# ACTIVE MOVEMENTS OF THE CHROMATOID BODY

## A Possible Transport Mechanism for Haploid Gene Products

MARTTI PARVINEN and LEENA-MAIJA PARVINEN

From the Institute of Biomedicine, Department of Anatomy, University of Turku, Turku 52, Finland

ABSTRACT

Recent data indicate that the chromatoid body typical of rat spermatogenesis may contain RNA synthesized in early spermatids by the haploid genome. Analyses of living step-1 and step-3 spermatids by time-lapse cinephotomicrography have shown that the chromatoid body moves in relation to the nuclear envelope in two different ways. Predominantly in step 1, the chromatoid body moves along the nuclear envelope on a wide area surrounding the Golgi complex and has frequent transient contacts with the latter organelle. In step 3, the chromatoid body was shown to move perpendicular to the nuclear envelope. It was seen located very transiently at the top of prominent outpocketings of the nuclear envelope with apparent material continuities through nuclear pore complexes to intranuclear particles.

The rapid movements of the chromatoid body are suggested to play a role in the transport of haploid gene products in the early spermatids, including probably nucleocytoplasmic RNA transport.

KEY WORDS rats · spermatids · chromatoid body (cytoplasmic inclusions) · electron microscopy · time-lapse cinephotomicrography · living cells

A considerable amount of new information has recently been accumulated about the origin, relationships, and chemical composition of the chromatoid body in mammalian spermatogenesis. The chromatoid body has been demonstrated to first appear in pachytene spermatocytes at stage VIII (9) of the rat seminiferous epithelial cycle (19). During the pachytene and diplotene stages of meiotic prophase it grows, and specific intranuclear membrane modifications may be involved in the transport of material from the nucleus to the chromatoid body (18). During meiotic reduction divisions, the chromatoid body has been found to be dispersed in the cytoplasm as small 30-nm particles (19), while its condensation into the definite shape typical of spermiogenesis takes place during step 1 of spermiogenesis (25). The chromatoid body has its most prominent structure during early spermiogenesis in round-nucleated spermatids (26). In these cells, the chromatoid body is apparently dependent on the function of the haploid genome (14, 21) which continues up to that stage of spermiogenesis in which the chromatin begins to condense (4, 6, 7, 10, 11, 22, 27). Radioactivity derived from tritiated uridine is incorporated into the chromatoid body clearly after the nuclear labeling, thus suggesting that RNA synthesized in the haploid nucleus is transported to this organelle (24). The mechanisms of this transport have not been analyzed in detail, although an increased occurrence of nuclear pore complexes has been reported on an area adjacent to the chromatoid body (2, 5, 17). Previous studies (13, 23) have led us to assume that the rapid nonrandom movements of the chromatoid

body on the nuclear surface during early spermiogenesis may play a role in the transport of material between the nucleus and the chromatoid body.

## MATERIALS AND METHODS

### *Living Cell Preparations and Cinephotomicrography*

Young adult male Sprague-Dawley rats were used in the experiments. After killing the animals, the testes were freed and the tunica albuginea was removed. The tubules were isolated from the interstitial tissue in Krebs-Ringer's solution by a technique described by Christensen and Mason (3), and subjected to transmitted light in a stereomicroscope. A characteristic variation in the light absorption of the isolated tubules allows the recognition of the stages of the seminiferous epithelial cycle (16). Stages I and III (9) were chosen for further analysis by phase-contrast microscope. Short segments (0.5–1 mm) were isolated and squeezed between glass slides to produce slightly flattened monolayers (12, 25). Time-lapse cinephotomicrographic analyses were performed

