela cells are on chromosomes 1, 9 and 16 (Steffensen *et al.* 1974), and these same genes occupy four bands, 56F 1-2 and 56F 6-7, on chromosome 2R of *D. melanogaster* (Szabo 1974). In *N. viridescens*, 300,000 sequences coding for 5S RNA are located on the four or five non-homologous chromosomes (Hutchison and Pardue 1975, Pukkila 1975).

Are all the genes for 5S RNA at the several loci on these chromosomes active in all cell types throughout development? Evidence from sequence analysis of 5S RNA isolated from *X. laevis* suggests that this is not the case and that there is differential transcription of the genes for 5S RNA. It has been shown that cultured somatic cells of *X. laevis* synthesize a 5S RNA which differs in base composition at seven sequence positions from the major 5S RNA synthesized in the oocyte (Brownlee *et al.* 1972, Wegnez *et al.* 1972, Ford and Southern 1973). In addition there are several distinct minor 5S RNAs detectable in the oocyte itself (Wegnez *et al.* 1972, Ford and Southern 1973). This implies that the repeated 5S genes in this organism are heterogenous. It remains to be shown that heterogeneity of 5S RNA is or is not a general feature among eucaryotic organisms with multiple 5S loci, and if so, whether the 5S genes comprising a particular locus are homogeneous or heterogeneous in sequence.

Results presented in a previous communication (Wen *et al.* 1974) indicate that there are probably several 5S gene loci on chromosomes of *G. barbipes*. It was of

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interest, therefore, for us to determine if all these sites are active in specific cells of this organism. Salivary gland chromosomes are ideal for such a study because transcription can be visualized directly as chromosomal puffs, or can easily be detected by labeling cells with ^3H -uridine and analyzing autoradiographs of the chromosomes. Furthermore, there are four larval instars during the development of *G. barbipes*, and if the activity of the several loci is stage-dependent, the salivary glands offer an easily manipulated, homogeneous system for tracing the cause of the change of activity in different cells.

We present evidence here confirming the presence of multiple 5S sites on the chromosomes of *G. barbipes* and showing that multiple gene sites on the same chromosome may arise by chromosomal inversion. We also present data indicating probable differential transcription from the several 5S loci on the polytene salivary gland chromosomes.

Materials and methods

Animals

Larvae of *G. barbipes* were collected either from sewage lagoons near Eugene, Oregon, or at Gavins Point National Fish Hatchery, Yankton, South Dakota. Animals from the latter source were kindly supplied by Mr. Dan B. Martin, Fisheries Research Biologist, U. S. Fish and Wildlife Service. *D. melanogaster* (Canton S $\times$ Oregon R) was cultured on cornmeal media for extraction of nucleic acids.

Preparation of ribosomal 5S RNA from D. melanogaster

All procedures were conducted at 4°C. Mixed age adult flies were collected and freeze-dried. Approximately 10 g (dry weight) of flies were homogenized in 100 ml of buffer: 50 mM Tris-HCl (pH 7.2), 7 mM beta-mercaptoethanol, 100 mM KCl, 6 mM Mg ($\text{C}_2\text{H}_3\text{O}_2$)₂, 1 mM Na₂EDTA, 1 mM 1-phenyl-2-thiourea and 250 mM sucrose. After homogenization, a further 80 ml of buffer was added, and the ribosomes were prepared following the procedure of Pelley and Stafford (1970) with some modifications. The homogenate was centrifuged at 25,000 $\times g$ for 30 minutes, and the buffer layer was decanted and recentrifuged at the same speed for 15 minutes. The final supernatant layer was transferred to polycarbonate centrifuge tubes of the Spinco Ti-60 rotor (Beckman Instruments, Inc., Palo Alto, Cal.). After two hours centrifugation at 40,000 rpm the ribosomal pellet was removed and extracted using the procedures of Monier (1971) for preparation of 5S RNA. RNA was extracted with buffered phenol, purified through DEAE-cellulose and fractionated by Sephadex G-100 column chromatography. Eighty-four A₂₆₀ units of RNA were obtained after EDTA-cellulose chromatography, and this separated into three peaks on Sephadex G-100 (Fig. 1).

Analytical gel electrophoresis

Composite gels of 4% acrylamide and 0.5% agarose were used to analyze RNA fractions. Gels were prepared, run, stained with methylene blue and destained according to methods of Dingman and Peacock (1973).

