MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXX, No. 3, Article 5 (Biology), 1963

Chromosome Behaviour in the Interkinesis

II. Observation of Archesporial and Spore Mother Cells in *Psilotum nudum*

By

Kazuo KATO

Botanical Institute, Faculty of Science, Kyoto University

(Received September 23, 1963)

Since the first paper of the series on the observation of the interkinesis in *Tradescantia* (KATO, 1935) being published, years have passed without publishing of this series, owing to the Second World War and under circumstances of the author. Observations made at that time in other plants will be reported in succeeding papers.

In the present investigation the nuclear structure in the interkinesis in *Psilotum* was observed in connection with the herical[1] structure of chromosomes. Some comparison of the interkinetic nucleus was also made with the nuclei in the interphases (last pre-meiotic and post-meiotic divisions) and with the periplasmodium nucleus.

Material and Method

As materials, archesporial and spore mother cells of *Psilotum nudum* were used. The cells were stained with the ordinary aceto-carmine method. In some cases SAX's ammonia-alcohol mixture (SAX and SAX, 1935) was also applied before staining with the aceto-carmine. In the observation of living state liquid paraffin and saccharose solution were used as media.

Observation

Last pre-meiotic metaphase to telophase. Archesporial cells in *Psilotum* are undergone a few pre-meiotic divisions before they enter into the meiotic division, and the cells originated from an archesporial cell are in groups. Of these grouped cells the maximum number was observed to be 8.

1) In place of spirals which have often been misused, the term helices are used in the present paper.

In the last pre-meiotic metaphase the chromosomes are slender as in ordinary somatic divisions. In Fig. 1, in a chromatid (half chromosome) chromonema helices with small somatic coils are seen in the matrical substance. In most chromatids the chromonemata appear in two helices lying side by side along the chromatid length and in others they appear as a single helix.

Fig. 2 shows a telophase nucleus, where the chromosomes take polar orientation leaving a clear space at the pole of the nucleus (pole field). In this figure the chromosomes are revealed as solid threads otherwise than internal structure. In these solid threads the two chromonema helices observed in the metaphase are concealed, so closely associated together to appear as a single helix. This chromonematal association might be caused by the lengthening out of chromosomes in late telophase, as pointed out by ATWOOD (1937) in the last pre-meiotic telophase of *Gaillardia aristata.*

Last pre-meiotic interphase. In the interphasic nucleus in the last pre-meiotic division, the thick solid helices observed in the preceding telophase are transformed into the form of herical chromonemata with small coils (somatic type). With these chromonema helices the nucleus is densely filled, the polar space and spaces between the chromosomes are lost. The chromonema helices are loosely relaxed and intricately expand in the nucleus, so that it is not possible to distinguish the chromonema herices which have been derived from a single chromosome (Figs. 3 and 4).

The diffuse interphasic nucleus in *Psilotum* presents a striking contrast to that in the same stage in *Bellevalia romana* (OEHLKERS and EBERLE, 1957), in which the chromosomes are less unravelled and the nuclear helices with different diameters along their length are disposed sparsely in the nucleus.

First meiotic metaphase to telophase. In *P. nudum*, about 50[1] thick and short chromosomes of somewhat different size are found in the first metaphase. In many organisms, especially in plants, compact and condenced chromosomes in the first metaphase are observed being composed of two distinct helices, major and minor; the minor helix is coiled again into the major helix with larger coils (KUWADA, 1932, 1935, 1939; KUWADA and NAKAMURA, 1933; OURA, 1936; GEITLER, 1938; MATSUURA, 1938; COLEMAN and HILLARY, 1941; DE ROBERTIS et al., 1960).[2] In the metaphase chromosomes in this plant the chromonema helices are coiled into helices of the major type, in which the minor helices are usually concealed (Fig. 5). The major helices can be recognized already in diakinesis, where a conspicuous shortening of chromosomes is taken place. The major helices can be relaxed, when ammonia-alcohol mixture is applied as pretreatment before staining with the aceto-carmine. In Fig. 6, the out-line of the chromosomes (chromosome matrix) is lost, and coiling of the major type is evident as continuous helices. In liquid paraffin, the chromosomes appear as homogeneous bodies, showing no internal structure. The same homogeneous appearance of the chromosomes is presented in a 15% saccharose solution. In this

1) According to OKABE (1929), n=52.