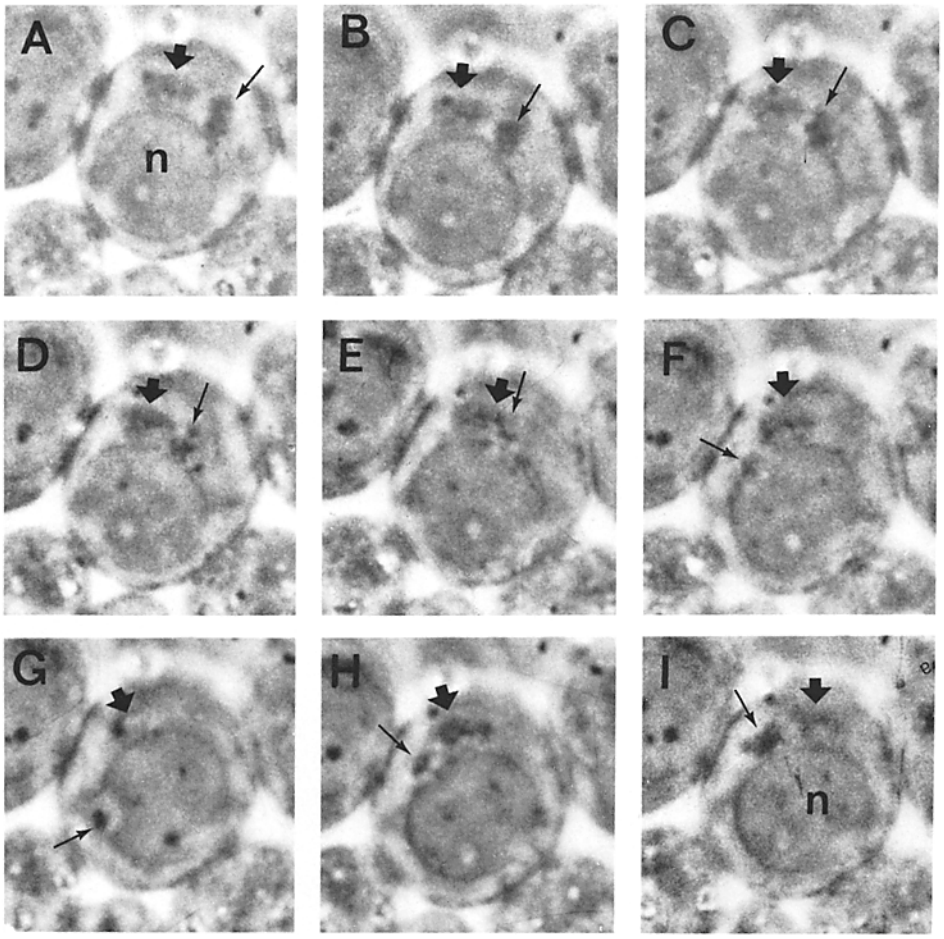



FIGURE 1 A series of Super-8 film frames of a living step-1 rat spermatid at selected time intervals after the situation depicted in frame *A*. The chromatoid body (cb, thin arrows) moves rapidly parallel to the nuclear (*n*) envelope. It has transient contacts with the Golgi complex (G, thick arrows). The time intervals and organelle situations are as follows: (*A*) 0, cb on right side of G; (*B*) 44 s, cb comes nearer G; (*C*) 1 min 36 s, cb is very close to G; (*D*) 2 min 12 s, cb has a contact with G; (*E*) 4 min 52 s, cb is superimposed by G; (*F*) 5 min 42 s, cb moves away from G on its left side; (*G*) 9 min 00 s, cb is located farthest away from G; (*H*) 11 min 16 s, cb has again come close to G; (*I*) 13 min 00 s, cb has a contact with G. During the excursions of the cb, the position of G remains relatively constant. During projection of the film, the intranuclear dense particles seem to follow the movements of the cb to some extent. × 2,100.

for 1 h after killing the animal, with a Beaulieu 4,008 ZM camera (Maison Brandt Frères, Charenton-le-Pont, France), Super-8 film (Kodachrome 40), and an automatic timer (Chinon Interval Timer, Chinon Interval Corp., Tokyo) that exposed the frames in 2-s intervals. Individual frames with specific information were selected and marked by using a viewer (Erno E-700 8-mm editor viewer, Sansei Koki Co., LTD, Goko, Japan) for photographic reproduction and serial analysis.

### *Electron Microscopy*

Stages I and III were recognized in the living, freshly isolated, unstained condition by a combined transillumination-phase-contrast-microscopic technique (15, 22). The tubular segments were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.2, for 3 h at 4°C. They were postfixed in osmium tetroxide, embedded in Epon, and sectioned at 70 nm with a LKB-Huxley ultramicrotome (LKB Instruments, Rockville, Md.). The sections were stained with uranyl acetate and lead citrate, and observations were made with a JEOL-JEM-T8 electron microscope.

