A STUDY OF THE NUCLEOLAR MATERIAL IN *SCIARA COPROPHILA*

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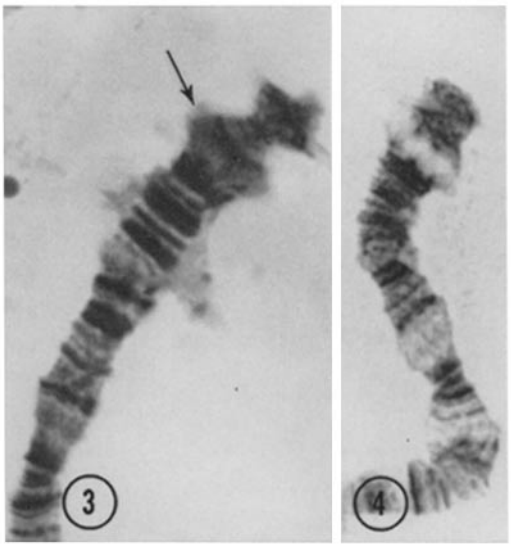
ABSTRACT

In the polytene chromosomes of *Sciara coprophila*, in addition to a nucleolus, large numbers of nucleolarlike structures or micronucleoli are formed. A detailed mapping localized the nucleolar organizer at one end of the X chromosome and revealed that approximately 18% of the bands of each chromosome are potentially capable of producing micronucleoli. Most of these sites are in regions known from a previous study to show asynchronous DNA replication: DNA puffs and certain heterochromatic regions. Micronucleoli are rarely found in association with bulbs. The RNA metabolism of the polytene chromosomes during late fourth instar was studied using radioautographic techniques. Isolated glands were incubated in tritiated uridine for 10 to 30 min, and radioautographs were made of squash preparations. Despite the wide range of variation found among different larval cultures, the following pattern was observed. Just prior to and at the beginning of DNA puff formation, a period of intense extrachromosomal nucleolar and micronucleolar RNA synthesis occurs. After maximal development of the DNA puffs, the synthesis of extrachromosomal RNA is at a low point, while incorporation into bulbs and DNA puffs remains high. With the onset of the prepupal stage, all nuclear RNA synthesis ceases.

INTRODUCTION

Evidence to date indicates that most, if not all, ribonucleic acid (RNA) synthesis occurs in the nucleus. Distinctive kinds and patterns of RNA synthesis have been described for nucleoli and chromosomes (1, 38, 48, 57–59, 79, 82) and within specific chromosomal regions (21–23), focusing attention on the functional and morphological relationships of the RNA-containing components of the cell. It has long been known that nucleoli, the most conspicuous of the RNA-containing organelles, are formed in association with specific chromosomal loci, the nucleolar organizer regions (31, 36, 46, 52, 53). Only recently have we begun to understand the function of the nucleolar organizer as the site of synthesis of ribosomal RNA (47, 66). However, many questions pertaining to the nucleolus and its organizer and ribosomal biogenesis remain unanswered. For example, it is not known whether all nucleolar RNA is ribosomal precursor, derived exclusively from the nucleolar organizer regions, or whether fractions of RNA from other chromosomal regions aggregate at the nucleolus and contribute to the nucleolar mass. The cistrons coding for ribosomal RNA appear to be generally localized at one or only a few sites; yet there are some indications that additional loci scattered throughout the genome may, on occasion, give rise to nucleolarlike bodies. Various examples of intranuclear RNA-containing bodies formed either in the course of development (40–42, 72) or as a consequence of altered physiological conditions (46, 75), or by mutations involving the nucleolar organizer (24) have been described. It would be of interest to know the relationship of these bodies to the true nucleolus.

The polytene salivary gland nuclei of the Diptera provide a particularly favorable system for studying metabolic patterns of specific chromo-



(Figs. 1 and 2 are charts, and appear opposite page 352.)

FIGURE 3 Bulb (RNA puff) near the free end (arrow) of chromosome III (map position 15B). From a smear preparation stained with toluidine blue. × 1750.

FIGURE 4 Bulb 15B (same as in Fig. 1) stained with the Feulgen reaction, showing Feulgen positive strands radiating out into the swollen portion. × 1750.

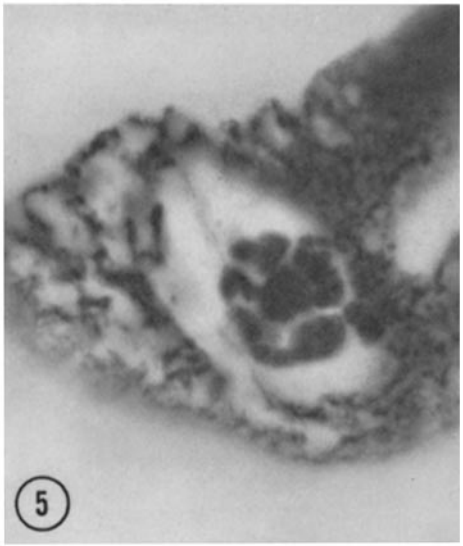


FIGURE 5 Section through a cell from the posterior part of the gland from a late fourth instar larva, showing a nucleus of low polyteny. Note a dense central nucleolus with several small vacuoles. Azure B. × 1750.

somal sites. In the salivary gland nuclei of various Sciarids, masses of extrachromosomal RNA-containing material in the form of numerous micronucleoli have been described (33, 76). These bodies are generally seen in close association with the chromosomes, and are readily distinguishable from the intrachromosomal RNA of puffs and bulbs.