2) For further literature on the double-coiled structure of chromosomes refer to MANTON (1950) and RUCH (1950).

case, however, the spore mother cells show a slight plasmolysis in a meantime.

The massive form of the chromosomes is maintained from the metaphase until the daughter nuclei are formed in the telophase. When the nuclear membrane is formed, the major helices begin to unravel and the shape of individual chromosomes become less evident (Fig. 7). With these changes one nucleolus makes its appearance. At early telophase the formation of a separating plate or wall of the cell is first visible in liquid paraffin. However, this plate formation is always non-visible in aceto-carmine preparations.[1)]

Interkinesis. In the interkinesis the major helices continue a further unravelling and the minor helices concealed in the major ones during previous stages become evident. A transitional stage from the major coiling into the minor one is shown in Fig. 8. In this nucleus not only the minor helices but also the major ones are seen. In Figs. 9 and 10 the major helices are not completely unravelled and they are recognizable as such here and there in the nucleus. In Fig. 10, in the lower part in the nucleus, it may be seen that two minor helices of a chromosome, to be separated in the second division, come loose from each other except for the attachment point of spindle fiber. The same feature has been observed in the same stage of *Tradescantia reflexa* (KATO, 1935). In Fig. 11, the unravelling of chromosomes is reached its maximum. In this nucleus the chromonema helices with minor coils are most evident. Although clear spaces are seen between territories which have been occupied by chromosomes, it is difficult to discriminate chromonema helices derived from a single chromosome.

In comparing with the interphasic nuclei in the pre- and post-meiotic (see blow) divisions, the chromonema helices in the interkinetic nucleus are coarsely distributed. In *Psilotum* a general appearance of the interkinetic nucleus, in its maximum chromosome unravelling, resembles to that in *Tradescantia reflexa* (KUWADA, 1932; KATO, 1936). However, with regard of presence of the relic major coils, the interkinetic nucleus in *Psilotum* might be classified as to be a member of *Lilium* type (SINKE, 1934; KATO and IWATA, 1935).

As the stage advances toward the end of the interkinesis, the nucleus situated closely to the cell periphery at the poles of the mother cell, moves toward the central in the daughter cells.

Fig. 12 shows an early prophase in the second division, in which the chromonemata begin to thicken and the minor coils become less evident, showing more or less larger coiling of prophasic nuclear threads like in the spiral stage in somatic mitoses (cf. KUWADA and NAKAMURA, 1934).

Second metaphase. The chromosomes in this stage are somewhat slender shape as compared with those in the first metaphase, and they appear to be composed of helices of the minor type (Fig. 13). In liquid paraffin the chromosomes appear to be homogeneous as in the first metaphase.

Post-meiotic interphase. In the post-meiotic interphase which follows the second

1) In the last pre-meiotic division, the separating wall can be seen in aceto-carmine preparations.

division, the nucleus is filled with the minor helices (Fig. 14). These minor helices are similar in coiling to those found in the last pre-meiotic interphase.

Periplasmodium nucleus. A tapetal periplasmodium nucleus, nature of which is nutritive, is shown in Fig. 15. In this nucleus the chromosomes are in the helical form with small somatic coils. Among these helices pycnotic bodies are observed. Such pycnotic bodies can not be observed in the interphasic nucleus in the last pre-meiotic division.

Summary

1) In *Psilotum nudum,* the nuclear structure in the interkinesis was observed, taking its structural connection to other stages into consideration.

