TRANSCRIPTION SITES IN SPREAD MEIOTIC PROPHASE CHROMOSOMES FROM MOUSE SPERMATOCYTES

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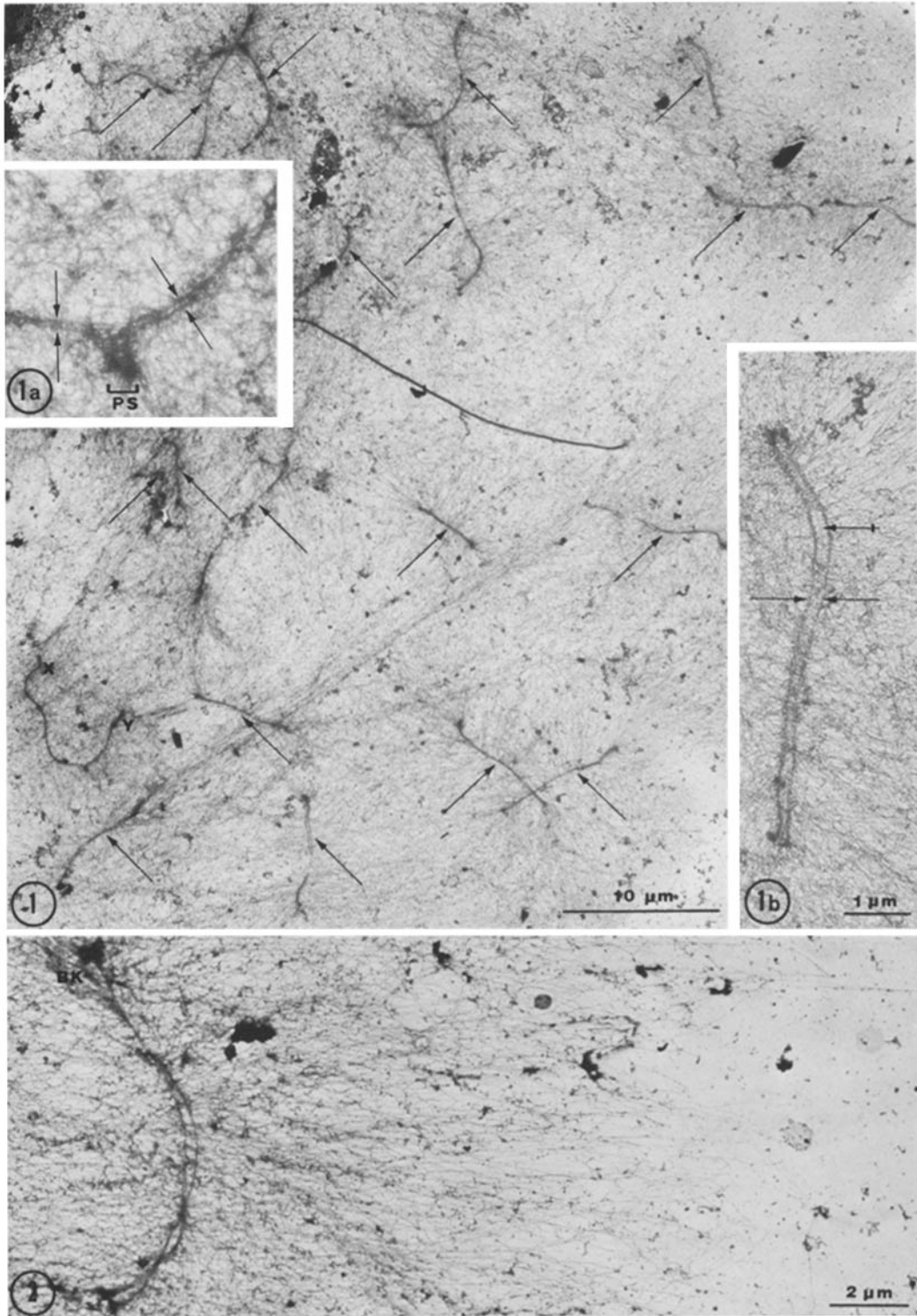
ABSTRACT

Mouse spermatocytes at pachytene stage have been examined by whole-mount electron microscope techniques complemented with autoradiography as an approach for visualizing their transcriptive activity. Structural elements of meiotic bivalents, such as synaptonemal complexes and chromatin fibers, have been satisfactorily displayed in the total set of autosomal and sexual bivalents in single spermatocytes. Adequate preservation of the entire set of bivalents has provided a basis for recognition of sites where presumptive preribosomal RNA and heterogeneous nuclear RNA species are being transcribed at different segments of autosomal bivalents. Nucleoli attached to the basal knob region where nucleolar organizer cistrons are assumed to be located and ribonucleoprotein fibrils associated with distinct chromatin loops have been recognized. These structural findings have been correlated with display of [³H]uridine incorporation sites in thin-section and whole-mount electron microscopy autoradiographic preparations. A low transcriptive activity of the sexual bivalent contrasted with extensive gene expression in autosomal bivalents. Each sex chromosome shows a double axial core. A short region of pairing with a synaptonemal complex joins the two chromosomes at one end. We conclude that variations in the rate of RNA synthesis throughout meiotic prophase stages in the mouse are expressed as fluctuations in the amount and distribution of distinct RNA species at specific segments of the bivalents.

Various regions of the genome in mammalian spermatocytes are transcribed into RNA at different rates during succeeding developmental stages. Some aspects of RNA synthesis throughout meiotic prophase in the mouse testis have recently been discovered by light and electron microscope autoradiographic studies with [³H]-uridine as a labeled precursor (Kierszenbaum and Tres, 1974). These investigations have revealed two different sites of RNA synthesis in synapsed autosomes during meiotic prophase;

a perichromosomal RNA synthesis related to heterogeneous nuclear RNA (hnRNA); and a nucleolar RNA synthesis linked with preribosomal species (prRNA). The condensed (heterochromatic) sex chromosomes displayed little or no RNA synthesis.

