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# A Colcemid-Sensitive Mechanism Involved in Regulation of Chromosome Movements During Meiotic Pairing

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**Abstract.** Active movements of the chromosomes may be needed in the process, where homologous chromosomes find each other during the meiotic pairing. Because the components of the cytoskeleton are generally believed to be responsible for all movements in living nonmuscle cells, we have analyzed the regulation of the movements of zygotene chromosomes in the male rat by using specific inhibitors of the assembly of the various components of the cytoskeleton. — Colcemid, an inhibitor of microtubule formation, completely inhibited the chromosome movements in vitro at a concentration of 1 μg/ml. This was associated with a damage of the nuclear envelope revealed by the electron microscopic analysis. Another inhibitor of microtubule formation, vinblastine, was ineffective below the level of general toxicity (100 μg/ml). A specific microfilament inhibitor, cytochalasin B was similarly ineffective. — The findings suggest the presence of a specific colcemid-sensitive mechanism in the nuclear envelope of the zygotene spermatocytes, which regulates the movements of the chromosomes during meiotic pairing.

## Introduction

The mechanisms which bring chromosomes together during the prophase of meiosis are not clearly understood. We have observed a maximum of rotatory and oscillatory movements in living zygotene chromosomes that may be associated with the formation of synapsis (Parvinen and Söderström, 1976). Fibrous processes have been shown to penetrate the nuclear envelope and to form fibrillar structures that radiate out into the perinuclear cytoplasm (Solari, 1970; Esponda and Giménez-Martin, 1972). This suggests that the attachment plaques of the meiotic chromosomes during the pairing process invoke contractile mechanisms, perhaps the components of the cytoskeleton (Bellvé, 1979). In order to further analyze the mechanism involved in the movements of the chromosomes during the pairing process, we have utilized specific inhibitors of the cytoskeletal components.

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## Materials and Methods

*Animals and Chemicals.* Young adult (2–5 months) male rats derived from the Sprague-Dawley strain were used in the experiments. They were killed by an overdose of ether, the testes were prepared free and decapsulated. Long segments of the seminiferous tubules were dissected free in Krebs-Ringer's solution and subjected to transillumination under a stereomicroscope (Parvinen and Vanha-Perttula, 1972). The zone of weak spots was recognized (Parvinen and Parvinen, 1978), and several tubules containing this zone were separated and transferred to small Petri dishes containing either pure Krebs-Ringer's solution or the following concentrations of drugs: Colcemid (N-deacetyl-N-methyl-colchicine, Ciba, Basel, Switzerland) 0.1, 0.5 and 1.0 µg/ml, vinblastine (Velbe®, Eli Lilly & Co., Indianapolis, Ind. 46206, USA) 1, 10 or 100 µg/ml of cytochalasin B (Serva Feinbiochemica, Heidelberg, Fed. Rep. Ger.) 1, 5, 25 or 100 µg/ml. In order to test the possible unspecific effects of colcemid, lumicolchicine was prepared by irradiating colchicine (Boehringer Mannheim GmbH, Fed. Rep. Ger.) in 94% ethanol for 60 min using a wavelength of 350 nm and an intensity of 8 W (Wilson, 1970). Lumicolchicine production was confirmed by its absorption maxima at 230 and 265 nm, compared with those of 247 and 350 nm of colchicine as measured by spectrophotometry (Wilson and Friedkin, 1966). Lumicolchicine was used in concentrations of 0.1, 0.5 and 1 µg/ml. The tubules were incubated in these solutions for 20–120 min before analyses by time-lapse cinephotomicrography or their fixation for electron microscopy.

*Cinephotomicrography.* Zygotene spermatocytes were found from stage XIII of the cycle of the seminiferous epithelium (Leblond and Clermont, 1952) using the transillumination-phase contrast microscopic method (Söderström and Parvinen, 1976). The tubular segment having the stage XIII was squashed between clean object- and cover glass slides to produce a monolayer of slightly flattened cells. The edges of the coverslip were sealed by paraffine oil and the living zygotene cells were observed under a 100 × phase contrast objective. Time-lapse cinephotomicrography was performed by exposing the frames manually in 2-sec intervals with an exposure time of 1/7 sec during 5–15 min using a Beaulieu 4008 ZM camera and super-8 film (Kodachrome 40).