2) In the interkinetic nucleus, the meiotic chromosomes composed of major and minor helices are transformed into their constituent helical chromonemata. At its culminating stage the nucleus is filled mostly with the minor helices, with some relics of the major helices.

3) In comparing the nuclear structure in the interkinesis with that in the somatic interphases (pre- and post-meiotic divisions), texture or pattern of the chromonema helices is coarser in the former than in the latter. In the periplasmodium nucleus pycnotic bodies are observed among the chromonema helices.

Literature Cited

ATWOOD, S., 1937. La Cellule, **46**: 189–410.
COLEMAN, L. C. and B. B. HILLARY, 1941. Amer. Journ. Bot., **28**: 464–469.
DE ROBERTIS, E. D. P., W. W. NOVINSKI and F. A. SAEZ, 1960. General Cytology (third ed.) Philadelphia and London.
GEITLER, L., 1938. Chromosomenbau. Protoplasma-Monographien, **14**. Berlin.
KATO. K., 1935. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, **10**: 251–262.
_____ and J. IWATA, 1935. Ibid., **10**: 265–273.
KUWADA, Y., 1932. Bot. Mag., Tokyo, **46**: 307–310.
_____ , 1935. Cytologia, **6**: 308–313.
_____ , 1939. Cytologia, **10**: 213–256.
_____ and T. NAKAMURA, 1933. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, **9**: 129–139.
MANTON, I., 1950. Biol. Rev., **25**: 486–508.
MATSUURA, H., 1938. Cytologia, **9**: 243–248.
OEHLKERS, F. and P. EBERLE, 1957. Cytologia, **8**: 351–363.
OKABE, S., 1929. Sci. Rep. Tôhoku Imp. Univ., Fourth Ser., **4**: 373–379.
OURA, G. 1936. Zeitschr. wiss. Mikros. Tech., **53**: 36–37.
RUCH, FR., 1950. Chromosoma, **3**: 358–392.
SAX, H. J. and K. SAX, 1935. Journ. Arbor., **16**: 423–439.
SINKE, N., 1934. Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, **9**: 367–392.

Explanation of Plates

Plate I

Figs. 1–15 are photomicrographs taken from aceto-carmine preparations, using ZEISS's apoch. imm. 2 mm. and comp. oc. 12.

Fig. 1. Chromosomes in the last pre-meiotic metaphase.

Fig. 2. Last pre-meiotic telophase.

Fig. 3. Interphase nucleus in side view.

Fig. 4. The same in polar view.

Fig. 5. I-metaphase chromosomes, showing major helices.

Fig. 6. Unravelling of I-metaphase chromosomes pretreated with SAX's mixture, showing major helices.

Plate II

Fig. 7. I-telophase, beginning of unravelling of major helices into minor ones.

Figs 8 and 9. Interkinesis. Major helices are more evident than minor ones.

Fig. 10. The same. Two minor helices of half chromosomes are seen in the lower part of the nucleus.

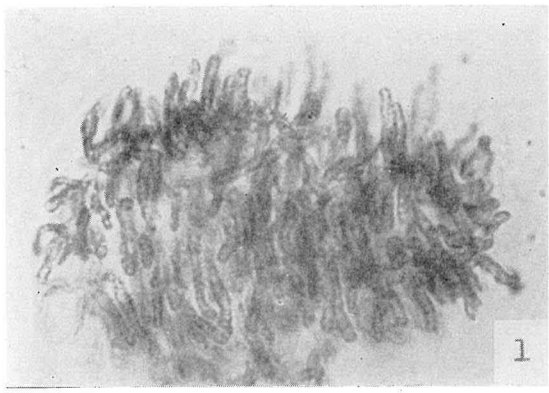
Fig. 11. Mid-interkinesis, major helices being unravelled to the maximum point.