Iodination of 5S RNA of D. melanogaster

Pooled RNA, characterized as 5S, was iodinated with carrier-free ^{125}I -labelled RNA as described previously (Wen *et al.* 1974). Specific activity of the ^{125}I -labelled RNA was calculated to be 9.62×10^6 dpm/ μg .

In situ hybridization

Five μg of ^{125}I -labelled RNA were dissolved in 0.5 ml of $2 \times \text{SSC}$ buffer (pH 7.2) containing 50% formamide (V/V). Twenty μl of this solution were applied to each slide for *in situ* hybridization. The details of this procedure have also been described previously (Wen *et al.* 1974). However, in the present study, yeast 4S RNA from Calbiochem, Inc. (La Jolla, Cal.) was purified on benzoylated DEAE-cellulose and was included in the annealing mixture to reduce non-specific binding of labelled molecules to the chromosomes.

Labelling of salivary gland chromosomes with ^3H -uridine

Salivary glands from different larval instars of *G. barbipes* were removed from the organism, placed in Robert's CR-Medium (Robert 1971) and labelled with ^3H -uridine (40 Ci/mole, 200 $\mu\text{C}/\text{ml}$ Robert's CR-Medium) for 10 minutes at 18–22°C. After incubation the glands were fixed in absolute ethanol and glacial acetic acid (3:1 V/V), squashed, washed three times with cold 5% trichloroacetic acid, and dipped into Kodak NTB-2 emulsion diluted with an equal volume of water. Autoradiographs were exposed for various periods as indicated in figure legends. When α -amanitin (Calbiochem, Inc.) was used to inhibit RNA polymerase activity, the salivary glands were incubated for 15 minutes in α -amanitin dissolved in CR-medium before addition of ^3H -uridine.

Results

Preparation of 5S RNA from D. melanogaster ribosomes

Results previously reported (Wen *et al.* 1974) indicated that genes for 5S RNA are present at six distinct loci in the polytene chromosomes of *G. barbipes*. However, the 5S RNA used in these earlier hybridization experiments was prepared from a total RNA extract of *G. barbipes*, and the 5S fraction was characterized only on criteria of size and capacity for hybridization to the 5S genes of *D. melanogaster*. It is possible, therefore, that the 5S RNA in this preparation was contaminated with other RNAs of the same size class. If such RNA contaminants exist and are specific for *G. barbipes*, they would be undetected in *D. melanogaster* control hybridizations. Since it was essential, for the experiments reported here, to be certain that the sites previously identified were truly 5S loci, we decided to confirm this by annealing 5S RNA from *D. melanogaster* ribosomes to *G. barbipes* salivary gland chromosomes.

RNA of *D. melanogaster* eluting from Sephadex G-100 in fractions 47–51 (Fig. 1) was pooled and a portion labelled with ^{125}I to localize the genes for 5S RNA in *G. barbipes*. This RNA fraction was first characterized for size by gel electrophoresis. Figure 2 illustrates the electrophoretic profile of labelled (^{125}I) and unlabelled 5S RNA of *D. melanogaster* as well as authentic 4S and 5S RNA of

E. coli. It is demonstrated in Fig. 2A and the first gel in Fig. 2B that there is only one size class of *D. melanogaster* RNA in the pooled fraction. The maximum concentration of RNAs of other sizes in this fraction was estimated as less than 0.5%. Since the RNA component of interest migrated, as does liver 5S RNA (Peacock and Dingman 1968), slightly behind the 5S of *E. coli* (gel 4, Fig. 2B), it was assumed to be the ribosomal 5S RNA species.

The putative 5S RNA was characterized for type by *in situ* hybridization. Figure 3 shows that the ^{125}I -labelled RNA of the pooled fractions hybridized to the 56F region and nucleolar organizer regions of *D. melanogaster*. The autoradiograph of Fig. 3 has been exposed for 29 days to prove the absence of radioactive RNAs hybridizing to other regions of the genome.