However, by electron microscope autoradiography of thin sections, it has not been possible to identify directly the active sites of perichromosomal labeling due to inadequate autoradiographic resolution and poorly defined boundaries of mei-

otic chromosomes. Therefore, isolation of mouse meiotic chromosomes has been attempted in an effort accurately to define active transcription sites.

Several aspects of meiotic chromosomes have been the subject of study since the development by Gall (1963) of a technique for the observation of isolated chromosomes in higher organisms. This procedure, based on whole mounts on electron microscope grids of the nuclear contents disspersed on an air-liquid interface, has been adapted and slightly modified by different authors for study of the chromatin fibers (Wolfe and John, 1965; Comings and Okada, 1970; Solari and Moses, 1973), of the synaptonemal complex (Comings and Okada, 1970; Solari and Moses, 1973), and of the kinetochore (Counce and Meyer, 1973) of meiotic chromosomes in several species. In general, these techniques have not permitted critical observations of RNA synthesis because of either insufficient spreading conditions or inadequate preservation of ribonucleoprotein (RNP) species, or of confusion of detail by precipitates of contaminating cellular aggregates on specimen grids along with the isolated chromosomes. Miller and Beatty (1969), Miller and Bakken (1973), and Hamkalo and Miller (1973), have overcome these difficulties by another method which is very useful for correlating chromosome structure and transcriptive activity.

In the present investigation we report structural and autoradiographic data obtained from whole-mount electron microscope preparations of pachytene mouse spermatocytes using spreads at an air-liquid interface and microcentrifugation techniques. We show that the perichromosomal labeling with [³H]uridine observed in thin sections of synapsed chromosomes corresponds to [³H]uridine incorporation at loosely extended chromatin projections along autosomal bivalents recognized in whole-mount preparations. Labeled nucleoli connected to terminal heterochromatin regions (basal knobs) of some autosomal bivalents have also been identified in these preparations. Structural features of entire sex chromosomes are well displayed.

MATERIAL AND METHODS

Adult male Swiss mice ($2n = 40$) (21-45-days old) were anesthetized with ether before injection of [³H]uridine ([5,6-³H]uridine, sp act 43 Ci/mmol, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y., lot no. YR-2413) used to label RNA. The labeled precursor was injected directly into both testes under the albuginea at a dose of 10 μCi per testis in a volume of 0.05 ml of sterile aqueous solution. The reasons for selecting an intratesticular route for isotope injection are discussed elsewhere (Kierszenbaum and Tres, 1974). After a postinjection interval of 3 h, testes were removed under anesthesia and placed on a watch glass kept on crushed ice. The tunica albuginea was removed. The seminiferous tubules were suspended in cold isotonic saline solution and the cells were dissociated from the seminiferous tubules by shearing forces produced by repeated aspirations and ejections with a syringe. The same procedure was used for control mice injected with the vehicle of the isotope and for untreated mice. Aliquots of the suspensions were transferred to centrifuge tubes (15 ml) precooled in ice and centrifuged at 800 g for 3 min in an International clinical centrifuge model CL. The supernate was discarded and replaced with the same amount of fresh isotonic saline solution. After the pellet had been resuspended, the tubes were centrifuged again at the same force for another 3 min. These procedures removed unincorporated labeled precursor. The pellet containing pieces of seminiferous tubules, cell aggregates, and isolated cells was gently resuspended with a Pasteur pipet. At this point, the

FIGURE 1   Electron micrograph of a spread mouse pachytene spermatocyte showing the complete set of autosomal and sexual bivalents. Each pair of synapsed autosomes is indicated by an arrow and the sexual pair by the letters X and Y. × 2,400. Fig. 1 a Detail of the sex chromosomes at the paired segment region (PS). Arrows indicate the double nature of the axial core of unpaired portions of X and Y chromosomes. × 17,400. Fig. 1 b Detail of an autosomal bivalent displaying the synaptonemal complex with a pair of lateral elements (between arrows) and a central element (→). × 10,500. Isolated in isotonic saline solution, dispersed in distilled water, microcentrifuged according to the procedure described by Miller et al. (1969, 1972), and positively stained with uranyl acetate.