In the present study of *Sciara coprophila*, a detailed mapping of the principal chromosomal sites associated with "nucleolar material" was prepared. (See Fig. 1 *a* to *c* and Fig. 2, which appear opposite page 352. The results of this initial study indicated the presence of a major nucleolar organizer site on the X chromosome and of a surprisingly large number of additional sites producing micronucleoli on each of the polytene chromosomes. Most, but not all, of these sites were localized at heterochromatic regions or areas which, in a previous study, had been characterized by asynchronous DNA replication (28). An attempt was made to evaluate the activity of these sites at different stages of development during the fourth larval instar. A wide range of variability in the ribonucleic acid metabolism of individual larval glands was encountered during all stages of development.

MATERIALS AND METHODS

Salivary glands of the late fourth instar larvae (from the time of appearance of eyespots to prepupa) of

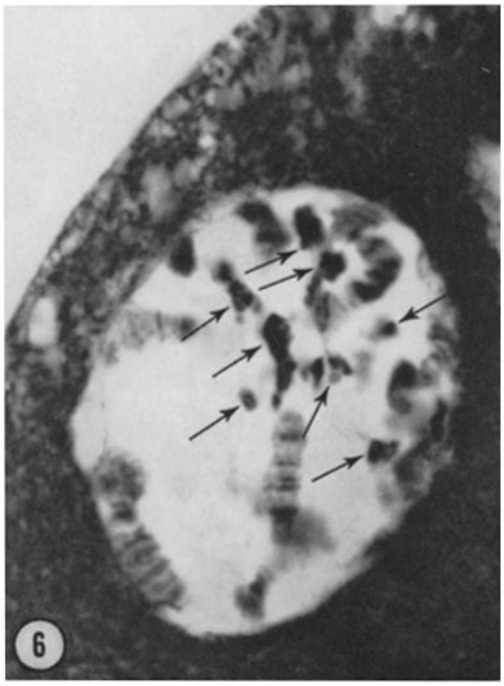


FIGURE 6 Section through a cell from the anterior part of the gland from a late fourth instar larva, showing a highly polytene nucleus. Several small aggregates of nucleolarlike metachromatic material are shown at arrows. Azure B. × 1750.

Sciara coprophila were used. For squash preparations, glands were dissected in Ringer's solution (51), fixed in 3:1 absolute ethanol-glacial-acetic acid for 1 to 2 min, and squashed in 45% acetic acid. Cover slips were flipped off by contact with dry ice, and the squashed preparations were promptly placed in cold 95% ethanol until ready for staining. Whole glands were fixed in 3:1 ethanol-acetic acid for 2 hr, dehydrated, and embedded in paraffin for sectioning. Routine acetoorcein squashes (51) were prepared when detailed morphological features of chromatin were to be studied.

Nuclear RNA was demonstrated by staining with the basic dyes, azure B and toluidine blue. A modification of the method of Flax and Himes (26) was used for azure B staining. Preparations were stained in a half strength solution of azure B (12.5 mg/100 ml in the McIlvaine buffer, pH 4.0) at 40°C overnight, rinsed briefly in distilled water, differentiated in tertiary butyl alcohol for approximately 6 hr, and mounted in Canada balsam. Toluidine blue staining was carried out by following the method of Pelling (58). RNA stains metachromatically with both procedures. Digestion with ribonuclease (Worthington Biochemical Corp.) for 1 hr at 37°C at pH 6.5 at an enzyme concentration of 0.2 mg/ml prior to staining, removed all metachromatically stained material from the nucleus and cytoplasm. Squashes stained with the Feulgen reaction (73) were made for DNA localization.

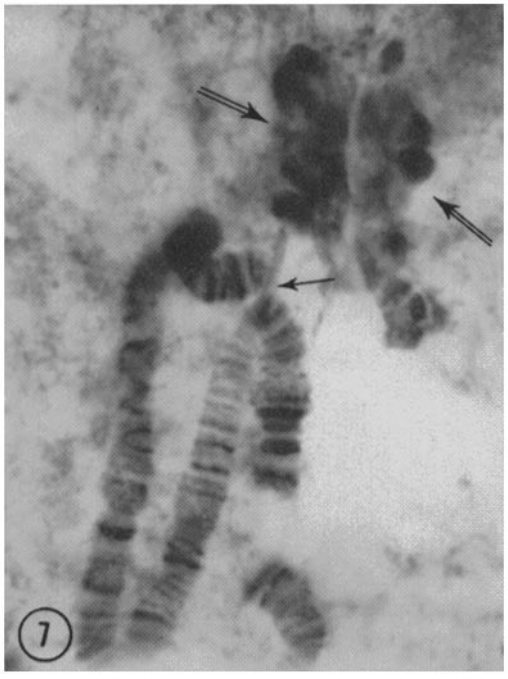


FIGURE 7 X chromosome with nucleolus, the latter slightly displaced. Single arrow points to the repeat regions, and double arrows to the nucleolus. Azure B. × 1750.

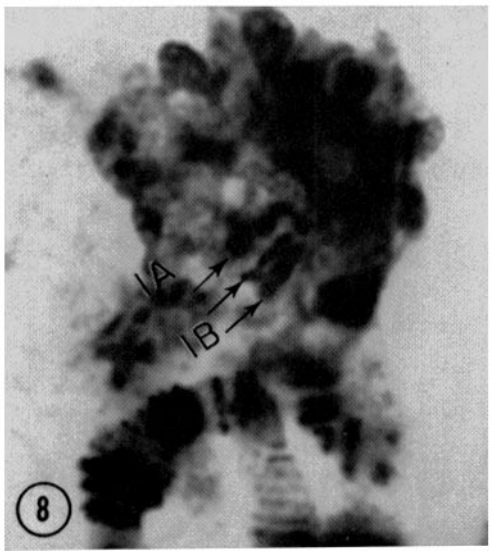


FIGURE 8 A portion of the X chromosome showing a large nucleolus. Several bands (arrows) are interpreted to represent part of the nucleolar organizer and adjacent bands. Note the grapelike appearance and the vacuolated nature of the nucleolus. Azure B. × 2500.

