Cytological Investigation on the Human Testis,  
with Special Reference to the Morphology  
of the Chromonema

By

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Introduction

Since around 1928, the structure of the chromonema has been studied on animals and plants by either Japanese or foreign research workers. The fact that the chromonema shows twisted and thread-like appearance was firstly reported by Japanese investigator.

In recent years, many workers have studied on the fine structure of the chromatin or chromosome by use of electron microscope, such as Yasuzumi (1955) who made an elaborated investigation in Japan. Nevertheless, the materials which were used for these studies were confined to the chromatin or chromosome of animals or plants. While, Tاناaka, Makino and Ishno (1969) reported an excellent work on the human chromatin and made a big contribution to the field of the morphology of the chromonema.

The present work deals with the change of the form of the human chromonema and of the feature of the cytoplasm during the course of the cell division.

Materials and methods

The materials used in this study was the human testis which was taken and fixed in the fixatives as quickly as possible after the death of the donor. The tissue was fairly normal and showed no pathological changes judging from the histological point of view.

The small blocks of the tissue were fixed with Champy's solution, Levi's solution, Flemming's solution, Kolatschev's solution as well as with Regaud's solution. The blocks were then dehydrated, and then embedded in paraffin. The serial sections were cut at 4-10μ in thickness and stained with iron-hematoxylin and eosin, or with PAS method.
Observation

As far as the spermatogonium and primary spermatocyte are concerned, the cells in the interphase are rather larger than those under division, have clear nucleolus and show no sign of the increase of the granular structure in the cytoplasm. In the early prophase, granular configuration appears in the nucleus, and the material which is stained well with hematoxylin is sparsely distributed in the cytoplasm. These granular materials in the cytoplasm are found to be mitochondria by the electron microscopic study. Though thread-like structure representing the chromonema is not yet seen in the cell, the nucleoplasmic pattern is quite different from that of the cells in the resting state. The PAS reaction is stronger in the cells of the prophase than those in the resting stage.

In the advancing prophase, the homogeneous chromonema begins to appear in the nucleus, the contours of the chromonema become clear with time, and the thread-like chromonema grows up to show the spiral configuration. This structure is considered to belong to the category of the large spiral of the chromonema. Though the double spiral structure of the chromonema can be observed in the plant cells after they are treated with KCN solution and stained with acetic carmine, the small spirals composing the large spirals are barely visible in the cells of the human testis within the limitation of the resolving power of the light microscope.

The granules which are fairly large in size and stained well with hematoxylin appear in the cytoplasm, arranging themselves regularly around the nucleus or sometimes in the periphery of the cytoplasm. Most of these are naturally mitochondria. The chromonemal threads become thicker, and are stained well with iron-hematoxylin. The number of the granular structure increases, and the nuclear membrane is not visible under the light microscope any more in this stage. The inner structure of human chromonema is too fine to look into with light microscope being quite different from the situation in the animal's and plant's chromonema.

In the prometaphase, the cells become smaller in size and have fewer granules in the cytoplasm than those in the resting stage.

In the metaphase, the chromatin turns into chromosomes, the cells demonstrate round and smaller size, and the granular structures are barely or hardly seen in the cytoplasm. The cells in the metaphase undergo repeated division and become further small in size.

Meanwhile, the granules in the cytoplasm gradually increase in number in the middle of the metaphase.
The cells approaching the anaphase have chromatin in the nucleus like in the prometaphase, and have increased number of the granular structure in the cytoplasm.

Turning from anaphase to metaphase, the number of the granular structure in the cytoplasm and the form of the chromonema are similar to those in the cells of the early prophase, the cells are larger in size in the early prophase than in the telophase. The PAS reaction is a little stronger in the cells in the telophase than in the early prophase.

The form of the chromonema is variable with the course of the nuclear division. The chromonema is hardly stained with hematoxylin and demonstrates anticlockwise spiral feature in the early prophase, while it becomes rather thick and has a high stainability to iron hematoxylin in the late prophase. As the small spirals are probably not seen and only large spirals are visible, chromonema is seemingly represented by thick, large spiral contours.