## RESULTS

At step 1 of spermiogenesis, a spermatid showing typical extensive movements of the chromatoid body on a wide area of nuclear envelope on both sides of the Golgi complex was selected for the first film analysis (Fig. 1). The chromatoid body in ~70% of all step-1 spermatids moves to an extent similar to that of this cell, while in others the movements are slower and not so extensive. The chromatoid body has typically frequent contacts with the Golgi complex, which is distinguished morphologically from the chromatoid body by its larger size, more solid structure, and its association with the acrosomic system. The chromatoid body moves three-dimensionally and principally over the hemisphere of the nucleus in which the Golgi complex and the early acrosomic system are located. The series of frames in Fig. 1 shows how the chromatoid body (thin arrows) is first located on the right side of the Golgi complex (thick arrows) on the nuclear (*n*) surface (Fig. 1*A*), then moves towards the Golgi complex (Figs. 1*B* and *C*) and has contact with it after 2 min 12 s (Fig. 1*D*). In Fig. 1*E*, the chromatoid body is superimposed, and possibly still in contact, with the Golgi complex. Then, the chromatoid body again appears in the plane of focus and is located on the left side of the Golgi complex (Fig. 1*F*). The distance between these organelles is largest in Fig. 1*G*, after which the chromatoid body again moves towards the Golgi complex and has contact with it (Fig. 1*I*).

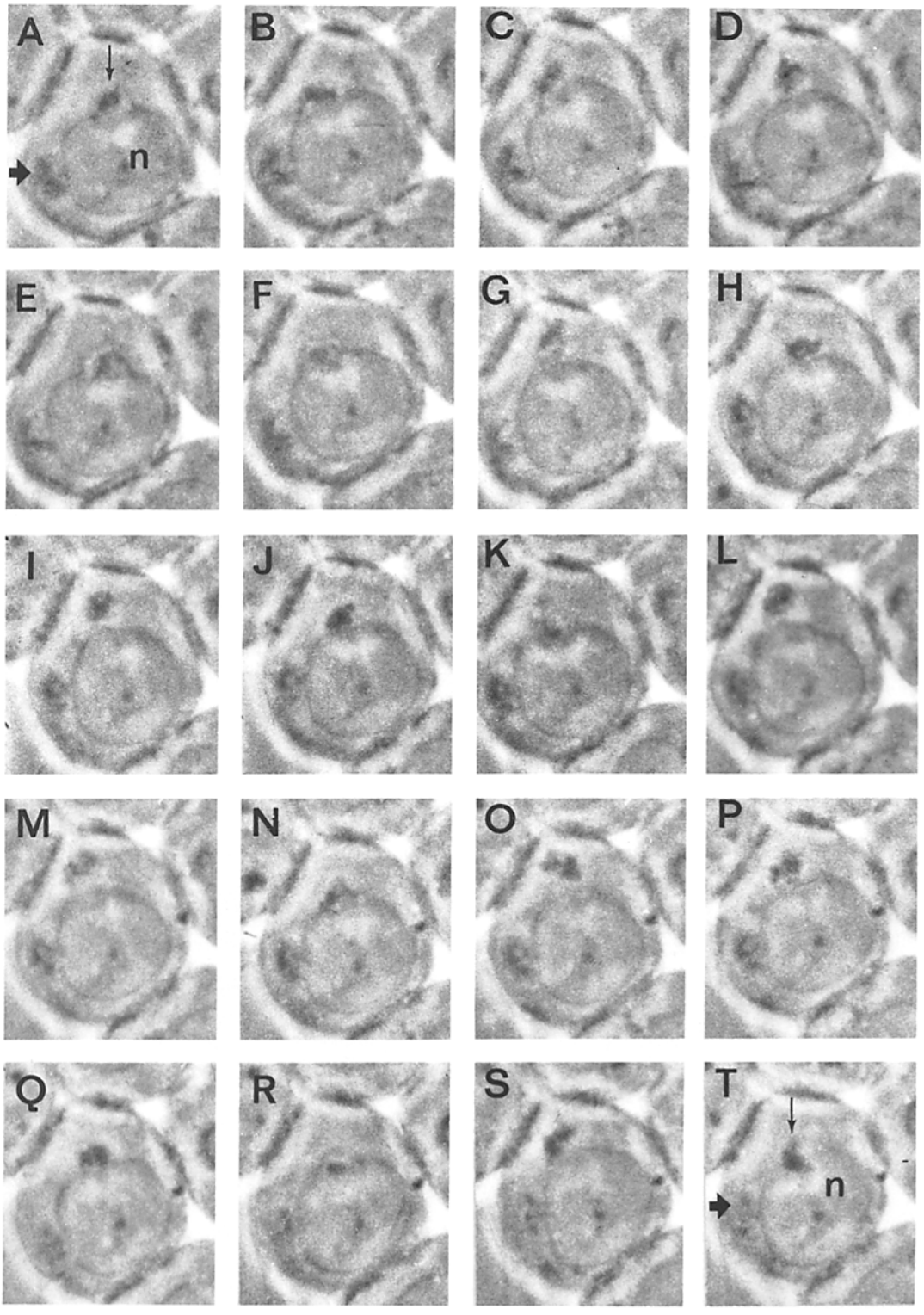
Another type of chromatoid body rapid movement was typical in step 3 of spermiogenesis (Fig. 2); this was directed perpendicular to the nuclear envelope. During these excursions, the chromatoid body is seen in nuclear-envelope inpushings (Fig. 2 *A*, *E*, *F*, *H*, *K*, *R*, and *T*), at the level of the nuclear envelope (Fig. 2*B*, *C*, *J*, *N*, *P*, and *Q*), or above the nuclear envelope (Fig. 2*D*, *G*, *I*, *L*, *M*, *O*, and *S*). When the film was projected on the screen, both rapid and slow components of the movements were distinguished. Outpocketings of the nuclear envelope at the proximity of the chromatoid body are rapid and transient phenomena, lasting only for a few seconds (Fig. 2*I*). During its excursions, the chromatoid body has continuously changing relationships with small particles which are mainly of cytoplasmic, but also of intranuclear, origin.