Annealing of radioactive 5S RNA *in situ* generally leads to the association of labelled molecules with nucleolus organizers as well as regions of the genome specific for authentic 5S ribosomal RNA. It is likely that hybridization to the nucleolar region results from the presence of 5.8S RNA in our preparation, for 5.8S RNA is also coded by the 18+28S ribosomal RNA genes of the nucleolus organizer (Ritossa and Spiegelman 1965, Darnell 1968, Pene *et al.* 1968). 5S ribosomal RNA will not separate from 5.8S RNA on Sephadex G-100, but one might expect to observe such a contaminant after gel electrophoresis of the iodinated 5S fraction. As previously pointed out, this was not the case (Fig. 2A). However, on hybridization with 5S RNA, label associated with the nucleolar organizer appeared only after lengthy exposure periods. The extreme repetition of the genes for 28+18S RNA may provide a very sensitive assay for the supposed 5.8S fraction.

Crytology of short arm of polytene chromosome III of G. barbipes and localization of 5S RNA Genes

Salivary gland chromosomes of *G. barbipes* have been studied previously by Basrur (1957), Meiseiko *et al.* (1971), Martin and Porter (1973), and Walter (1973). The short arm of chromosome III was designated as arm F by Martin and Porter (1973), and this designation is followed here. These same authors have characterized four sequences of this chromosome arm in populations from North America. We have identified these, as well as other sequences occurring less frequently, in the natural population from Yankton, S.D. (unpublished observations). Band sequences F2 and F4 differ from the F1 sequence by single inversion rearrangements and F3 is derived from F2 by simple chromosomal inversion also (Martin and Porter 1973). Regions illustrated by brackets around homozygous chromosomes of the F1/F1 and F2/F2 sequences (Fig. 4) indicate inversions required to form the F3 and F4 sequences. The heterozygous F1/F2 sequence shown in Fig. 4 illustrates the

Figs. 1-2. 1, chromatography of *D. melanogaster* RNA on Sephadex G-100. 2.5 ml fractions were collected and fractions 47-51 were pooled for analysis by gel electrophoresis and iodination. 2A and B, gel electrophoresis of *D. melanogaster* RNA purified on Sephadex G-100. (A) Radioactivity profile of gel electrophoresis of ^{125}I -labelled RNA (fractions 47-51, Fig. 1) illustrated in B-1 below. (B) Gel 1, co-electrophoresis of unlabelled and ^{125}I -labelled *Drosophila* RNA; Gel 2, 5S RNA of *E. coli* (contaminated with an unknown RNA species); Gel 3, 4S and 5S RNA of *E. coli*; Gel 4, 5S RNA of *E. coli* and *Drosophila* RNA pool. Gel. 4 was not run simultaneously with the first three.

Figs. 3-4. 3, autoradiograph of *D. melanogaster* salivary chromosomes hybridized with ^{125}I -labelled RNA fraction prepared from *Drosophila* ribosomes. Small arrow, labelling over band 56F; Large arrow, labelling over nucleolus. Exposure time 29 days. Bar=20 μm . 4A-C, band sequences of chromosome arm F of *G. barbipes*. (A) F1/F1 homozygote. (B) F2/F2 homozygote. (C) F1/F2 heterozygote. Inversions are indicated by brackets above or below the chromosomes. F8a, $\blacktriangle$; F9o, $\uparrow$; and probably F11L, $\blacktriangledown$ are the gene loci of 5S RNA. Bands F8a to F11v are paired in the heterozygote. Bar=10 μm .

bands between F8a and F11V involved in the F1-F2 transformation.

In our previous report (Wen *et al.* 1974), evidence of six loci for 5S RNA in the polytene chromosomes of *G. barbipes* was presented. However, *in situ* hybridi-

Figs. 5-6. 5, autoradiograph of *G. barbipes* chromosomes hybridized with ^{125}I -labelled 5S RNA of *D. melanogaster*. Three gene loci of 5S RNA $\blacktriangle$, F8a; $\uparrow$, F9o; $\blacktriangle$, F11L; (see also Fig. 8 and 9 for band recognition) are labelled as well as three 18+28S RNA sites (triangles). Exposure time 20 days. Bar $\times 20 \mu$. 6, three gene loci for 5S RNA (F8a $\blacktriangle$; F9o $\uparrow$; F11L $\blacktriangle$) on the homozygous F2/F2 chromosomes of *G. barbipes*. Distal end of chromosomes (band F8a) is heavily labelled. Exposure time 15 days. Bar $= 10 \mu\text{m}$.

zation of *G. barbipes* chromosomes with ^{125}I -labelled *D. melanogaster* RNA in the present study confirms only three of these sites (Fig. 5), and these are limited to the short arm of chromosome III. The position of these loci has been analyzed, and