In the late prophase, the chromonemal threads become thicker and the twist becomes more remarkable. Though the twist seems to be caused by the arrangement of fine granules, chromomere, attached on the surface of the straight thread. But in fact, the chromonema looks twisted, and there was no sign of the attachment of the granules on the surface of the normal human chromonema. The distribution pattern of the granular structure in the cytoplasm showed the variety as cell division develops. Namely, the mitochondria arrange themselves around the nucleus or in the peripheral part of the cytoplasm. The various pattern of the arrangement of the cell organelles was also noticed in the cytoplasm. The PAS reaction becomes stronger with the development of the course of the cell division. But the PAS reaction gets weaker as the cell division proceeds toward the anaphase.

Discussion

The investigation on the human chromosomes has been carried out on their number or on the form of XY chromosomes, and makes a big advance recently. Nevertheless, no report has been done on the structure of the chromonema of human spermatogenic cells.

As regards to fine granular structure attached on the surface of the chromonema, more than 2000 granules, which were supposed to correspond to genes, were found in the plants chromosome in around 1928 or 1935. Furthermore, the number of the granules was found to be almost identical with that of the stripes on the chromo-
some of Drosophila melanogasta. Judging from the fact mentioned above, the granules, or the chromomere were thought to have an intimate relationship with the heredity.

Later, K u w a t a (1937) and other research workers studied on the form of the chromosome of the plant cells, and proved that the chromonema demonstrates the spiral structure.

The human chromonema are twisted and give the impression of the convoluted threads. The twists give the false appearance that the threads have granules on their surface. The grade of the twisting of human chromonema is not always identical with that of animal or plant chromosome. But, the pattern of the twisting is not quite different from the chromonema of animal or plant. Thinking from the fact that T a n a k a et al. (1969) have recently observed the branching of human chromonemal threads, the chromonema might have the double spiral structure.

The granular structures which appear in the cytoplasm during the course of the cell division are mostly mitochondria. It is widely known that mitochondria and Golgi apparatus change their position during the cell division depending upon the condition of the nucleus.

During the metaphase, mitochondria decrease their number, and only chromosomes are seen in the cell. This phenomenon is not specific only for the spermatogenic cells, but is noticeable in every kind of cells in the body. The cells are filled with small vacuoles in the prophase, like in the case of the amniotic epithelial cells which are occupied by vacuoles of various sizes as observed by A b e (1956).

In the metaphase, the vacuoles enlarge their size, and decrease in number together with that of mitochondria. Though the pattern of appearance and disappearance of the granular structure in the spermatocyte is not as clear as in the amniotic epithelial cells, the number of the granule decreases in the metaphase and increases again in the anaphase.

Conclusion

1. The morphology of human chromonema is not too much different from those of animals and plants. They demonstrate the spiral appearance and have twisted contours.
2. With the alternation of the configuration of the chromonema during the course of the cell division, the granular structure as well as other cell components changed their feature. Specifically, mitochondria increase their number in the early prophase, and decrease in number in the metaphase together with the reduction of the cell size.
3. The reason why the chromonema has double spiral structure is unknown, but is supposed to reduce the volume of the chromosome and to confine as many threads as possible within the cell which has only a definite narrow space.

The formation of the spiral and twisted structure might possibly be due partly to the influence of the gravitation of the earth.

The abstract of this work was already reported in the Japanese Association of Anatomists held at Osaka City University Medical School in 1950. The material used in this study was kindly collected for me by Dr. T. Ito during his stay abroad.

References


Explanation of figures

Fig. 1. The cells of human testis in the early prophase. Fixed in the Champy's solution. 90 x 1.25
Fig. 2. Ibidem. A number of granules arranged themselves around the nucleus. 90 x 1.25
Fig. 3. Human testis, fixed with Kolatschev's fixative. The cell in the same stadium of cell division as Fig. 1, shows a different pattern of distribution of the granules within the cytoplasm. 90 x 1.25
Fig. 4. The cells in the late prophase, show the disappearance of both nuclear membrane and granular structure in the cytoplasm. The chromonema demonstrate themselves the twisted feature. Human testis, fixed with Champy's solution. 90 x 1.25
Fig. 5. The cell in the same stadium of cell division as Fig. 4. The twisted thread-like appearance of the chromonema is noticed. 80 x 1.25
Fig. 6. The enlarged image of the chromonema.
Fig. 7. The feature of the chromosome in the metaphase. Human testis, fixed with Champy's solution. 90 x 1.25
Plate I

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