The ultrastructural analyses were focused on the transient outpocketing phenomenon of the nuclear envelope. Figs. 3 and 4 show a step-3 spermatid in which a nuclear outpushing in the vicinity of the chromatoid body has an apparent material continuity with the body through a nuclear pore complex. In the outpocketing, several 20-nm particles are seen. There may also be a 20-nm particle in the central part of the nuclear pore complex.

## DISCUSSION

Time-lapse cinephotomicrography of living cells has proven to be a valuable tool in directing ultrastructural analyses to organelle constellations which because of their transiency in vivo are rarely seen but which probably have significance for the functions of the haploid genome and the chromatoid body. Although the continuous interaction between the nucleus and the chromatoid body was also observed in our previous films (13), the main attention was focused on the rapidly changing relationships of the chromatoid body and the Golgi complex during steps 1 and 2, and the perpendicular movement was not observed because it is typically found in step 3 of spermiogenesis.

Numerous recent investigations support the view that the chromatoid body has several functions during spermiogenesis. There is evidence of its role in the early formation of the acrosomic system through contacts with the Golgi complex (1, 2, 13, 17, 23) and in the formation of a subacrosomal rodlike structure which possibly has a function in oocyte activation (17).


A
n
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
n

Perhaps the most interesting function of the chromatoid body, however, is its participation in the RNA metabolism of developing male germ cells. During early spermiogenesis, the nuclear pore complexes in the vicinity of the chromatoid body are more numerous than elsewhere (2, 5), and material continuities through them into the intranuclear organelles have been demonstrated (23). Radioactivity derived from tritiated uridine, which was first incorporated into the spermatid nucleus, has been shown to incorporate later into the chromatoid body (24). The intranuclear particles with an apparent material continuity through nuclear pore complexes to the chromatoid body have a diameter of 20 nm, which corresponds to the size of nuclear informofers (20). These structures have been shown to be involved in the transport of RNA from the nuclei of dragonfly oocytes to cytoplasmic structures analogous to the chromatoid body (8).

The role of the chromatoid body in the RNA metabolism of spermatogenic cells is still far from clearly understood. Recent data indicate that a considerable proportion of the RNA synthesized in pachytene spermatocytes is preserved through spermatid development until late spermiogenesis (6). An interesting possibility is that the chromatoid body is a storing organelle for this long-lived RNA which is suggested to direct the protein synthesis during late spermiogenesis when the genome of the spermatid is inactive (11). Continued investigation is required to determine the significance of the movements of the chromatoid body for the metabolism of this RNA. Because it is a clearly visible organelle in the light microscope, the chromatoid body may serve as a new aspect for research on the general mechanisms of nucleocytoplasmic RNA transport.