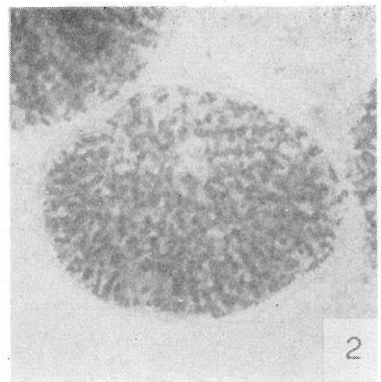
Fig. 12. Early II-prophase, showing thickening of nuclear helices.

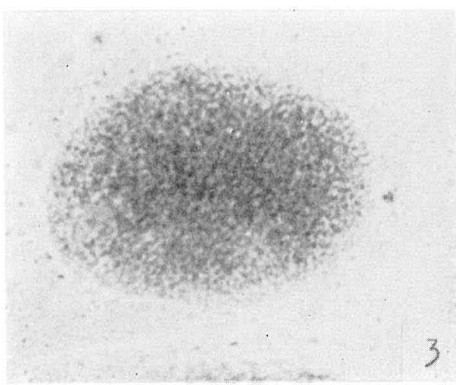
Fig. 13. Chromosomes in II-metaphase.

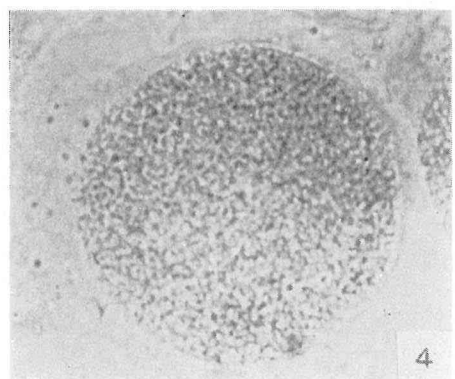
Fig. 14. Interphase in post-meiotic division.

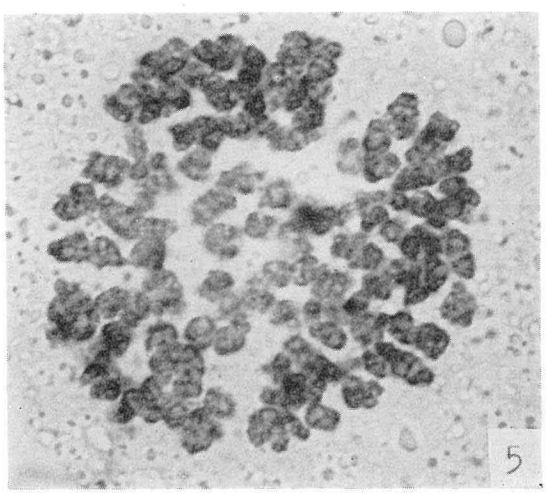
Fig. 15. A periplasmodium nucleus, showing diffuse structure of metabolic stage.