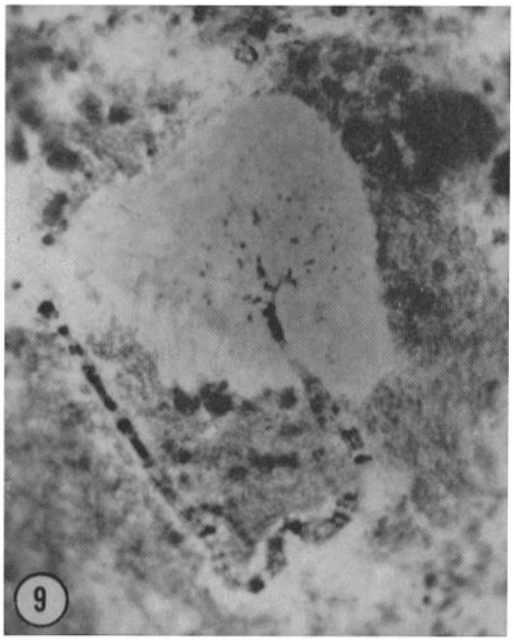


FIGURE 9 A compact nucleolus from a nucleus of low polyteny. Note the ramification of the chromosomal strands of the organizer region. Toluidine blue. × 1050.

RNA metabolism of the salivary gland nuclei was studied by following the incorporation of uridine-H^3 into RNA by radioautography. Glands were dissected out in Ringer's solution transferred to a fresh

FIGURE 10 X chromosome from an orcein squash preparation, showing the organizer (arrow) in a compact state. A small amount of nucleolar material is present but unstained. × 1050.

FIGURE 11 X chromosome from an orcein squash preparation, showing the organizer chromatin (arrow) in an uncoiled, extensively ramified condition. Nucleolar material unstained. X′ inversion present. × 1050.

solution containing uridine-H^3 (2 μc/ml, specific activity 3.2 c/mmole), and incubated for 10, 20, or 30 min. The paired glands were then separated into two halves, fixed and squashed on separate slides, and placed in cold 95% ethanol as described above. The squashed preparations were brought down to water. One half of each gland was left unextracted (rinsed in cold 5% trichloroacetic acid for 2 min to remove low molecular weight nucleotides), while the partner half was subjected to ribonuclease digestion (then rinsed in cold 5% trichloroacetic acid) to remove RNA. All preparations were then coated with Kodak AR10 stripping film or Ilford L-4 liquid emulsion. The ribonuclease-treated squashes showed a loss of almost, but not quite, all radioactivity.

RESULTS

In polytene nuclei, chromosomal RNA accumulates within specific regions of the chromosome structure, enlarging the chromosomal width to form a puff. Extensive literature describing the morphological, metabolic, and fine structural characteristic of puffs is available (2–4, 6, 8, 16, 39, 49, 54–56, 64, 65, 77). A typical metachromatic RNA puff (bulb) of *S. coprophila* is shown in Fig. 3. When the same puffed chromosome region is stained with the Feulgen reaction (or acetoorcein), the swollen portion is barely stained, due to the extreme "dilution" of the DNA by RNA and protein (Fig. 4). In contrast, nucleolar RNA, also associated with specific chromosomal regions, tends to aggregate into discrete masses which are more or less separate from the chromosomal structure.

Nucleoli are readily recognized as dense, spherical, refractile, frequently vacuolated RNA-containing bodies which have a distinctive fine structure. In the salivary gland cells of *Sciara*, the nucleolar material of fourth instar larvae varies greatly in form, amount, staining properties, and RNA metabolism. In the smaller, more compact nuclei of the posterior part of the gland, a single, dense, vacuolated nucleolus is frequently found (Fig. 5), whereas in the larger, more highly polytene nuclei of the anterior part of the gland, dispersed masses with the same staining properties are

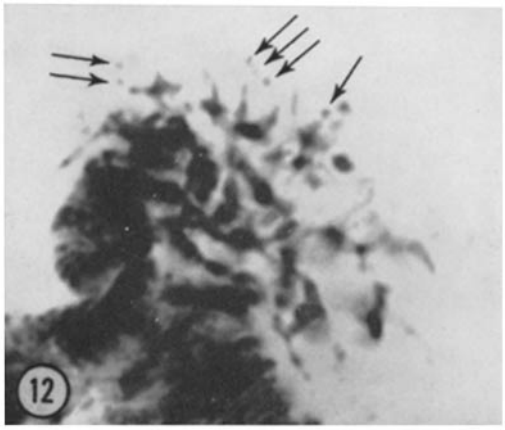


FIGURE 12 Enlargement of Fig. 9, showing uncoiled chromatin strands and granules (arrows) of the nucleolar organizer region. × 2500.

scattered throughout the nucleus (Fig. 6). At some stages, no nucleolar material can be detected. However, when nucleolar material is present at all, a major aggregate is always found in association with a particular chromosomal region, suggesting the presence of a major nucleolar organizer site (Figs. 7, 8, and 9). This aggregate may vary in form from a grapelike cluster of deeply metachromatic bodies (Fig. 8) to a rather weakly stained more homogeneous structure (Fig. 9).