*Measurement of the Chromosome Movement.* Individual frames were analyzed using an Erno E-700 editor viewer and marked for photographic reproduction and serial analysis. Single frames were analyzed at 20 sec intervals and the path of a single well identifiable chromosome was traced. The total length of the broken line pathway of the moving chromosome was measured during 5 min. The average speed of five cells was calculated in each experiment.

*Electron Microscopy.* Seminiferous tubular segments containing the cellular association XIII were isolated by the transillumination-phase contrast microscopic method and fixed in 3% glutaraldehyde in cacodylate buffer, pH 7.2 for 3 h at room temperature. They were postfixed in osmium tetroxide, embedded in Epon and sectioned at 70 nm using a LKB-Huxley ultra-microtome. The sections were stained with uranyl acetate and lead citrate, and the observations were made with a Jeol-JEM-T8 electron microscope.

**Table 1.** Inhibiting effect of colcemid on chromosome movements during zygotene stage of rat spermatogenesis

| Treatment | Number of rats | Number of cells analyzed | Number of cells with moving chromosomes | Number of cells with quiet chromosomes |
|---|---|---|---|---|
| Control | 17 | 65 | 60 | 5 (7%) |
| Colcemid: | | | | |
| 0.1 µg/ml | 4 | 16 | 14 | 2 (13%) |
| 0.5 µg/ml | 4 | 22 | 8 | 14 (63%) |
| 1.0 µg/ml | 4 | 14 | 0 | 14 (100%) |