FIGURE 2   Autosomal bivalent showing a twisted synaptonemal complex with a basal terminal knob (BK) differentiating the two ends of the bivalent. Tiny lateral loops are exposed at one side of the bivalent in this illustration. × 6,600. Microcentrifugation technique.
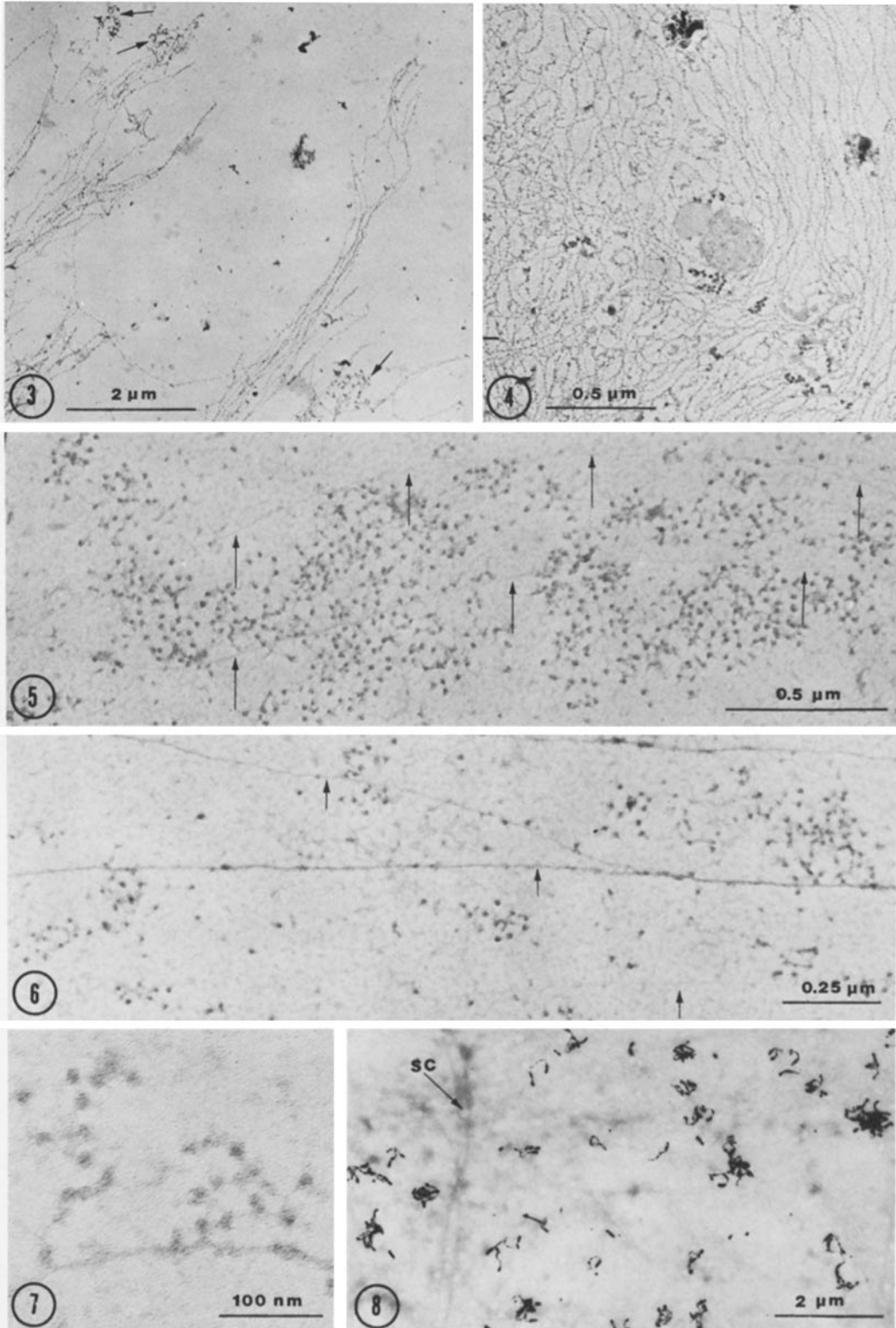
cellular suspension was processed by one of two different methods.

Microcentrifugation Method

For electron microscope examination of whole meiotic chromosomes and transcriptive sites, we used the method described by Miller and Beatty (1969), and Miller and Bakken (1973). A detailed description of this method has recently been published (Hamkalo and Miller, 1973).

Liquid-Air Interface Spreading Method

This is refered to here as the "spreading method." A drop of the cell suspension was allowed to spread on a surface of 0.9% aqueous NaCl in a glass crystallizing dish (125-mm diameter and 65-mm depth), after the liquid surface had been cleaned with a sheet of optical lens tissue. During this step, surface tension forces burst the cells and nuclear envelopes, spreading the nuclear content into a surface film. Immediately after spreading, the material was picked up by touching the liquid surface with Formvar-carbon-coated copper electron microscope grids (150 mesh). The grids were then stained for 10 min in a freshly prepared and filtered 0.5% aqueous solution of uranyl acetate, rinsed with distilled water, and air dried. This procedure was repeated several times after the liquid surface had been cleaned to remove cytoplasmic lipoproteins and other contaminating substances which might interfere with spreading by reducing the surface tension.

Some preparations were treated with pancreatic RNase (freed of DNase by boiling) in a concentration of 100 µg/ml in standard saline citrate (SSC). After enzymatic digestion, the grids were washed in SSC, and then stained with uranyl acetate.

Electron Microscope Autoradiography of Whole-Mount Preparations

Cellular suspensions of [³H]uridine-treated mice were spread and picked up on electron microscope grids supporting a Formvar-carbon film and stained in uranyl acetate, as cited above. Once stained, the grids were coated with a thin layer of carbon by vacuum evaporation. A monolayer of Ilford L4 emulsion was applied to the grids by a rectangular platinum wire loop, exposed for 30-60 days, and developed with Microdol-X or the gold latensification-Elon ascorbic acid procedure (Wisse and Tates, 1968; Salpeter and Bachmann, 1972; Salpeter and Szabo, 1972). The autoradiograms were fixed with Kodak Fixer (Eastman Kodak Co., Rochester, N. Y.), rinsed in distilled water, air dried, and examined in the electron microscope.

Air-Dried Preparations

Microscope slides for histochemical observations were obtained for light microscopy according to the method already described (Kierszenbaum and Tres, 1974). Slides were stained with 0.5% toluidine blue in 0.05 M potassium biphthalate buffer (pH 4) or with methyl green-pyronine. Some preparations were digested with RNase and afterwards stained with toluidine blue or methyl green-pyronine.

Electron Microscopy of Embedded Specimens

Small pieces of adult mouse testes were fixed in 2.5% glutaraldehyde (biological grade) in purified s-collidine buffer (Bennett and Luft, 1959) (pH 7.4), and embedded in Maraglas according to standard procedures. Sections yielding silver-gray interference-re-

FIGURE 3 High-magnification view of loops with attached RNP material (arrows). × 20,000. Microcentrifugation technique.

FIGURE 4 Area of chromatin fibers close to the pairing region. Presumptive chains of polyribosomes are superimposed at different points. × 34,200. Microcentrifugation technique.

FIGURE 5 Segments of chromatin fibers from a mouse pachytene spermatocyte. Arrows indicate the path of aggregated chromatin fibers coated by RNP chains of different length. × 50,400. Isolated and spread in an isotonic saline solution-air surface, picked up on electron microscope grids, and positively stained with uranyl acetate.

FIGURE 6 Chromatin axes of another spermatocyte under different aggregation forms (arrows). RNP transcripts are short, some are attached to the axes and others are free. ×60,000. Spreading method.

FIGURE 7 Detail of RNP chains associated with tightly joined chromatin fibers. × 160,000. Spreading method.