Figs. 7-8. 7, three gene loci (arrows) of 5S RNA on the heterozygous F1/F2 chromosomes of *G. barbipes*. Both homologous chromosomes above scale bar on the photograph are heavily labelled because of presence of band F8a (see Fig. 4C). Exposure time 25 days. Bar=10 μ m. 8, the main gene locus for 5S RNA of *G. barbipes* on band F8a (arrow). Exposure time 80 hours. Bar=10 μ m.

they are indicated as bands F8a, F9o, and probably F11L in Fig. 5. (See also Fig. 4 for band localization.) These three loci were demonstrated on the homozygous F1/F1 (Fig. 5) and F2/F2 (Fig. 6) sequences as well as the heterozygous F1/F2 sequences (Fig. 7). The gene locus at F8a is shown most clearly in Fig. 8, and the locus at F9o in Fig. 9. The third locus is tentatively assigned to F11L because the cytology of the preparations was not adequate for absolute correlation of the silver grains with the band. It was repeatedly observed in these polytene chromosome preparations that the number of silver grains accumulated over the 5S gene loci was unequal, with the greatest number of grains over F8a and the least number over F9o (Figs. 5, 6 and 7). Since the number of silver grains is roughly proportional to the number of DNA sequences complementary to the labelled RNA (Szabo 1974), the autoradiographs indicate that most of the 5S RNA genes are located in band F8a, while band F9o contains the fewest copies of 5S RNA genes.

In Fig. 5 one may see that the three gene loci specific for 18+28S RNA (Wen *et al.* 1974) are also labelled by the 5S preparation. This again suggests the presence of 5.8S RNA in the 5S iodinated fraction.

There is significant and consistent accumulation of silver grains only in the regions described above. Two loci on the long arm of chromosome II and one on chromosome IV, tentatively identified as 5S RNA transcription sites in our previous report (Wen *et al.* 1974), were not labelled by iodinated 5S ribosomal RNA from *D. melanogaster*.

In optimal preparations of inverted segments of the short arm of chromosome III, a fourth 5S locus was observed (Fig. 10). Analysis of the banding pattern indicates that the new 5S locus probably arises by a break within the F8a tandem repeat (see Discussion).

Transcription of 5S RNA on salivary gland chromosomes

Transcription of 5S genes in the salivary gland chromosomes of *G. barbipes* is not accompanied by distinct puffing of the bands, but it can be detected by labelling with ³H-uridine as described in Materials and methods. Autoradiography of the chromosomes after ³H-uridine uptake permits a comparison of the relative transcriptional activity at the several 5S loci. Figures 11 A and B (chromosomes from the third and fourth instar stages) indicate that F8a, the locus containing the greatest number of 5S RNA gene sequences, is not labelled, while F9o, thought to contain the fewest gene copies, has probably incorporated some ³H-uridine. The site tentatively identified as F11L appears to be active also, but diffuse labelling in this area prevents certainty.

The same results were obtained when salivary glands were preincubated with low concentrations (5 µg/ml) of α -amanitin (Fig. 11C), a differential inhibitor of RNA polymerases (Egyhazi *et al.* 1972, Serfling *et al.* 1972). In the presence of this low concentration of inhibitor the total label associated with the chromosomes is greatly reduced as indicated by the lower number of silver grains over Balbiani rings on chromosome IV (unpublished observation). Polymerases transcribing heterogeneous RNA appear to be most sensitive to α -amanitin as evidenced by studies in *Chironomus* (Egyhazi *et al.* 1972, Serfling *et al.* 1972), while polymerases

transcribing 1) transfer RNA and 5S RNA and 2) 18+28S RNA are less susceptible (Weinman and Roeder 1974). This appears to be in keeping with the results found here. When the concentration of inhibitor is increased to 100 $\mu\text{g/ml}$, subsequent autoradiographic analysis of the 5S sites indicates that these are inactivated. This is demonstrated in Fig. 12, an autoradiograph from a third instar

Fig. 11A-C. Autoradiographs of arm F of polytene chromosomes from third (A) and fourth (B and C) larval instar labelled with tritiated uridine in the presence (C) or absence (A and B) of 5 $\mu\text{g/ml}$ α -amanitin. Exposure time: A, 42 hr; B, 48 hr; C, 86 hr.

