H³-thymidine Autoradiographic Studies on the Heteropycnosis, Heterochromatin and Euchromatin in *Spiranthes sinensis**

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Autoradiographic studies on the pattern of DNA synthesis in heteropycnotic bodies have been reported in *Rumex acetosa*¹⁾, in *Melanoplus differentialis*²⁾, in Chinese hamsters³⁾, in *Equus hybrid*⁴⁾ and in human leucocytes and skin cultures⁵⁻¹¹⁾. In all of these materials the heteropycnotic bodies were found to synthesize DNA at later stage of DNA synthetic phase. It is known that the heteropycnotic bodies of these materials originate from sex chromosome. No report was presented on the DNA synthesis in the heteropycnotic bodies originated from autosomes, except that in the large semi-condensed chromatin block of *Secale cereale* by Lima-de-Faria²⁾ and that in the heterochromatic regions in interphase nuclei of *Fritillaria lanceolata* by Pelc and La Cour¹²⁾, where asynchronous DNA replication was reported.

The interphase nuclei in the meristematic cells of the root tips of *Spiranthes sinensis* ($2n=30$), Orchidaceae, show several heteropycnotic condensed bodies which are large enough for autoradiographic study. The present report concerns the earlier replication of DNA in the heteropycnotic bodies of this species. It also deals with the pattern of DNA synthesis in the chromosomes of this species.

Material and Method

A clone of *Spiranthes sinensis* was obtained from Hatsukaichi, Hiroshima, Japan. The external morphology of the clone was similar to that of common form. Somatic chromosomes of the clone were counted to be $2n=30$. The clone was divided into 15 plantlets. Each of the plantlets was grown in moistened sphagnum at the temperature 18-20°.

Roots developed from the plantlets were immersed in the H³-thymidine (TRA. 61, Thymidine-6-T(n), Batch 34, obtained from the Japan Radiochemical Centre) solution diluted with 1/2 Knop's solution for 1/2 hr. at 22°. The specific activity of H³-thymidine used was 2 μ C/ml. A preliminary test showed that there is no apparent rhythm of the appearance of mitosis in the root tip cells of this species. The immersion treatment of root tips was done at 6 p.m. The roots on plantlets were washed thoroughly in water after the treatment with H³-thymidine, and were left to grow in moistened sphagnum.

The root tips treated with H³-thymidine were fixed in acetic-alcohol (1:3) at 15°. Five root tips were fixed immediately after the 1/2 hr. pulse treatment with H³-thymidine. Two root tips were fixed at each 1 hr. interval up to 32 hrs. posttreatment. The root tips collected were hydrolyzed in 1 N HCl at 60° for 8 min. and were stained by usual Feulgen's nuclear reaction. The processes of preparation: the root tip was squashed under cover glass on the slide glass which was covered by dried gelatin film. After the removal of cover glass by dry ice method (about 1 min.), the Sakura stripping

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film NR M1 was applied on squashed material. Slides were air-dried and then were stored in a dark box. They were stored in the ice box at 4°–8° for 14 days. The slides were developed in the Sakura Konidol X for 5 min. at 20°. After washing in tap water the slides were immersed in 50% alcohol solution with 2% glycerol for 10 min., and finally they were air-dried. Observations were made in Cargille's immersion oil Type B under microscope.

Usual squashed preparations using aceto-orcein were also made for the observation of the morphology of interphase nuclei.

Results

1. *Morphology of interphase nuclei:* In interphase nuclei of meristematic cells of root tips there were several heteropycnotic condensed bodies of chromatin. The condensed bodies varied in number, size and form in both preparations prepared by aceto-orcein staining and those by autoradiography. The number of condensed bodies varied from 0 to about 25 per nucleus. The form of condensed bodies was approximately spherical showing fusiform, crescent shape, or astral shape with irregular uneven surface. Some of the interphase nuclei without condensed body were observed. The interphase nuclei were classified into five categories by the characteristics of condensed bodies: 1) *micro-condensed nucleus*. This nucleus showed small condensed fusiform bodies of chromatin. Coiled chromonemata were observed in most of the condensed bodies. The size of nuclei was small. Most of the nuclei of this type were observed in pair showing similar characteristics of chromatin (Fig. 1 a). 2) *extended nucleus*. This nucleus had no condensed bodies of chromatin. All of the chromatin were of extended state. The size of nuclei was small. Most of nuclei were found in pair (Fig. 1 b). 3) *partly condensed nucleus*. This nucleus had partly condensed bodies of chromatin. The condensed bodies were of astral or crescent form with exserted chromatin threads which gradually transformed into extended chromatin. Some of the condensed bodies were observed attaching to nucleolus forming a ring of chromatin surrounding the nucleolus. The nuclei of this type was larger than the above two types of nuclei (Fig. 1 c). 4) *major condensed nucleus*. The condensed bodies of chromatin in this nucleus were larger than those of partly condensed nuclei. They were of irregular form showing astral or globular shape with uneven surface. The condensed bodies, attached to nucleolus, partly disjoined from the nucleolus. The size of the nuclei of this type was larger than the nuclei described above (Fig. 1 d). 5) *macro-condensed nucleus*. Some of the condensed bodies of chromatin were fused each other forming large globular blocks with relatively smooth surface. The size of the nuclei of this type was the largest of all the nuclei in meristematic cells (Fig. 1 e).