FIGURE 8 Autoradiogram of a whole-mount preparation made by the spreading technique 3 h after [³H]-uridine intratesticular injection. Silver grains are deposited in the lateral regions of the bivalent. The synaptonemal complex (SC) is free of labeling. × 8,500.

flected colors were stained with an 0.5% aqueous uranyl acetate solution for 10 min at room temperature.

All the specimens were examined in a JEM 100B electron microscope with a "cool beam" gun system operated at 60 or 80 kV with an objective aperture of 50-μm diameter. The instrument was calibrated with a cross-lined carbon grating replica having 1,350 lines per millimeter.

RESULTS

Sexual and Autosomal Bivalents in Whole-Mount Preparations

Meiotic chromosomes of the mouse testis observed by the microcentrifugation method display fine fibers radiating almost symmetrically at both sides of the axis of each homologue (Fig. 1), forming lateral loops (Figs. 2 and 3). Depending on the degree of spreading and on the meiotic prophase stage, sexual and autosomal bivalents of a single spermatocyte at pachytene stage can be identified (Fig. 1, 1 a, and 1 b) and measured. During pachytene stage, X and Y chromosomes are recognized as two associated chromosomes of unequal length, paired over a short portion at one end, displaying a paired segment much shorter than either chromosome (Figs. 1 and 1 a, 12, and 13). By contrast, the autosomal bivalents display a complete synapsis between homologues of the same length (Fig. 1 and 1 b). Synaptonemal complexes and basal knob regions are seen in these preparations. These features are helpful for recognizing the several meiotic prophase stages as well as the size and orientation of bivalents. The two lateral elements of the synaptonemal complex are easily identified but the central element and transverse fibers crossing the central region between two lateral elements can be recognized only if the tripartite synaptonemal complex is not overlapped by chromatin fibers (Fig. 1 b). When the spreading method is used, meiotic chromosomes are