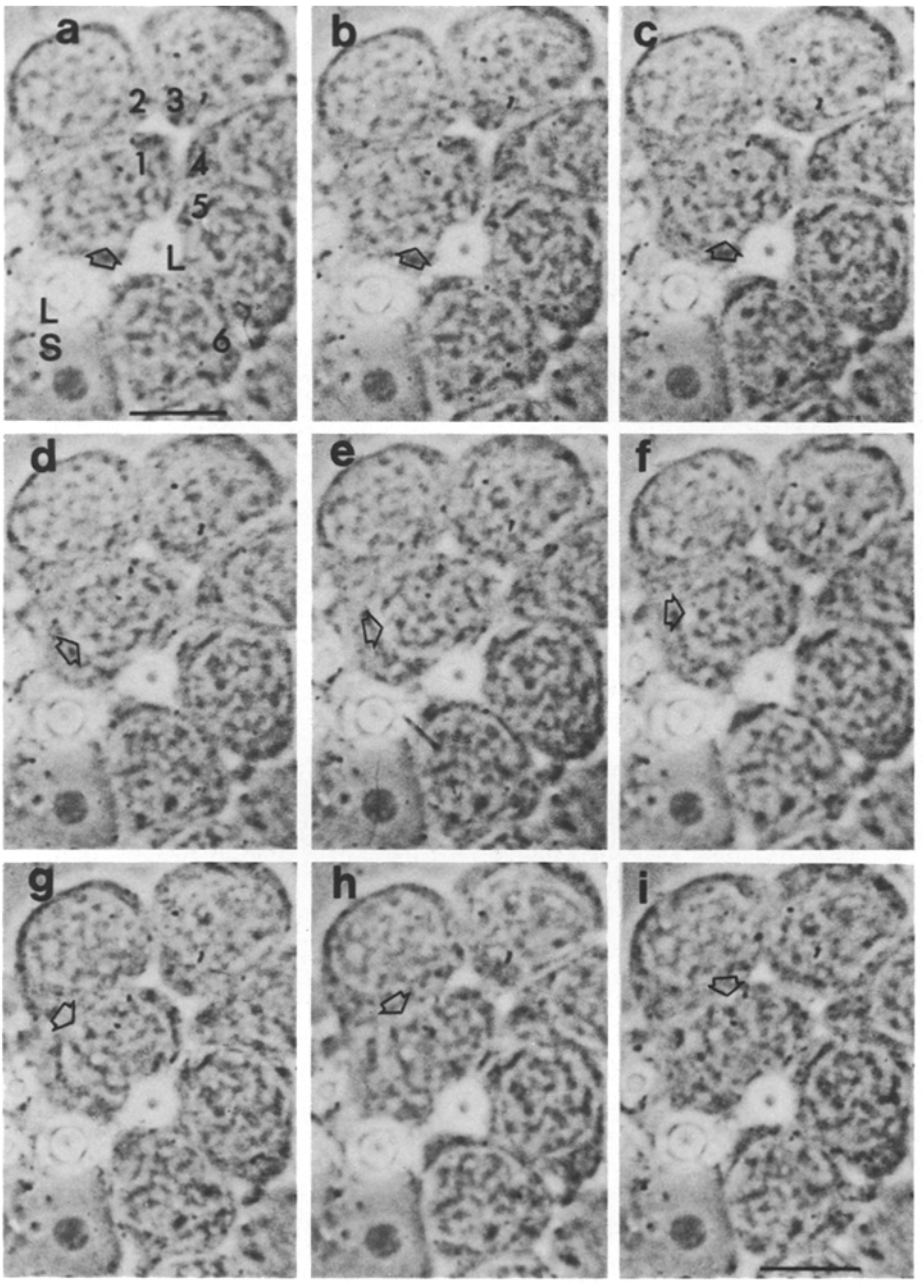


**Fig. 1.** A series of super-8 film frames of living control zygotene spermatocytes at stage XIII of rat spermatogenesis. The six cells (consecutive arabic numerals in a) were followed in 20 sec intervals in frames **a–g** and in 60 sec intervals in **h–i**. The open arrow in cell 1 shows the movement of a definite single chromosome fragment. It rotates approximately half a circle during 4 min observation time. *L* lipid droplet in the Sertoli cell (*S*) cytoplasm. Bar represents 10 μm

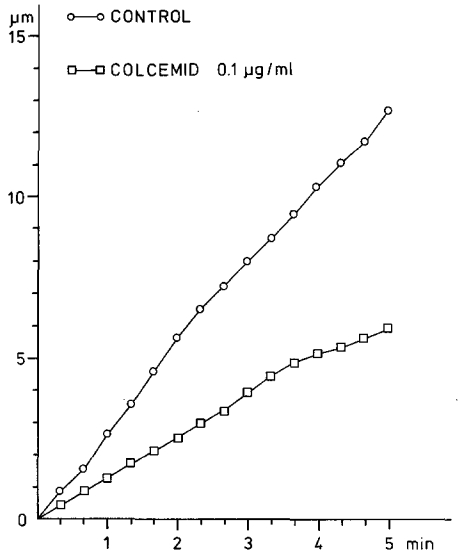


**Fig. 2.** The average speed of individual selected chromosome components from five cells during movements in zygotene stage of rat spermatogenesis. The ordinate shows the length of the path followed by the chromosome in µm, related to the time indicated on the abscissa. In control cells, the chromosomes move in a rotatory-oscillatory fashion in various directions at an average speed of 2.5 µm/min. The speed is reduced to half after incubation with 0.1 µg/ml of colcemid and to zero with 1.0 µg/ml of colcemid

## Results

In the control preparations, 93% of the zygotene chromosomes showed rotatory movements (Table 1) at an average speed of 2.5–3 µm/min (Figs. 1 and 2). The direction and axis of the movement as well as the speed varied continuously. Usually, the chromosomes moved as a whole unit, but sometimes also relative to each other. The resolution of the super-8 film is not sufficient for judgment, if the nuclear envelope participates in the movements or not.

Colcemid in concentrations of 0.1 and 0.5 µg/ml slowed down the average speed of the chromosome movements (Figs. 1 and 3). Increasing number of cells showed quiescent chromosomes (Table 1), and at the concentration of 1.0 µg/ml of colcemid, the zygotene chromosomes were not moving. Lumicolchicine in the same concentration had no effect on the chromosome movements (Fig. 3).

Vinblastine and cytochalasin B in concentrations close to the lethal dose (100 µg/ml) had little if any detectable effect on the rotatory movements of the zygotene chromosomes.

Electron microscopy revealed extensive alterations in the nuclear envelope after colcemid treatment (Fig. 4); in large areas the nuclear envelope was completely missing after incubation in 1 µg/ml of colcemid. However, other structures of the cell including the cytoplasmic microtubules showed no major alterations. The nuclear envelope was considered normal after treatment with 100 µg/ml of vinblastine or cytochalasin B.