The nucleolar organizer was localized at the proximal heterochromatin of the X chromosome (Figs. 8 to 10). This corresponds to region 1A consisting of three heavily staining (heterochromatic) bands, as sketched in the previously published chromosome maps of *S. coprophila* (28). The nucleolar organizer region is occasionally found in a condensed, banded state (Fig. 10). When large amounts of nucleolar material are present, the organizer shows some degree of uncoiling and branching. In cases of extreme uncoiling, as seen in Figs. 11 and 12, the chromatin of region 1A is extensively ramified, resembling, in some aspects, a puffed region. As will be seen later, the majority of the micronucleoli is also formed at condensed, heterochromatic bands. Here too, localized uncoiling during micronucleolar formation may occur. In a few favorable preparations, one can resolve strands of chromatin which emanate from the band and extend into the micronucleolus where they terminate in chromatin granules (Fig. 13).

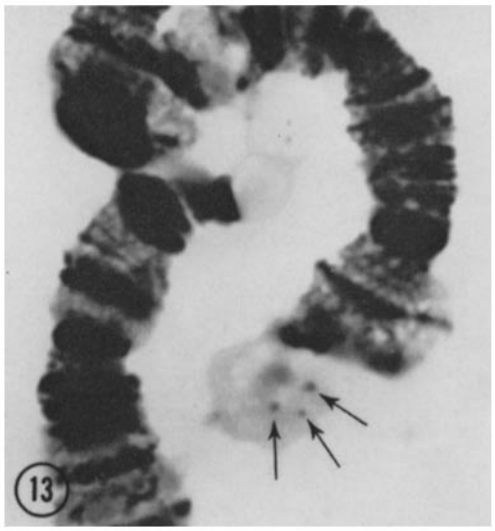


FIGURE 13 Right end of chromosome IV (map position 20C) from an orcein squash preparation, showing a large micronucleolus associated with the terminal band. Note several orcein-positive granules (arrows) similar to those seen in Fig. 12. × 2500.

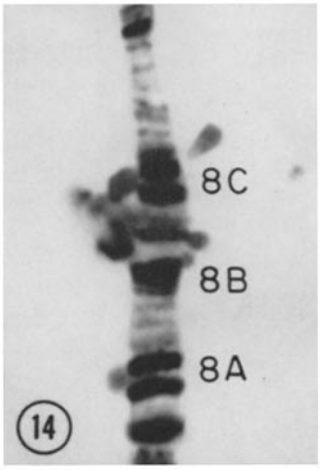


FIGURE 14 A segment of chromosome IV from a squash preparation, showing micronucleoli associated with condensed bands (map regions 8A, 8B, and 8C). Azure B. × 2000.

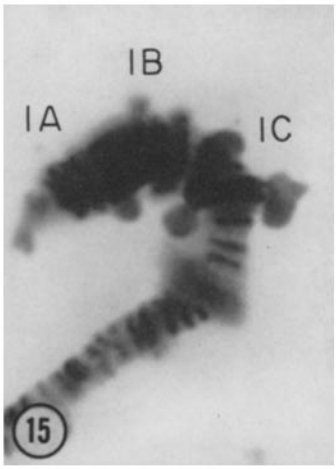


FIGURE 15 Centromere end of chromosome III from a squash preparation, showing micronucleoli associated with heterochromatic bands of regions 1A, 1B, and 1C. Azure B. × 2000.

The study of sites of micronucleolar formation was made on squash preparations of salivary glands taken from three different larval cultures. A minimum of 50 chromosomes of each type, II, III, and IV, were analyzed in detail. The X chromosome, which also possesses such sites, was omitted from this study, since the banding pattern is difficult to analyze due to the X′ inversion and the triple repeat regions present (18, 19). When squash preparations are made, physical displacement of material occurs. This, plus the tendency of nucleolar material to fuse and stick together, seemingly would make the analysis of the localization of the sites of origin of micronucleolar bodies

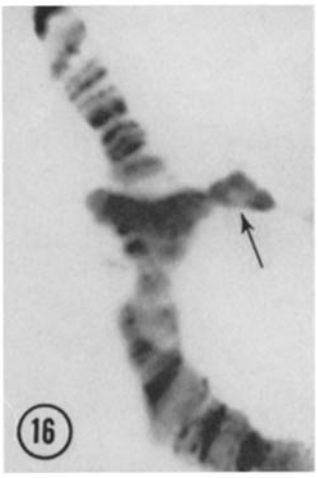


FIGURE 16 A cluster of micronucleoli (arrow) associated with a DNA puff (11A on chromosome III). Squash preparation, azure B. × 2000.