usually not well separated. The lateral chromatin fibers appear as random aggregates (Fig. 10). This prevents the identification of loops, probably due to uneven spreading and mechanical breakdowns. However, despite the mechanical action, structures such as the nucleoli associated with autosomal basal knob regions (Fig. 10) remain at their attachment sites.
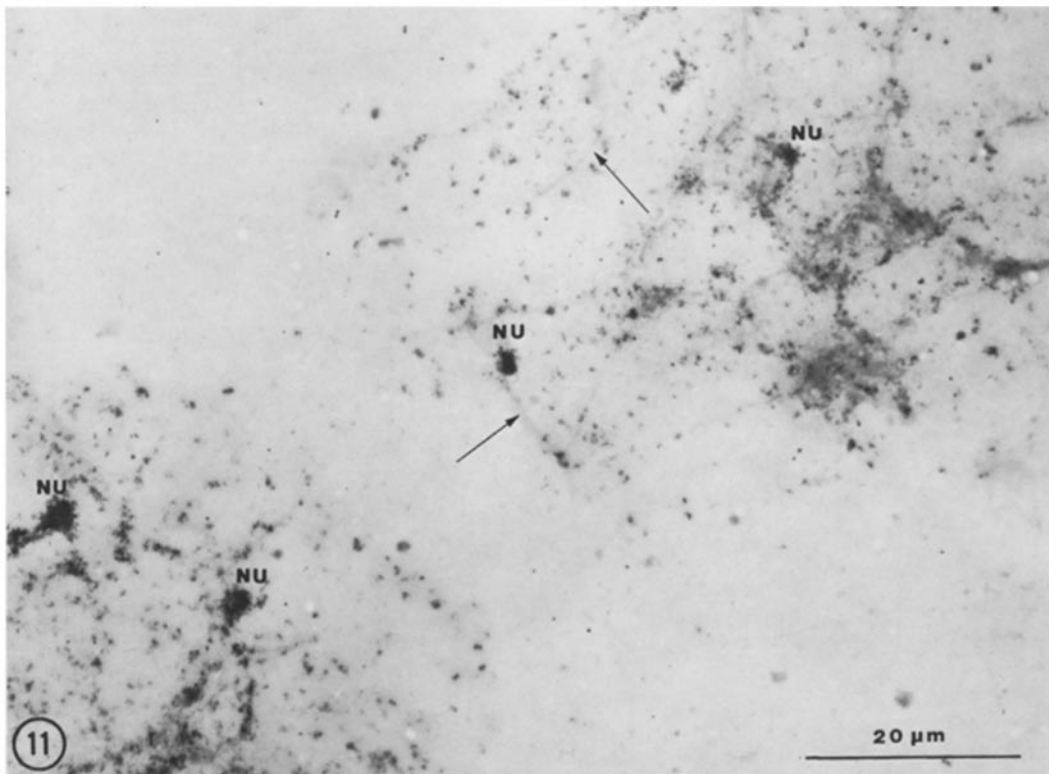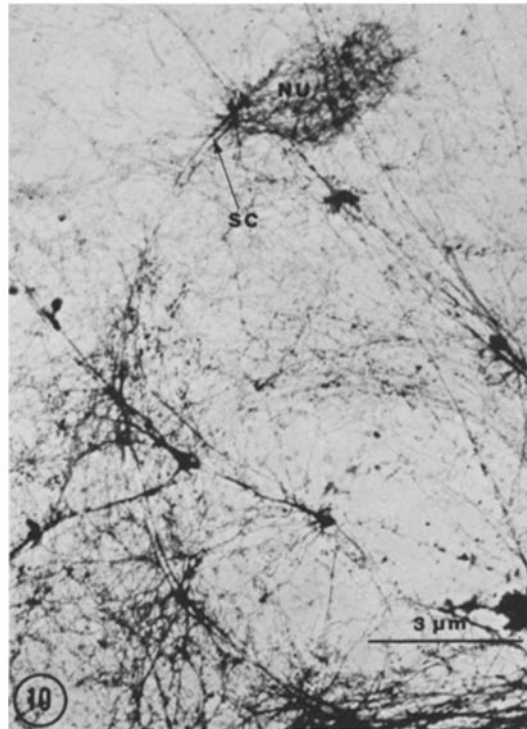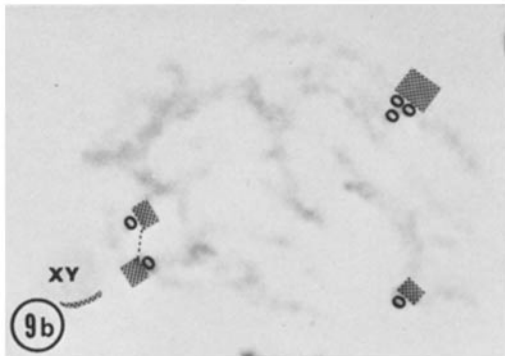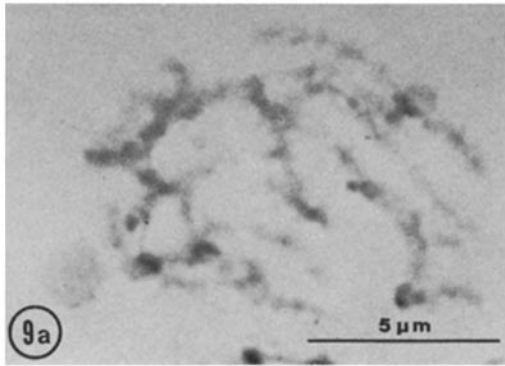
Structural details of the sexual bivalent observed at pachytene in whole-mount preparations coincide in general with three-dimensional reconstructions of the sex chromosomes derived from serial sections in the same species (Solari, 1970). Figs. 1 a and 12 show a partial synapsis in the XY bivalent where a synaptonemal complex is usually observed. The axial elements or cores representing chromosomal axes of each sex chromosome show thickened portions alternating with distinct double axial cores at the unpaired region of each chromosome (Fig. 1 a). A central element in a short synaptonemal complex of the Y chromosome can be recognized (Figs. 12 and 13). RNP chains can be identified interspersed among and close to chromatin projections of the sex chromosomes (Fig. 14). Since these chains are not seen attached to the sex chromosome fibers, we assume that RNP chains become detached from autosomal transcriptive regions and are relocated close to the sex pair during preparative manipulations.
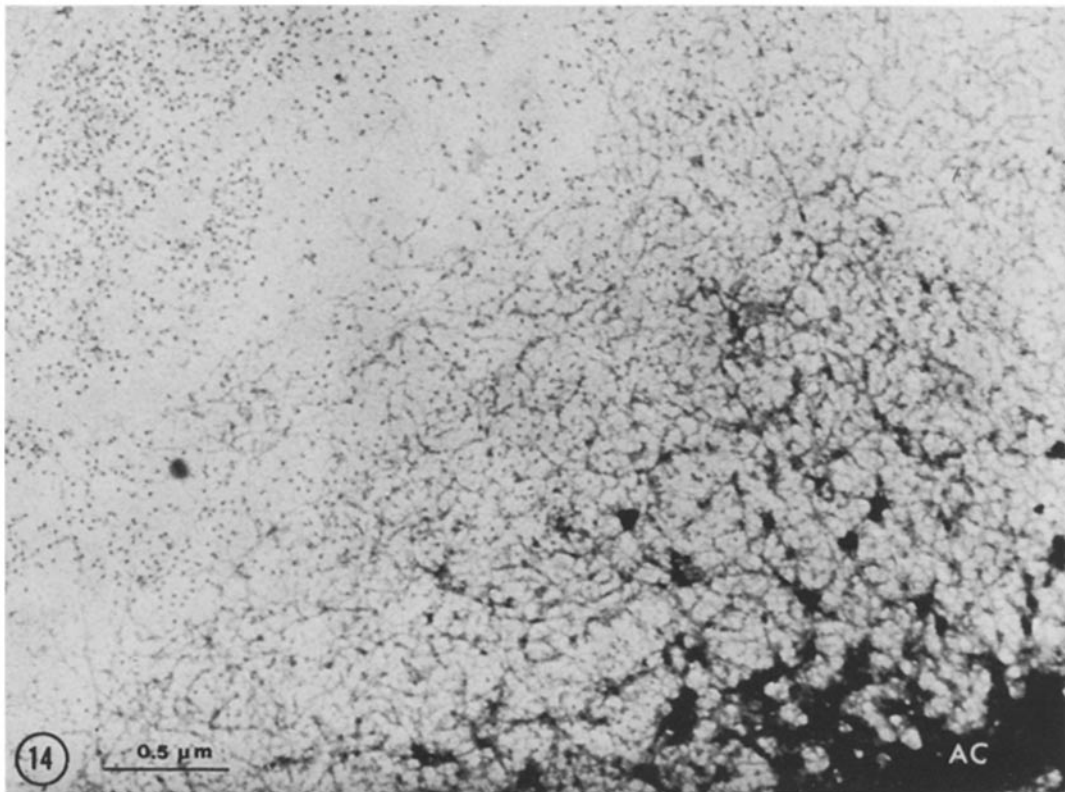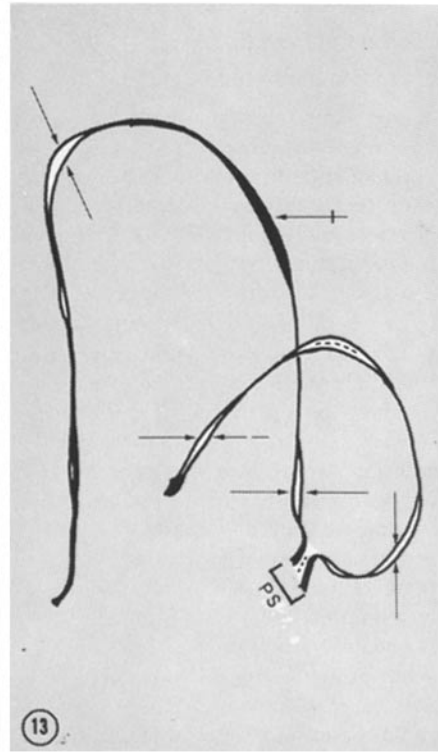

Figure 9   Air-dried preparation of a mouse pachytene spermatocyte. Stained with methyl green-pyronine. Fig. 9 b indicates the heterochromatin regions of the basal knobs (O) and some associated nucleoli as depicted in Fig. 9 a. Small clumps of nucleolar material are close to the XY chromosomes. A nucleolar connective piece joins the nucleolar regions (dotted line in Fig. 9 b). Printed from a Kodakchrome II transparency taken with light microscope. × 5,200.