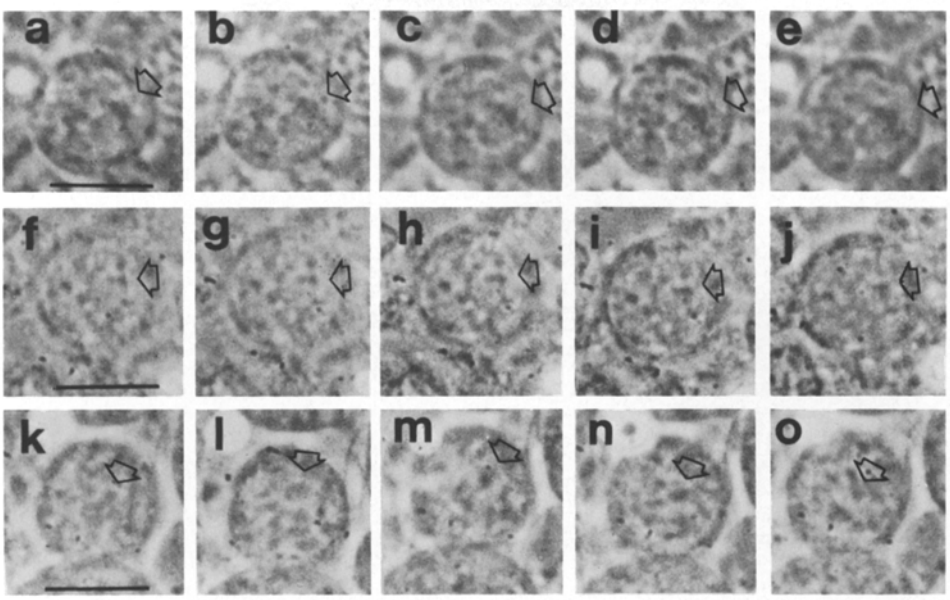


**Fig. 3.** A series of super-8 film frames of living zygotene spermatocytes treated with 0.1 μg/ml of colcemid (**a–e**), 1.0 μg/ml of colcemid (**f–j**) and with lumicolchicine (**k–o**). Slight movement of the chromosomes can be seen in **a–e** and **k–o**, whereas in **f–j** all of the frames are similar. The *open arrow* shows the movement of a definite single chromosome fragment. Frame interval 1 min. Bar represents 10 μm

## Discussion

The regulation of the development of the male germ cells is critically dependent on the Sertoli cells; after onset of meiosis the germ cells become completely surrounded by these elements (Ritzén et al., 1981). All drugs affecting the meiotic and postmeiotic germ cells must therefore first pass the barrier created by the Sertoli cells. Colchicine has been shown to exert an inhibitory effect on the meiotic divisions (Roosen-Runge, 1951), and the vinca alkaloids vincristine and vinblastine also cause a metaphase arrest and chromosome clumping during meiotic divisions (Parvinen et al., 1978). These observations are considered as evidence for the ability of colcemid and vinblastine to reach the germ cells through the Sertoli cells.

When applied at zygotene, colcemid but not vinblastine and cytochalasin B effectively terminates the chromosomal movements believed to be required for juxtapositioning of homologous chromosomes and subsequent formation of the synaptonemal complex. An explanation for the lack of the effect of the specific microfilament inhibitor, cytochalasin B may simply be its inability to penetrate the barrier formed by the Sertoli cells. The difference between the effects of microtubule polymerization inhibitors, colcemid and vinblastine is highly interesting. The inhibitory effect of colchicine on the chromosome pairing and chiasma frequency is well known (Bennett and Smith, 1979). This is obviously associated with a colchicine-binding protein in the nuclear envelope