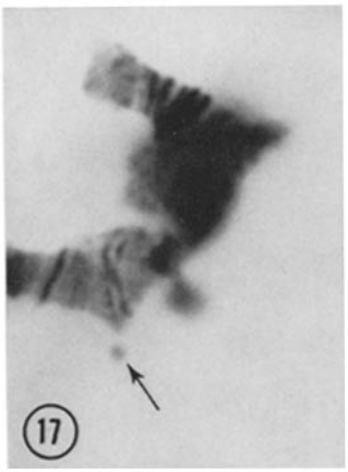


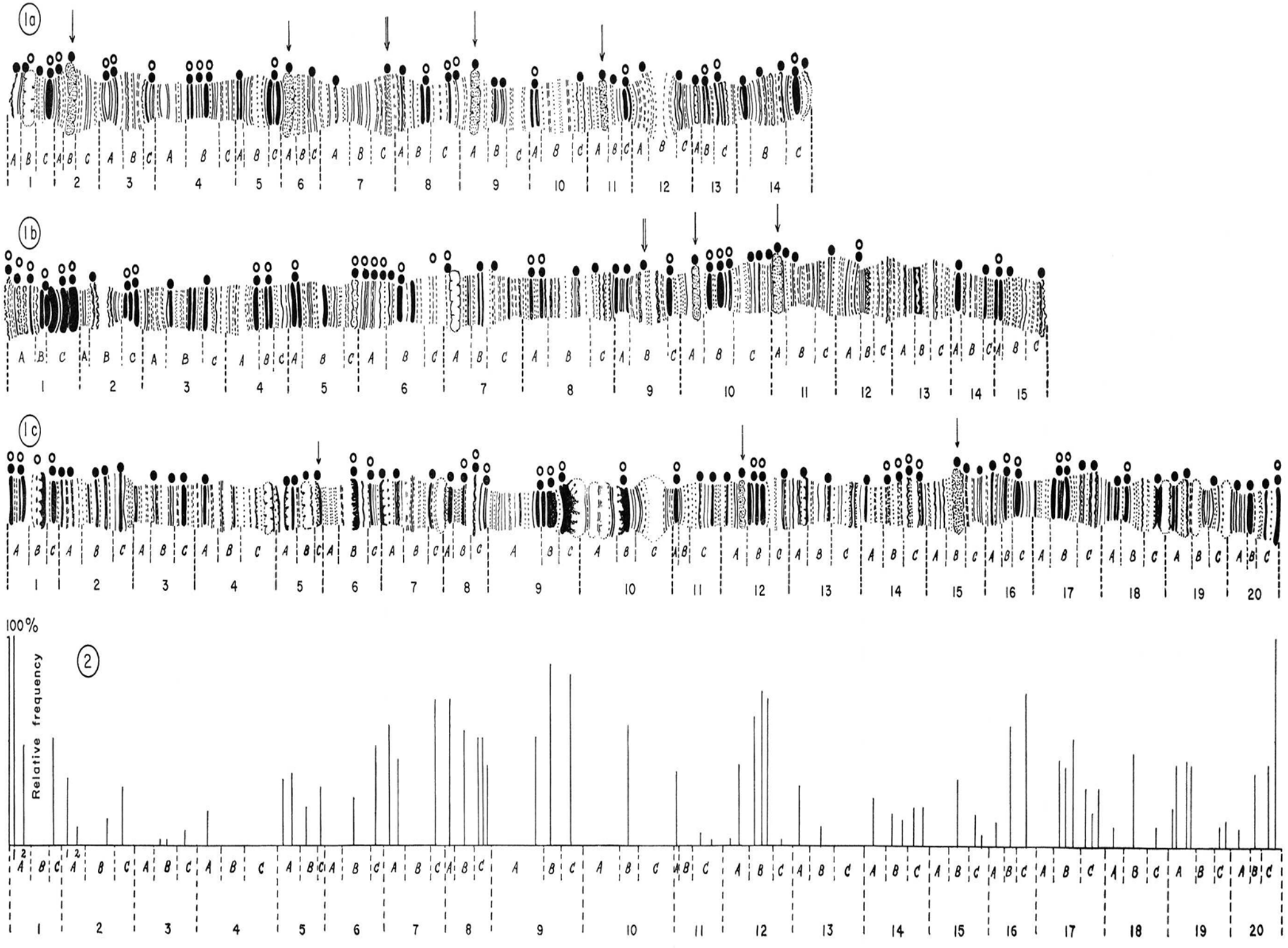
FIGURE 17 A micronucleolus (arrow) associated with a bulb (7C on chromosome II). Squash preparation, azure B. × 2000.

subject to error. In practice, however, a fairly high degree of resolution was obtained in localizing these sites. The micronucleoli, even when fragmented, appear to remain connected rather firmly to their specific chromosomal regions (Figs. 14 and 15). Sites were mapped as positive only when 2 or more of the 50 chromosomes studied showed micronucleoli at any given position. As may be seen in Fig. 1, the total number of micronucleolar sites is impressive: chromosome II has 43 to 50 out of a total of 240 to 250 bands; chromosome III has 52 to 55 out of a total of 290 to 300 bands; and chromosome IV has 68 to 70 out of a total of 340 to 350 bands. Thus, approximately 18 to 20% of the bands on each of the chromosomes are potentially capable of producing micronucleoli. Micronucleoli are most frequently found in contact with condensed bands (Figs. 14 and 15); they are found also at DNA puffs (Fig. 16) but rarely at a bulb (Fig. 17). The frequency with which micronucleoli are found at a particular site gives a rough estimate of the relative activity of that site. A detailed comparison of the "relative activities" of the sites on chromosome IV, based on a sample of 30 individual chromosomes from larvae at early DNA puff stages, was made (Fig. 2). As Fig. 2 shows, the most active sites are both ends ($1A_1$, $20C_2$) and the centric region (9B, 9C) with a frequency above 80%. Other condensed bands have a frequency range of 10 to 75%. The frequency at DNA puffs is approximately 30%.

In a previous study of *S. coprophila* salivary chromosomes, the heterochromatic regions were found to show asynchronous DNA replication (28). In Fig. 1, all heterochromatic, asynchronously replicating sites are compared with the sites of micronucleolar formation. It may be noted that almost all of these (with the exception of two) are

FIGURE 1 *a*, *b*, and *c* Cytological maps of chromosomes II, III, and IV of *Sciara coprophila*. Sites of micronucleolar formation are shown at solid circles. The heterochromatic bands known to show asynchronous DNA replication are marked by hollow circles. Note that all these bands, with the exception of 6C on chromosome III and 1B on chromosome IV, are also sites where micronucleoli are formed. Note also the presence of micronucleoli at DNA puffs (arrows) and a few bulbs, 7C on chromosome II and 9B on chromosome III (double arrow).