Figure 10   Autosomal bivalent with an ovoid-shaped nucleolus (NU) connected to a basal knob region. The synaptonemal complex (SC) also is shown. × 6,500. Spreading technique.

Figure 11   Autoradiogram of a whole-mount spread preparation 3 h after [³H]uridine. Two spermatocytes show different bivalents defined by dense axial regions corresponding to synaptonemal complexes (→), some of them showing silver grain depositions at one of the terminal ends. Aggregates of chromatin fibers do not display the "hot spots" indicated as nucleolus (NU). Silver grains are also located at the lateral regions of bivalents, which are not very well resolved. × 1,400.

9a

5 µm

9b

XY

NU

SC

10

3 µm

NU

NU

NU

NU

11

20 µm

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X

Y

2 μm

⑫

⑬

PS

⑭ 0.5 μm

AC

Visualization of Transcription on the Lampbrush Loops of Autosomal Bivalents

The lampbrush character of mouse meiotic chromosomes observed by light microscopy (Monesi, 1965) is confirmed here in whole-mount microcentrifugation preparations examined by electron microscopy (Fig. 2). Moreover, lateral chromosomal loops of pachytene spermatocytes are associated with units of nascent RNP chains (Figs. 3, 5, and 6) of variable length that are distributed at random points along the chromatin axes. These chains are composed of granules ranging from 13 to 20 nm in diameter linked by thinner segments 15–25 nm in length. Granules and intervening segments are presumed to be formed of RNA, as they disappear after RNase treatment. The arrangement of RNP chains complicates the task of measuring them. An approximate estimation, however, may be obtained by counting the number of granular and fibrillar segments in uniformly stretched RNP chains. As determined from these figures and the average size of granules and fibrils, most of the RNP chains appear to range in length from about 350 nm to more than 900 nm. Presumptive polyribosome chains that overlap chromatin fibers are frequently seen also in the preparations (Fig. 4).

Autoradiographs of whole-mount spreads show that [³H]uridine is incorporated at the lateral loops projecting from both sides of the unlabeled synaptonemal complex (Fig. 8). This labeling almost certainly is equivalent to the perichromosomal [³H]uridine labeling seen in electron microscope autoradiographs of thin sections (Kierszenbaum and Tres, 1974), in which it was shown that nuclear labeling reaches a maximum during middle pachytene. Thus, it would be expected that the nascent RNP chains would vary in number in different pachytene substages. In fact, a dense population of side chains is de-

tected in loops of some pachytene spermatocytes (Fig. 5) whereas sparser chains occur in others (Fig. 6).

The numbers of silver grains along the basal knob, nucleolus, and lampbrush segments of autosomal bivalents are depicted in Fig. 18. The distribution curve for the three regions in the bivalent diagrammed reflects the average value for each region scored in different whole-mount bivalents (with and without nucleolus) and corrected for background values. Therefore, the ratios of grain density do not accurately reflect the transcriptive activity in a given bivalent. Significantly enough, the distribution curve per region shows a localized prRNA synthesis somewhere at the basal knob region, whereas RNA synthesis along the lampbrush segment is more dispersed.

Nucleoli in Whole-Mount Preparations

Single nucleoli connected to the paracentromeric heterochromatin region of the mouse acrocentric bivalents in pachytene can be seen in the light microscope (Fig. 9 a and b). As pointed out earlier (Kierszenbaum and Tres, 1974), the use of low temperatures (about 4°C) during manipulation of testicular suspensions favors preservation of nucleoli. Toluidine blue and methyl green-pyronine staining, tested with RNase digestion, reveals round or ovoid-shaped RNA-containing nucleoli at their autosomal attachment sites. Moreover, small patches of nucleolar material are recognized adjacent to the condensed mass of the sexual bivalent (Fig. 9 a and b). A satisfactory correlation can be established with an electron micrograph of a spread autosomal bivalent bearing a nucleolus (Fig. 10). Nucleoli can also be recognized by dense accumulations of silver grains at one of the terminal ends of the bivalent in autoradiograms of spreads made 3 h after [³H]uridine administration (Fig. 11). The

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Figure 12 Whole X and Y chromosomes showing the axial cores against a background of spread chromatin fibers. × 13,800. Microcentrifugation technique.

Figure 13 Diagram corresponding to Fig. 12. *PS*, paired segment. Arrows indicate the pair of cores for each chromosome. Dashed lines indicate synaptonemal complex regions. Thick lines (→) indicate tightly joined cores.

Figure 14 High magnification of part of a sexual bivalent. *AC*, axial core. Accumulations of RNP granules, some of them arranged in small chains are close to and interspersed with peripheral regions of the bivalent. × 32,000. Spreading technique.

specific region of the basal knob involved in the molecular organization of prRNA has not yet been identified.

Interchromatin Granules

Aggregates of granules in interchromatin spaces in pachytene spermatocytes can be identified clearly in thin sections of *s*-collidine-glutaraldehyde-fixed testis stained with aqueous uranyl acetate alone (Figs. 15 and 16). It is apparent from our results that staining of interchromatin granules is somehow influenced by the specific constitution of the buffer, since comparative studies with phosphate and veronal-acetate buffers show different contrasts with otherwise identical staining procedures. Available data (Swift, 1962; Nebel and Coulon, 1962 *b*; Busch and Smetana, 1970; Fakan and Bernhard, 1973) indicate that interchromatin granules contain RNA which presumably is surrounded by a protein coat protecting it from RNase degradation (Fakan and Bernhard, 1973). A chainlike structure of the interchromatin granules can be observed in thin sections (Fig. 15) and in whole-mount preparations (Fig. 17). The diameters of these granules in sections and spreads ranges between 18 and 25 nm. Our whole-mount autoradiographic preparations yielded insufficient resolution for a more precise characterization of the weakly [³H]uridine-labeled interchromatin granules (Fakan and Bernhard, 1973; Kierszenbaum, 1974). This matter deserves further study.