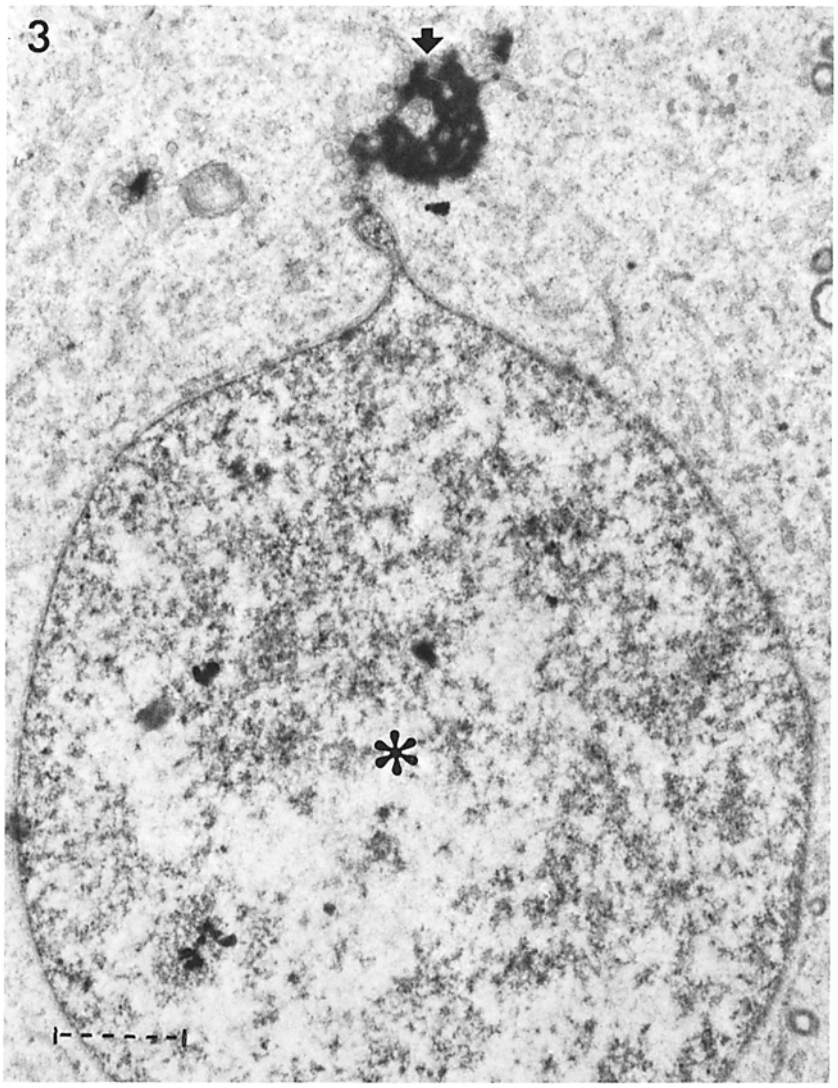
The authors are grateful to Drs. Liisa and Olli Halkka and to Dr. Peter Engelhardt for stimulating discussions and advice, to Ms. Raija Andersen, Mr. Rolf Björkroth, and Mr. Kauko Marttila for skillful technical assistance, and to the Paulo Foundation for financial support.