FIGURE 2 Graph showing the relative frequencies of micronucleoli at particular sites on chromosome IV. Thirty individual chromosomes from two different larvae, staged at early DNA puff formation, were analyzed. Map regions are represented on the abscissa. The frequency of micronucleoli seen at each site, expressed as per cent, is graphed on the ordinate. Regions with more than one active band are numbered consecutively on the map, i.e., $1A_1$, $1A_2$, etc.


1a
1b
1c
2
100%
Relative frequency

also associated with micronucleoli. In this context, it is interesting to note that DNA puffs also show asynchronous DNA replication, but form a group apart from the heterochromatic sites; while the heterochromatic sites probably replicate at the end of the DNA synthesis period, the DNA puffs show a more complex pattern.

A feature complicating the analysis of chromosomal RNA metabolism is the extreme variation encountered among larvae even when great care is taken to time precisely the developmental stages. In some preparations, we invariably found an almost complete lack of metachromatic staining, i.e., bulbs were barely stained, and chromosomes were free of micronucleoli. The vagaries of basic dye binding, particularly of intrachromosomal RNA (74), always raises the question of whether one is observing staining artefacts or variations in the amount of nucleic acids. An experimental series was run in which one of the paired glands of each larva was fixed, squashed, and stained, while the other one was prepared for radioautography after incubation in uridine-H^3. In all cases, the presence of good metachromatic RNA bulbs and micronucleoli in one gland was accompanied by a high rate of precursor incorporation into its partner. In glands where little or no metachromasy could be demonstrated, the partner gland invariably showed very low levels of incorporation. This variability may reflect the wide margin of asynchrony characteristic of larval development in *S. coprophila* as well as in other Diptera, e.g., *Chironomus*. It has also been reported that the rate of chromosomal RNA synthesis in *Chironomus* shows wide and unpredictable fluctuations from larva to larva (58).

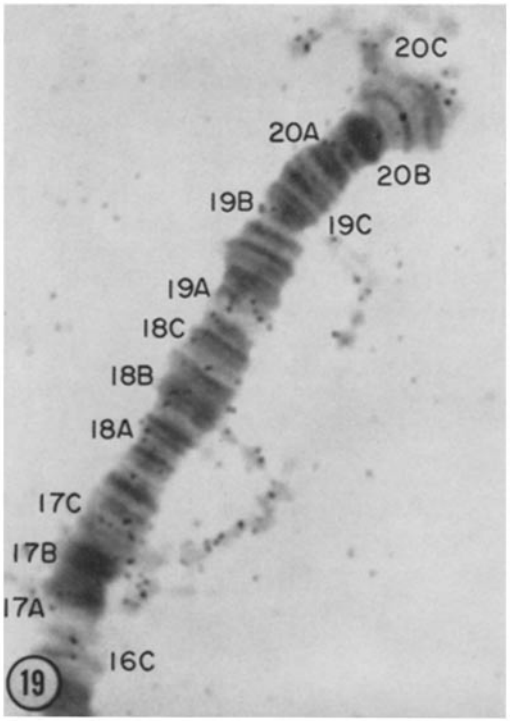


FIGURE 19 Radioautograph of chromosome IV originating from the same nucleus as chromosome III shown in Fig. 18. Labeled micronucleoli are associated with regions 17B, $17C_1$, 18B, $19C_1$, and $20C_2$. Grain density is comparable to that over a bulb (19A). × 1750.

FIGURE 18 Radioautograph of chromosome III slightly before DNA puff formation, following a 10 min incubation in uridine-H^3. Centromere end (map regions 1A, 1B, 1C) with associated labeled micronucleoli is shown. Cf. with Fig. 15. Smear preparation. × 1750.

Apart from the over-all fluctuation in RNA metabolism among larvae, there are characteristic differences in the rates of intrachromosomal and nucleolar RNA synthesis at different stages of development. During the period between eyespot formation and the initiation of DNA puff formation, a period of intense extra- and intrachromosomal RNA synthesis occurs. Bulbs, nucleoli, and micronucleoli all show high rates of precursor incorporation. Figs. 18 and 19 show portions of chromosomes III and IV following a 10 min pulse in uridine-H^3. The grain density per unit area over bulbs is roughly comparable to that over micronucleoli. At the time of maximal development of the DNA puffs, indicative of the approaching prepupal stage, a low point in the synthesis of extrachromosomal RNA occurs. Labeling of nucleoli and micronucleoli at this stage is very low, while

incorporation into bulbs and DNA puffs is still high. Figs. 20 and 21 show the X chromosome with its nucleolus at two different stages. At the time when DNA puff formation starts (Fig. 20), the nucleolar and intrachromosomal labeling is high. In a nucleus where DNA puffs reached their maximum size, incorporation into the nucleolus is greatly reduced, while incorporation into chromosomal RNA remains comparatively high (Fig. 21). With the onset of the prepupal stage, nuclear RNA synthesis of all fractions comes to a halt.

A characteristic of DNA puffs is that at these sites both intrachromosomal RNA synthesis and micronucleolar formation occur. A correlation between DNA puff regions and micronucleoli in *Sciara coprophila* was first reported by Swift (76). At the beginning of DNA puffing, RNA synthesis at these sites is predominantly micronucleolar (Fig. 22). At stages of maximal development, precursor incorporation into intrachromosomal RNA takes place; the entire puff area is labeled (Fig. 23), and micronucleoli are not found.