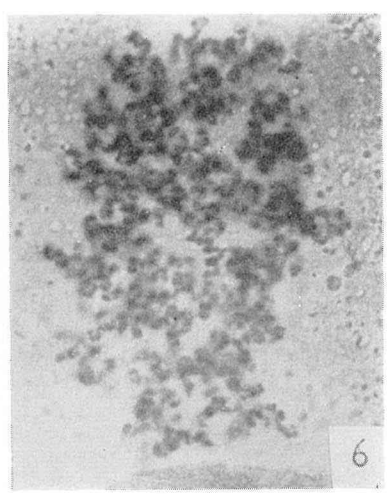




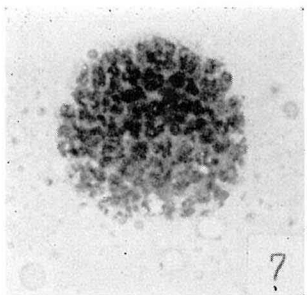


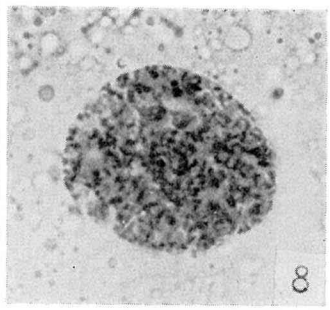


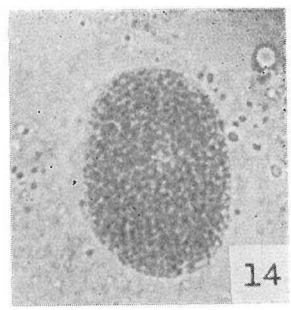


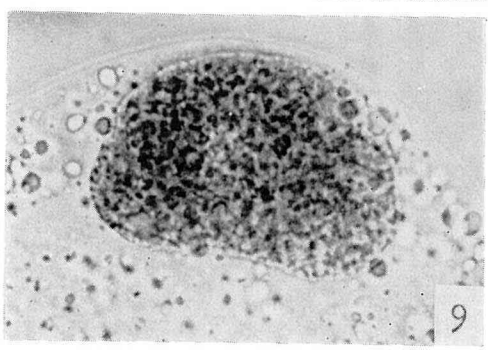


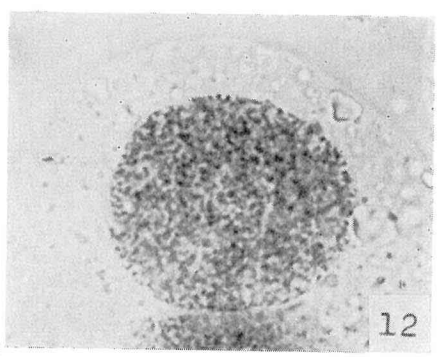
K. KATO

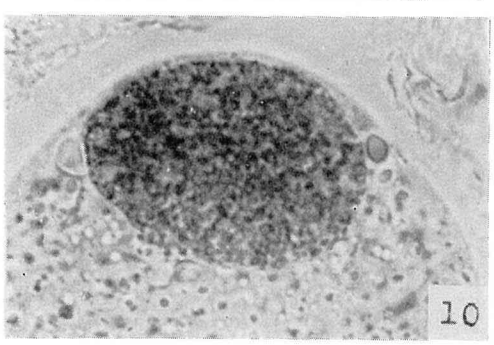

7

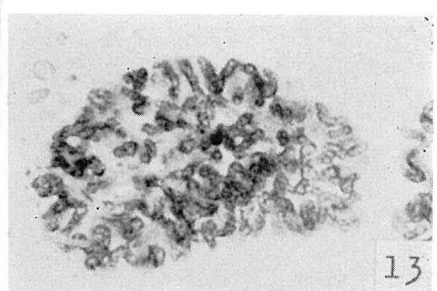

8

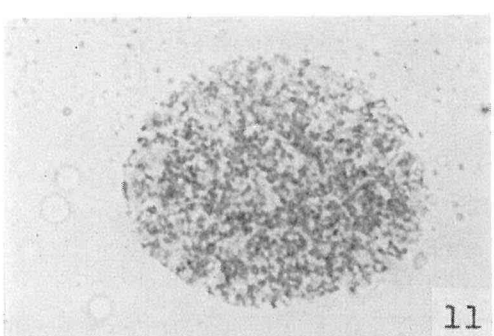

14

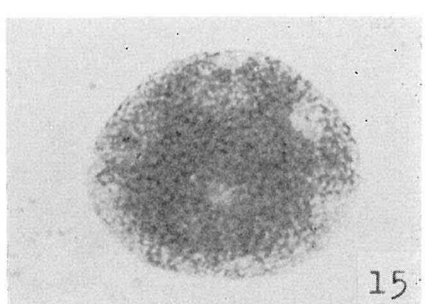

9


12


10


13


11


15