*Received for publication 23 May 1978, and in revised form 17 October 1978.*

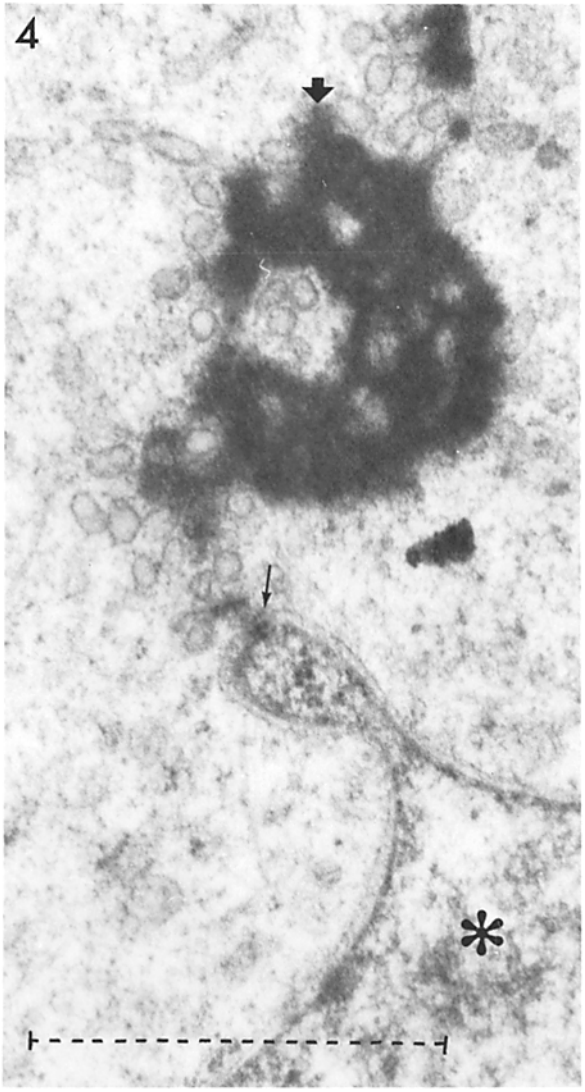
## REFERENCES

1. ALVESTAD-GRAEBNER, I., and H. ADAM. 1976. Relationship between the chromatoid body and the acrosomal system in early spermatids of *Myxine glutinosa L. Cell Tissue Res.* **174:**427–430.
2. BAWA, S. R. 1975. Comparative studies on the origin of the chromatoid body. *Biol. J. Linn. Soc.* **7**(Suppl. 1):275–278.
3. CHRISTENSEN, A. K., and N. R. MASON. 1965. Comparative ability of seminiferous tubules and interstitial tissue of rat testes to synthesize androgens from progesterone-4-$^{14}$C in vitro. *Endocrinology.* **76:** 646–656.
4. D'AGOSTINO, A., R. GEREMIA, AND V. MONESI. 1978. Post-meiotic gene activity in spermatogenesis of the mouse. *Cell Differ.* **7:**175–184.
5. FAWCETT, D. W., E. M. EDDY, AND D. M. PHILLIPS. 1970. Observations on the fine structure and relationships of the chromatoid body in mammalian spermatogenesis. *Biol. Reprod.* **2:**129–153.
6. GEREMIA, R., C. BOITANI, M. CONTI, and V. MONESI. 1977. RNA synthesis in spermatocytes and spermatids and preservation of meiotic RNA during spermiogenesis in the mouse. *Cell Differ.* **5:** 343–355.
7. GEREMIA, R., A. D'AGOSTINO, and V. MONESI. 1978. Biochemical evidence of haploid gene activity in spermatogenesis of the mouse. *Exp. Cell Res.* **111:**23–30.
8. HALKKA, L., and O. HALKKA. 1975. Accumulation of gene products in the oocytes of the dragonfly *Cordulia aenea L.* I. The nematosomes. *J. Cell Sci.* **19:**103–115.
9. LEBLOND, C. P., and Y. CLERMONT. 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. N. Y. Acad. Sci.* **55:**548–573.
10. MONESI, V. 1965. Synthetic activities during spermatogenesis in the mouse. RNA and protein. *Exp. Cell Res.* **39:**197–224.
11. MONESI, V. 1971. Chromosome activities during meiosis and spermiogenesis. *J. Reprod. Fertil. Suppl.* **13:**1–14.
12. PARVINEN, M. 1973. Observations on freshly isolated and accurately identified spermatogenic cells of the rat. Early effects of heat and short-time experimental cryptorchidism. *Virchows Arch. Abt. B Zellpathol.* **13:**38–47.
13. PARVINEN, M., and P. T. JOKELAINEN. 1974. Rapid movements of the chromatoid body in living early spermatids of the rat. *Biol. Reprod.* **11:**85–92.
14. PARVINEN, L.-M., P. T. JOKELAINEN, and M. PARVINEN. 1978. Chromatoid body and haploid gene activity: Actinomycin D induced morphological alterations. *Hereditas.* **88:**75–82.
15. PARVINEN, L.-M., and M. PARVINEN. 1978. A "living cell method" for testing the early effects of antispermatogenic compounds: Model