larva prepared under the same experimental conditions as that used for the low concentration α -amanitin experiments (Fig. 11). Only the three 18+28S RNA sites have conspicuously incorporated label at the high inhibitor levels (Fig. 12). Polymerases transcribing large ribosomal RNAs have been shown to be most

Figs. 9-10. 9A and B, gene locus of 5S RNA of *G. barbipes* at F9o (arrow). (A) Exposure time 67 hours. (B) Exposure time 29 days. Bar=10 μm . 10, demonstration of four 5S RNA sites on arm F of *G. barbipes* in the F1/F2 configuration (arrows). Exposure time is 25 days. In Fig. 8 of our previous communication (Wen, *et al.* 1974), these four sites are also displayed on arm F of the F1/F1 configuration. Bar=10 μm .

resistant to α -amanitin (Weinman and Roeder 1974).

Discussion

When 5S RNA is purified from total RNA extracted from *G. barbipes*, iodinated with ^{125}I and annealed to the polytene chromosomes of this organism, a significant number of silver grains are consistently found over six discrete positions (Wen *et al.* 1974). We wanted to confirm that these sites are all truly specific for ribosomal

Fig. 12. Autoradiograph of polytene chromosomes from third larval instar labelled with ^3H -uridine in presence of α -amanitin (100 $\mu\text{g}/\text{ml}$). Three 18+28S RNA sites are labelled (triangles), whereas genes coding for 5S RNA (short arrows) as well as genes in Balbiani ring (long arrow) are not. Exposure time 72 hrs. Bar=10 μm .

5S RNA. Since it is difficult to purify any nucleic acid fraction to homogeneity, it is possible that the *G. barbipes* 5S RNA contained a contaminant of similar molecular size. If such contaminating molecules are transcribed from repetitive sequences unique to the *G. barbipes* genome, they would not hybridize to chromosomes of the control organism but would anneal to chromosomes of *G. barbipes*. Such an occurrence would lead to erroneous identification of labelled sites as 5S loci.

It has been shown that the genes of 5S RNA are conserved during evolution, for the nucleotide sequences of 5S RNAs isolated from different Orders are very similar (Payne *et al.* 1973, Sanhoff *et al.* 1973). We thought a most exacting way to verify the alleged 5S loci was to challenge *G. barbipes* chromosomes with 5S RNA prepared from *D. melanogaster* ribosomes. *G. barbipes* and *D. melanogaster* are both in the Order *Diptera* and are expected to have similar sequences for 5S RNA. Furthermore, it seemed unlikely that any contaminating RNA molecules in the *D. melanogaster* 5S RNA would anneal to *G. barbipes* chromosomes. Our results indicate that only three of the six previously identified sites are verifiable as loci of 5S RNA transcription. These three true 5S loci are all confined to the short arm of chromosome III: F8a, F9o and, tentatively, F11L.

Multiple gene loci for 5S RNA are common but not universal among eucaryotic organisms. For example, *Rhynchosciara angelae* (Szabo *et al.* 1972), *Zea mays* (Wimber *et al.* 1974) and *Taricha granulosa* (Leon 1976) have only single loci for transcription of 5S RNA. Exchange of DNA between non-homologous chromosomes is a possible mechanism by which repeated genes may be spread to different regions of the genome. For example, in *X. laevis* during zygotene of spermatocyte meiosis all the genes for 5S RNA form a tight cluster on one side of the nucleus ("bouquet conformation"), and it has been suggested that exchange of 5S RNA could occur between all the chromosomes as meiotic crossing-over proceeds (Pardue *et al.* 1973). In polytene chromosomes of *G. barbipes*, rDNA of non-homologous chromosomes forms chromatin bridges between nucleolar organizers (Wen *et al.* 1974). This suggests that meiotic or mitotic chromosomes may behave in a similar way and lead of exchange of genetic material.