DISCUSSION

The structural organization of transcription sites in meiotic prophase chromosomes of mouse spermatocytes, particularly at pachytene, has been studied by whole-mount electron microscope techniques combined with autoradiography. The available data from electron microscope autoradiographic study of whole-mount preparations indicate conspicuous [³H]uridine-labeled masses attached to the terminal condensed region of autosomal bivalents (basal knobs). Labeled regions extending along the sides of the paired autosomes are also evident. These findings confirm and extend our earlier light and electron microscope autoradiographic studies of nucleolar and perichromosomal labeling with [³H]uridine throughout meiotic prophase stages in the mouse testis (Kierszenbaum and Tres, 1974).

A lampbrush pattern of chromosomes in mouse pachytene spermatocytes is well displayed in our whole-mount preparations. Although differences in the organization of oocyte (Callan, 1969) and spermatocyte meiotic chromosomes have been described (Henderson, 1971; Nebel and Coulon, 1962 *a*), it appears that RNA synthesis on lampbrush loops is one of the common functional features in both cell types (Muramatsu et al., 1968; Callan, 1969; Henderson, 1971; Miller and Bakken, 1973). It is now possible to simplify and unify terminology, since it is clear that the RNA transcription referred to previously as "perichromosomal" actually occurs on DNA-containing loops which are best regarded as part of the chromosome. In thin sections, the boundaries of the chromosomes are not clearly recognized, so that transcription actually occurring on loops was assigned a perichromosomal localization.

We can assume that the RNP fibrils represent the primary product of the loops and, therefore, that they may contain hnRNA molecules complexed with proteins. These assumptions are based on the following biochemical, autoradiographic, and structural evidences: (*a*) a rapid labeling of hnRNA molecules in pachytene nuclei of hamster testis demonstrated by sedimentation and base composition analyses (Muramatsu et al., 1968); (*b*) an early perichromosomal labeling with [³H]uridine of autosomal bivalents in hamster (Muramatsu et al., 1968) and mouse spermatocytes (Monesi, 1965; Kierszenbaum and Tres, 1974), revealed by autoradiography; (*c*) a nucleolar localization of [³H]uridine at the basal knob region of the synapsed autosomes (Kierszenbaum and Tres, 1974), where prRNA cistrons are presumed to be located; (*d*) a striking similarity between the RNP chains and similar RNP fibrils seen both free and attached to portions of the HeLa genome, probably representing hnRNA molecules (Miller and Bakken, 1973); (*e*) a lack of RNP chains attached to the chromatin of the sex chromosomes which are almost inactive in RNA synthesis (Monesi, 1965; Kierszenbaum and Tres, 1974); and (*f*) a progressive decrease of RNP chains in late spermiogenic stages, where transcription diminishes (unpublished observations). Besides this, cytochemical studies on meiotic prophase chromosomes of oocytes (Callan, 1969; Miller and Beatty, 1969; Miller and Bakken, 1973; Hamkalo and Miller, 1973) and spermatocytes (Nebel and Coulon, 1962 *b*) con-
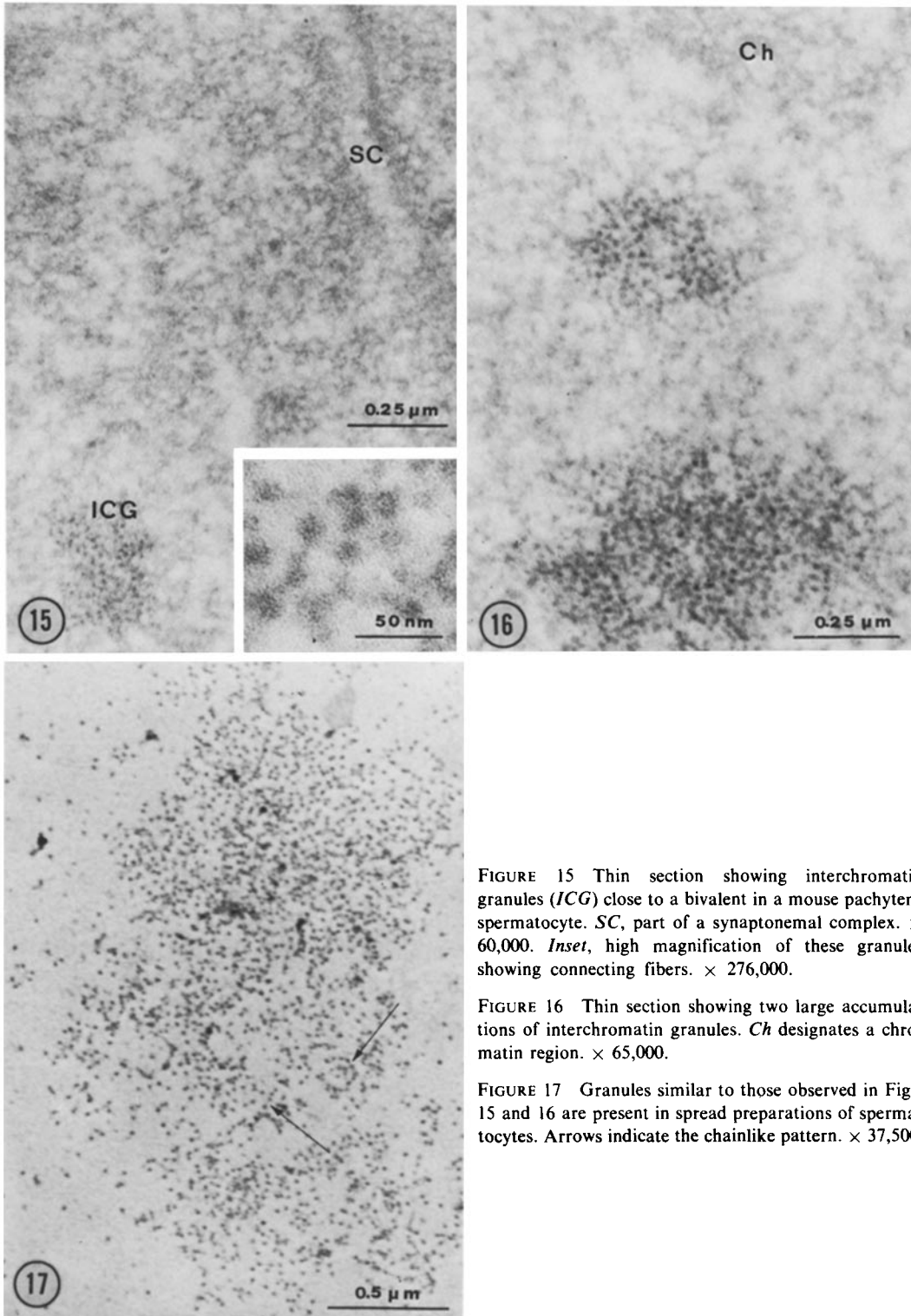
FIGURE 15 Thin section showing interchromatin granules (*ICG*) close to a bivalent in a mouse pachytene spermatocyte. *SC*, part of a synaptonemal complex. ×60,000. *Inset*, high magnification of these granules showing connecting fibers. × 276,000.