FIGURE 2 A series of Super-8 film frames of a living step-3 spermatid showing rapid movements of the chromatoid body (thin arrows) in direction perpendicular to nuclear (*n*) envelope. The Golgi complex is indicated by thick arrows. The time intervals (after *A*) and the positions of the cb are as follows: (*A*) 0, cb in a shallow inpushing of the nuclear envelope (ne); (*B*) 56 s, cb at the level of ne; (*C*) 1 min 40 s, cb slightly elevated from ne; (*D*) 2 min 44 s, cb above ne; (*E*) 3 min 02 s, cb in a shallow inpushing of ne; (*F*) 3 min 46 s, cb in a deep inpushing of ne; (*G*) 4 min 12 s, cb high above ne, probably at a top of an outpocketing of ne; (*H*) 8 min 08 s, cb in a shallow depression of ne; (*I*) 8 min 24 s, very shortly after (*H*), cb is located high above ne and clearly at a top of a ne outpocketing; (*J*) 9 min 42 s, cb at the level of ne, probably in a slight depression; (*K*) 11 min 10 s, cb in the deepest location of this study; (*L*) 12 min 14 s, cb above ne, probably with an outpocketing; (*M*) 12 min 32 s, cb still high above ne; (*N*) 12 min 54 s, cb at the level of ne; (*O*) 13 min 30 s, cb above ne, without outpushing of ne; (*P*) 14 min 34 s, cb at the level of ne; (*Q*) 18 min 44 s, cb at the level of ne; (*R*) 19 min 34 s, cb in a depression of ne, slightly out of the focal plane; (*S*) 24 min 20 s, cb at the top of a slight outpushing of ne; (*T*) 26 min 00 s, cb in a definite inpushing of ne. × 2,100.




FIGURES 3 and 4 Two magnifications (Fig. 3, × 17,000; Fig. 4, × 55,000) of a step-3 spermatid, where both nuclear membranes form a prominent outpocketing in the proximity of the chromatoid body. Several 20-nm particles are accumulated in the sac. Through a nuclear pore complex (thin arrow) at the top of the outpocketing there is a material continuity with the chromatoid body. The corresponding time-lapse film shows that this phenomenon is a very transient one in vivo and therefore difficult to find in sectioned material. Bars, 1 μm.


4

experiments with two alkylating agents thiotepa and nitrogen mustard. *Int. J. Androl. Suppl.* **2:**523–537.
16. PARVINEN, M., and T. VANHA-PERTTULA. 1972. Identification and enzyme quantitation of the stages of the seminiferous epithelial wave in the rat. *Anat. Rec.* **174:**435–450.
17. REGER, J. F., M. A. FAIN-MAUREL, and P. CASSIER. 1977. The origin, distribution, and fate of the chromatoid body (germ plasm) during spermatogenesis and spermiogenesis in two Peracaridae. *J. Ultrastruct. Res.* **60:**84–94.
18. RUSSELL, L. 1977. Membranous structures within nuclei of spermatocytes in the adult male rat. *Biol. Reprod.* **17:**184–191.
19. RUSSELL, L., and B. FRANK. 1978. Ultrastructural characterization of nuage in spermatocytes of the rat testis. *Anat. Rec.* **190:**79–98.
20. SAMARINA, O. P., E. M. LUKANIDIN, and G. P. GEORGIEV. 1973. Ribonucleoprotein particles containing mRNA and pre-mRNA. *Karolinska Symp. Res. Methods Reprod. Endocrinol.* **6:**130–160.
21. SÖDERSTRÖM, K.-O. 1977. Effect of actinomycin D on the structure of the chromatoid body in the rat spermatids. *Cell Tissue Res.* **184:** 411–421.
22. SÖDERSTRÖM, K.-O., and M. PARVINEN. 1976. RNA synthesis in different stages of rat seminiferous epithelial cycle. *Mol. Cell. Endocr.* **5:**181–199.
23. SÖDERSTRÖM, K.-O., and M. PARVINEN. 1976. Transport of material between the nucleus, the chromatoid body and the Golgi complex in the early spermatids of the rat. *Cell Tissue Res.* **168:**335–342.
24. SÖDERSTRÖM, K.-O., and M. PARVINEN. 1976. Incorporation of $^3$H-uridine by the chromatoid body during rat spermatogenesis. *J. Cell Biol.* **70:**239–246.
25. SÖDERSTRÖM, K.-O., and M. PARVINEN. 1978. Observations on living rat spermatogenic cells in different developmental stages. *Acta Anat.* **100:**557–572.
26. SUSI, F. R., and Y. CLERMONT. 1970. Fine structural modifications of the rat chromatoid body during spermatogenesis. *Am. J. Anat.* **129:**177–192.
27. UTAKOJI, T. 1966. Chronology of nucleic acid synthesis in meiosis of the male Chinese hamster. *Exp Cell Res.* **42:**585–596.