DISCUSSION

In *Sciara coprophila*, in addition to a major nucleolar aggregate, a large number of nucleolarlike bodies or micronucleoli are found to be intimately associated with the chromosomes. Assuming that such an association is not a fortuitous one, a study was made of the frequency of micronucleoli at specific chromosomal regions. The results show that most micronucleoli are localized at condensed or heterochromatic regions. The concept has developed that, during transcription, the chromosome segments involved are in a relatively uncoiled state (4, 7, 14, 29, 32, 76). Substantial evidence shows that condensed heterochromatic portions of some genomes are genetically inactive (13, 45, 68, 69) and, in general, fail to incorporate RNA precursors (9, 27, 44). This has been interpreted to mean that condensed, heterochromatic areas represent DNA templates which have become more or less inaccessible to transcription (9, 44). Heterochromatin is a difficult term to define in a conceptual sense (for reviews, see 17, 30, 67, 70). It differs from euchromatin in staining characteristics, due to a higher degree of condensation, and, in general, shows a tendency towards asynchronous DNA replication (28, 37, 43, 78). When referring to heterochromatic regions in *Sciara* polytene chromosomes, these are the two distinguishing criteria used.

The high frequency with which micronucleoli are found in association with condensed or heterochromatic regions raises the question of the relative synthetic activity at these sites. Since it is difficult to determine the exact site of synthesis of micronucleolar RNA, the possibility exists that the

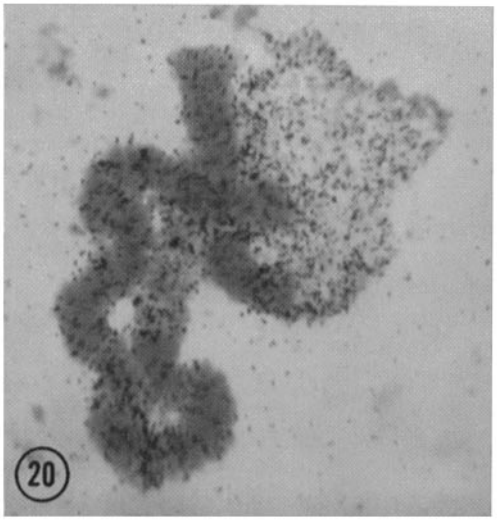


FIGURE 20 Radioautograph showing the X chromosome from a late fourth instar larva at the beginning of the DNA puff formation, following a 20 min incubation in uridine-H^3. Note intense labeling of the nucleolus and of most chromosomal bands. × 1050.

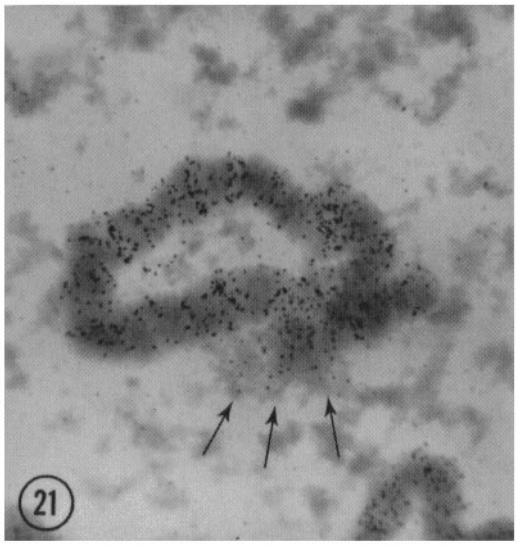


FIGURE 21 Radioautograph showing the X chromosome from late fourth instar larva at the stage of maximum expansion of the DNA puffs. Note low label and small size of the nucleolus (at arrows). × 1050.

material attached to a heterochromatic band is actually formed at immediately adjacent, noncondensed regions. However, what seems at least as likely a possibility is that localized uncoiling of a restricted segment of the heterochromatic band takes place. This is suggested by the presence of chromatin strands, often seen within micronucleoli, which terminate in chromatin granules (Fig. 13). It may be relevant to note that localized configurational changes have been reported to occur during the transformation of a heterochromatic band into a puff in *Chironomus* (84).

Why certain bands uncoil and expand to form puffs during periods of activity while others do not, remains to be explained. Why the RNA product elaborated at some bands tends to aggregate into bodies outside of the chromosome structure and why at other bands it tends to accumulate within the chromosome structure is not known. In general, puffing is associated with intrachromosomal RNA accumulation, while bands that give rise to extrachromosomal bodies show little morphological alteration during activity. The nucleolar organizer region shows both aspects, i.e., extrachromosomal aggregation and intrachromosomal puffing. At some stages, region 1A shows little expansion (Fig. 10), while at other times,

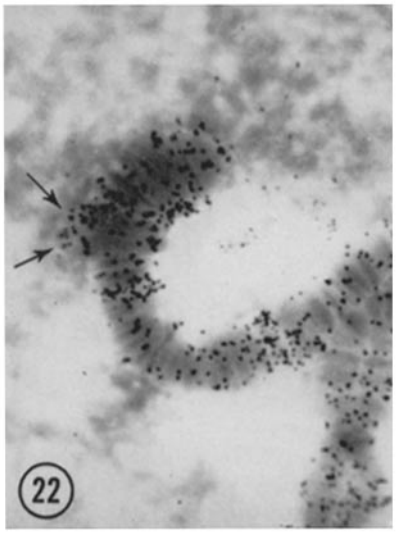


FIGURE 22 Radioautograph showing a portion of chromosome II with the DNA puff at 2B, after 20 min incubation in uridine-H^3, from a late fourth instar larva at the beginning of DNA puff formation. Note that at this stage label at the DNA puff is associated mainly with micronucleoli (arrows) and not with the central portion. × 1050.