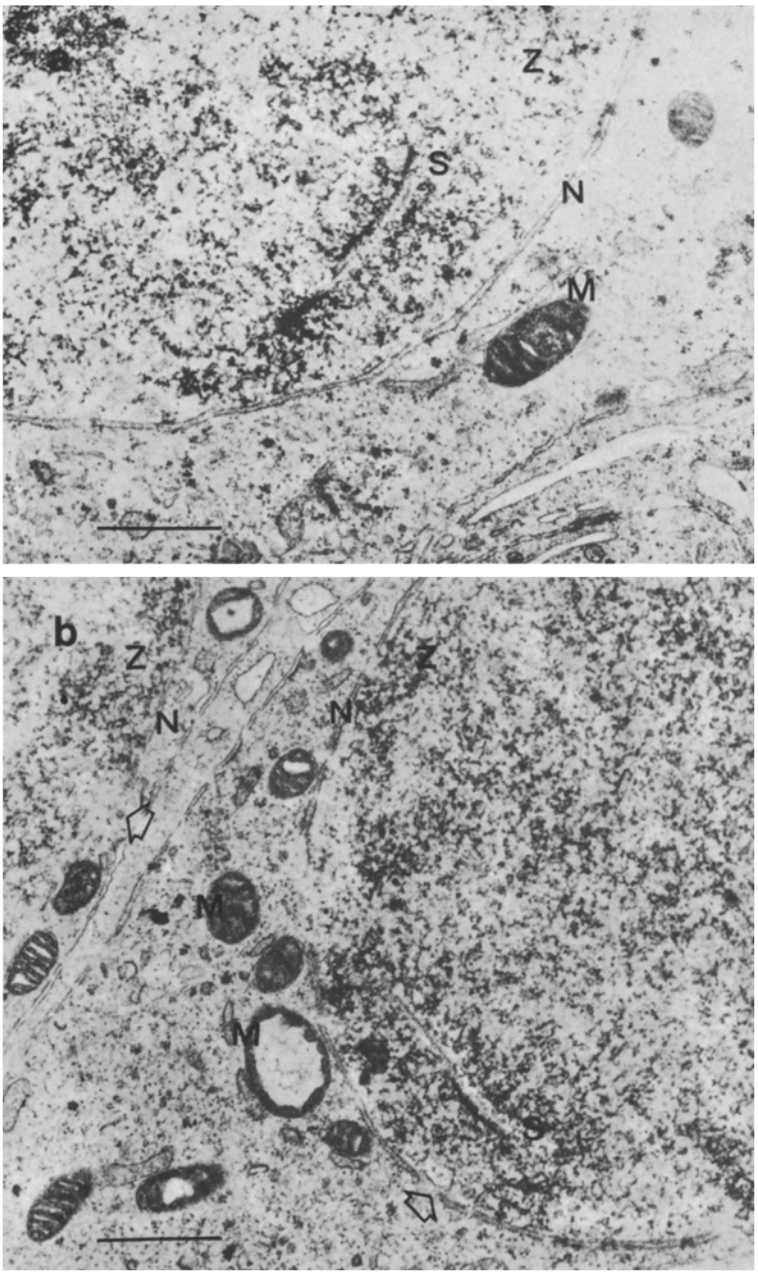


**Fig. 4.** Electron micrographs of control (**a**) and colcemid treated (1.0 μg/ml) (**b**) zygotene spermatocytes. The nuclear envelope is continuous in the control cell (*N*) whereas a marked instability reflected by numerous openings can be seen in the nuclear envelope of the colcemid-treated cell (*open arrows* in **b**). Other structures do not show any marked alterations. *M* mitochondria with typical location close to the nuclear envelope, *Z* zygotene nuclei, *S* synaptonemal complex. Bar represents 1 μm

of the meiotic cells, which increases in amount when cells pass from leptotene until pachytene (Hotta and Shepard, 1973). The protein is distinguishable from its cytoplasmic counterpart by several physicochemical criteria, and especially by its behavior toward vinblastine, which selectively precipitates only the cytoplasmic, but not nuclear colchicine complex (Hotta and Shepard, 1973). This is in good agreement with our observation about the marked alterations in the nuclear envelope that are induced by a colcemid concentration not having any obvious effect on other cellular components seen in the electron microscope. Vinblastine, even in high concentrations, did not induce such alterations.

The nuclear envelope obviously plays an important role in the pairing of the meiotic chromosomes during the zygotene stage of the meiotic prophase (Rasmussen and Holm, 1980). It may have an orientational role for the chromosome movements during the pairing, probably through its specific colchicine-binding protein. The force producers for the movements may be associated with the chromosomes themselves, as can be suggested on the basis of recent immunocytochemical demonstration of contractile proteins in the meiotic chromosomes (De Martino et al., 1980).

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