FIGURE 16 Thin section showing two large accumulations of interchromatin granules. *Ch* designates a chromatin region. × 65,000.

FIGURE 17 Granules similar to those observed in Figs. 15 and 16 are present in spread preparations of spermatocytes. Arrows indicate the chainlike pattern. × 37,500.
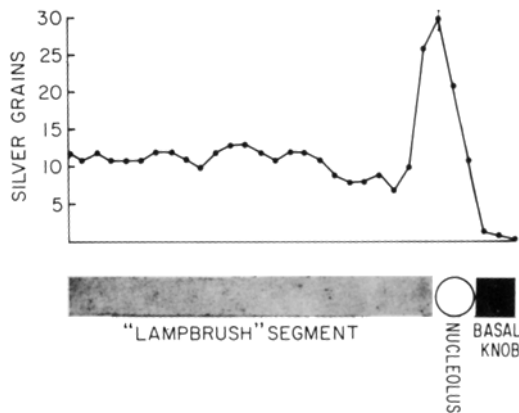
FIGURE 18 Mean values of silver grains per segment of an average autosomal bivalent with nucleolar organizing function. The values are designated for lampbrush, nucleolus, and basal knob segments. The nucleolus close to the basal knob shows a peak distribution. The bar indicates SD. The basal knob itself is not labeled with [³H]uridine. For details see text.

vincingly indicate that the lateral loops can be broken by DNase, whereas the associated RNP can be removed by proteases and RNase.

It was shown in the present study that discrete aggregates of RNP fibrils can also be detected free from chromatin attachment points. This observation presumably accounts for part of the persistent nuclear labeling 7-8 days after [³H]uridine injection (Kierszenbaum and Tres, 1974), since the newly synthesized hnRNA is to a large extent degraded to acid-soluble products within the nucleus after its synthesis (for a recent review, see Weinberg, 1973).

We have not recognized spacer-matrix units in our preparations, as have been shown in chromosomal (nucleolar organizer) (Miller and Bakken, 1973; Hamkalo and Miller, 1973) and extrachromosomal (amplified) ribosomal cistron loci in oocytes of diverse amphibian species (urodeles) (Miller and Bakken, 1973; Hamkalo and Miller, 1973), and in the house cricket (*Acheta domesticus*) (Trendelenburg et al., 1973). These features, if present in pachytene stage, may be obscured in our preparations, which are only partially spread, so that the chromatin and RNP-associated species are clumped together in aggregates. More complete spreading results in the disruption of the synapsed regions of the bivalents. It is helpful to preserve these regions, as they aid in the recognition of meiotic prophase stages in our whole-mount preparations.

Accumulations of RNP interchromatin granules have been described in pachytene spermatocytes (Nebel and Coulon, 1962 *a*, *b*; Tres et al., 1972) and in Sertoli cells (Tres et al., 1972; Kierszenbaum, 1974). In agreement with in vitro studies, one of us (Kierszenbaum, 1974) has shown that those polysome-like granules are weakly labeled in vivo after 3 h of [³H]uridine administration and are completely unlabeled 8-12 days after isotope injection. The data are still insufficient to determine whether these granules contain slowly labeled nuclear RNA (Fakan and Bernhard, 1973), or whether they represent persisting nuclear pools of RNP species originating elsewhere in the nucleus. Interestingly enough, interchromatin granules are very conspicuous in the nucleoplasm of Sertoli cells (Kierszenbaum, 1974) and of middle pachytene spermatocytes (Tres et al., 1972), cells which are very active in RNA synthesis.

In a previous study (Kierszenbaum and Tres, 1974), we noted no evidence of significant transcription activity in the sex chromosomes after different times of labeling with [³H]uridine. Our present results, based on whole-mount preparations combined with autoradiography, support the validity of inactive templates in mouse sex chromosomes. Another remarkable finding in the whole-mount display of the sexual bivalent is the double axial element or core of each mouse sex chromosome. Similar findings have been reported in three-dimensional reconstructions of the XY pair in human spermatocytes (Solari and Tres, 1970). The biological significance of these observations resides in the presence of synaptonemal complex structures at variable points along the unpaired segments of the X and Y chromosomes ("anomalous complexes," Moses, 1968; Solari, 1970) depending on the developmental stage of this bivalent during the meiotic prophase. From the foregoing account, we assume that the axial cores become the lateral elements of the complex at the unpaired segments of the sex chromosomes.

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