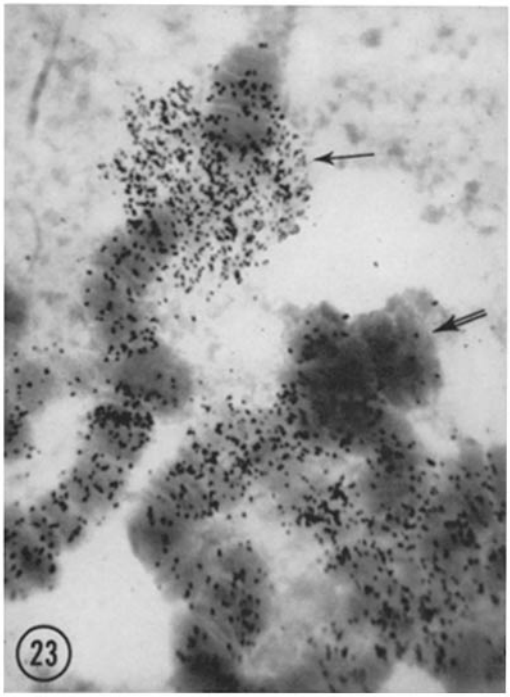


FIGURE 23 Radioautograph showing portions of chromosome II and the X chromosome, after a 20 min incubation in uridine-H^3, from a larva at the stage of maximum DNA puff expansion. Note label incorporation over the entire DNA puff area (single arrow). On the X chromosome, note low label and small size of the nucleolus (double arrow). × 1050.

particularly when the nucleolus is greatly enlarged, extensive ramification resembling a puff occurs (Figs. 11 and 12).

The presence of nucleolarlike bodies at many chromosomal sites other than the nucleolar organizer seems to be of widespread occurrence (5, 24, 25, 81). In studying the "fragmented" nucleoli of *Sciara*, Swift has reported that they have a fine structure similar to that of a typical nucleolus (76). In another Sciarid, *Bradysia mycorum*, Jacob and Sirlin (33) have described "elementary nucleoli." These consist of small aggregates, within and on the surface of chromosomal bands, and have a fine structure like that of the nucleolus. The authors suggest that these bodies coalesce, detach from the chromosome, and become free nucleoli. Micronucleolarlike bodies have also been described in the genus *Chironomus* (34). Here too, even the smallest body (0.3 μ) has been found to contain both the granular and amorphous elements typical of nucleoli.

In several plant and animal cells, concomitant with nucleolar formation in telophase, the socalled prenucleolar bodies appear at numerous sites on the chromosomes (40–42, 72). Since these bodies are structurally similar to the nucleolus and tend to fuse with it, they have been equated with nucleolar material. Some investigators have questioned this interpretation (75). In a recent study of interphase and prophase nuclei of meristematic cells, Lafontaine (41) has described spherical bodies which are structurally indistinguishable from the amorphous zones of the nucleolus, but lack the typical granular component. In summary, the ultrastructure of extrachromosomal bodies or micronucleoli, in general, resembles that of a typical nucleolus, but it may vary in the distribution of the granular and amorphous components.

There are now numerous studies to support the hypothesis that the nucleolus is the site of ribosomal RNA synthesis (10, 11, 20, 60–63, 66), and that the DNA complementary to ribosomal RNA tends to be clustered at the nucleolar organizer region. In bacteria (83), a plant (15), the insect *Drosophila* (66), and mice (Hoyer, quoted in reference 10), it has been found that approximately 0.3% of the genome is complementary to ribosomal RNA. Thus, the nucleolar organizer, it has been postulated, represents thousands of gene loci clustered together which are probably transcribed as a unit (10, 12). In *Sciara*, the number of bands that may produce micronucleoli amounts to about 18% per chromosome. On the other hand, taking the total number of bands of the entire complement as roughly 1000, the three dense bands of region 1A would represent about 0.3%. This is of the same order of magnitude as the 0.3% of the genome associated with ribosomal RNA reported from studies using molecular hybridization techniques, as mentioned above. Admittedly, counting bands is far removed from knowing the DNA content of any chromosomal segment, but the point to be noted is that if 18% of the bands are coding for ribosomal RNA, this would represent a far larger fraction of the genome than what has thus far been reported. Our studies do not enable us to draw any conclusions about the possible identity or diversity of the micronucleoli and the nucleolar aggregate in *Sciara*. What seems significant is that, in those genomes where decisive experiments could be performed, the evidence has been in favor of the clustered, as opposed to an extensively scattered, arrangement of ribosomal RNA codons.

There is at present no reason to believe that all material within the nucleolus is ribosomal RNA precursor derived exclusively from the organizer region. A number of authors have reported the presence of several RNA fractions in isolated nucleoli (50, 71, 80). There are many observations showing that extranucleolar material synthesized at various chromosomal loci, e.g. prenucleolar bodies, elementary nucleoli, spherical bodies, micronucleoli, and blob material in *Xenopus* and other amphibia (35), in general resembles the nucleolus structurally and tends to fuse with it. This suggests that there may be more to the nucleolus than can be defined from the codons of the organizer alone. Considering the information available so far, the following tentative picture of the nucleolus emerges. The nucleolar organizer is the site of synthesis of ribosomal RNA precursors. How these precursors and other components are organized to form the complete and functional organelle is not known. Other classes of RNA, produced elsewhere in the nucleus and released to the nuclear sap, may coalesce with the nucleolus. We rather doubt that, in *Sciara*, all sites capable of micronucleolar formation are equivalent to the nucleolar organizer region, although the material produced there may contribute to the nucleolar mass. Since our knowledge of the sequence from synthesis of ribosomal precursors to the formation of mature ribosomes and their subsequent transport to the cytoplasm is still incomplete, speculation about the functional significance of this aggregation would be premature.

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