Chromosomal inversions are of common occurrence in the *Diptera* and have been well documented in different species of *Drosophila* (Novitski 1946), *Tendipes* (Tothfels and Fairlie 1957), *Glyptotendipes* (Fig. 4), etc. Inversions may provide another way for spreading repetitive DNA sequences and producing multiple gene clusters on the chromosome. In Fig. 4, band F8a is seen to be slightly separated from F8b. This band separation is not observable in Fig. 8, but in this figure the silver grains have accumulated only on the proximal side of the observed band, presumably over F8a. Although the labelling of the distal 5S gene cluster in the F2/F2 homozygote is always diffuse (Fig. 6), the banding pattern seen in Fig. 4 shows that F8a is included in the inverted region of the F2/F2 chromosome. This observation implies that it is band F8a which is labelled. This was confirmed by *in situ* hybridization of ¹²⁵I-labelled RNA to F1/F2 heterozygotes. In this case, labelling of F8a is observed on both of the paired homologous chromosomes (Fig. 7). When F8a moves from the distal to the proximal position of arm F, by chromosomal inversion involving bands F8a through F11v (F2→F1 conversion), four gene loci on arm F are labelled with 5S RNA prepared from either *G. barbipes* or *D. melanogaster* (Fig. 10). This suggests that one of the tandemly-repeated sequences for 5S on arm F has been cleaved within the repeated region, resulting in an additional locus after inversion. It is tempting to speculate that all three gene loci of 5S RNA on arm F may have been generated in a similar way from a primordial locus.

It has been demonstrated (Fig. 11) that 5S locus F8a is not labelled with tritiated uridine. This indicates that the synthesis of 5S RNA at this site is too slow to be detected under the conditions of the experiment. However, there is rapid synthesis of 18+28S RNA on the same chromosomes during this period as indicated by the large number of silver grains covering the three rDNA sites (unpublished observation). In ribosomes, 18+28S RNA and 5S RNA occur in a one:one ratio, and one might expect, contrary to the evidence, that the 5S locus at F8a would be active, for this is probably the largest 5S cluster in this organism. However, there are several cases in which non-coordinate synthesis of 5S and 18+28S RNA has been observed (Ford 1971, Weinmann 1972). Two possible interpretations for the slow rate of 5S synthesis in *G. barbipes* salivary glands are: 1) There exists a pool of 5S RNA in the salivary gland which makes unnecessary to transcribe these molecules at a rapid rate for ribosome assembly; and 2) there are more copies of 5S RNA than rRNA in the salivary glands, and a relatively slow rate of 5S RNA synthesis in F8a is sufficient to satisfy the required one:one stoichiometry of products.

The ratio of 5S to 18+28S RNA genes can be increased during polytenization. For example, in the salivary gland polytene chromosomes of *D. melanogaster*, rDNA is under-replicated relative to euchromatic DNA (Spear and Gall 1973). In fact, even in diploid tissues there may be more copies of genes for 5S RNA than rRNA. Such is the case in *Xenopus* (Brown and Weber 1968), and in Hela cells (Hatlen and Attardi 1971). Further, in *X. laevis* only 3% of the total 5S genes of somatic cells are active in RNA transcription (Brownless *et al.* 1974). Therefore, in this organisms also, genes for 5S RNA appear to be subject to differential transcriptional control.

Genes coding for rRNA commonly undergo intensive extrachromosomal replication or amplification during oogenesis (Brown and David 1968, Gall 1968, Gall and Rochaix 1974). On subsequent synthesis of rRNA from these genes, it may be necessary to have "extra" copies of the 5S genes on the chromosomes themselves for coordinate synthesis and assembly of ribosomes. After this period of rapid synthesis, further transcription may be terminated at many of the 5S genes. It would be of interest to determine if 5S genes on F8a are active in other tissue of this organism.

In further work it is also important to analyze the nucleotide sequence of 5S RNA from the salivary gland and other tissues of *G. barbipes*. If more than one sequence of 5S RNA is indicated by fingerprint analysis, the occurrence of several loci would lead one to suspect that each locus may be specific for a particular sequence. With such an arrangement, the expression of all genes could be regulated by a simple mechanism such as that suggested by Ford and Southern (1973). On the other hand, if the multiple 5S clusters originated by breakage within tandem gene repeats and subsequent inversion, the suggestion that the several loci have distinct DNA sequences becomes less compelling. Of course, gene mutation at the several sites might have altered these distinct loci even after the inversion events.

Abstract

Three loci transcribing 5S RNA have been verified on the short arm of chromosome III of *G. barbipes*. Evidence is also presented which indicates that a fourth site may arise on this same arm by chromosomal inversion following a break within the major 5S locus. Autoradiography after ³H-uridine incorporation indicates that the major 5S locus on chromosome III is relatively inactive in transcription in nuclei of salivary gland cells while the minor sites actively transcribe 5S RNA.

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