2. *Autoradiography of the interphase nuclei fixed immediately after 1/2 hour pulse labelling:* Activated silver grains in the root tips which were fixed immediately after the 1/2 hr. treatment with H³-thymidine were found over extended nuclei, over partly condensed nuclei and over major condensed nuclei. No grains were observed over micro-condensed nuclei and over macro-condensed nuclei. Of the 120 extended nuclei studied, 76 (63%) nuclei had grains over them. Of the 96 partly condensed nuclei studied, 45 (47%) nuclei had grains over them. Of the 110 major condensed nuclei, 56 (51%) nuclei had grains over them.

Grains were observed scattering over extended chromatin in extended nuclei (Fig. 1 f). The number of grains over the nuclei varied from about 5 to about 400 per nucleus. In some extended nuclei the accurate count of grains was not possible,

because of the large number of confluent grains.

In partly condensed nuclei grains were observed over extended chromatin (Fig. 1 g). No grain was found over the condensed chromatin. It was observed that peripheral regions of some partly condensed bodies were found labelled. The number of grains over the nuclei ranged from about 5 to about 200 per nucleus.

In major condensed nuclei grains were observed only over extended chromatin (Fig. 1 h). The number of grains over the nuclei varied from about 5 to about 300 per nucleus.

Differential labelling of extended chromatin was found in all of the interphase nuclei fixed immediately after 1/2 hr. pulse labelling. The extended chromatin in the three types of nuclei would have been active in the DNA synthetic phase at the time the cells were exposed to H^3 -thymidine. The condensed bodies in interphase nuclei, on the other hand, did not incorporate H^3 -thymidine during the exposure of root to the tritiated precursor. It is presumed that the chromatin in condensed bodies would have completed the synthesis of DNA prior to the H^3 -thymidine was available.

3. *Duration of interphase nuclei:* Observations were made on the interphase nuclei fixed every 1 hr. interval after 1/2 hr. treatment with H^3 -thymidine. The first appearance of grains over condensed bodies was noted in the partly condensed nuclei fixed at 2 hrs. after pulse labelling. The grains were observed over the peripheral regions of condensed bodies (Fig. 1 i). The number of grains over condensed bodies varied from 0 to approximately 10 per body. Most of grains on these nuclei were found over extended chromatin. Restricted localization of all of the grains over the condensed bodies was noted for the first time in the partly condensed nuclei fixed at 4 hrs. after pulse labelling (Fig. 1 j). The labelling of condensed bodies in a nucleus was found to be uneven. The number of grains over the condensed bodies varied from 0 to approximately 20 per body. The larger condensed bodies had more grains than the smaller condensed bodies. Some of the condensed bodies had no grains over them.

The first appearance of grains over condensed bodies in major condensed nuclei was noted in the cells fixed at 4 hrs. after pulse labelling (Fig. 1 k). More grains were found over larger condensed bodies than the smaller condensed bodies. The number of grains over condensed bodies varied from 0 to about 20 per body. On these nuclei grains were also observed over extended chromatin. Localized labelling of condensed bodies in major condensed nuclei was noted for the first time in the cells fixed at 6 hrs. after pulse labelling (Fig. 1 l). The number of grains over condensed bodies in these nuclei varied from 0 to about 30 per body. Uneven labelling was found among condensed bodies in a nucleus.

The first appearance of grains over macro-condensed nuclei was noted in the cells fixed at 2 hrs. after pulse labelling. The grains on the nuclei were observed over

Fig. 1. Interphase nuclei and their autoradiographs in the root tip meristematic cells of *Spiranthes sinensis* ($2n=30$). a-e, nuclei stained with aceto-orcein; f-n, autoradiographs of nuclei. a, two micro-condensed nuclei; b, two extended nuclei; c, a partly condensed nucleus; d, a major condensed nucleus; e, a macro-condensed nucleus; f, g, h, an extended nucleus, a partly condensed nucleus and a major condensed nucleus, respectively, which were fixed immediately after 1/2 hr. pulse labelling with H^3 -thymidine; i, j, partly condensed nuclei fixed at 2 hrs. and at 4 hrs., respectively, after pulse labelling; k, l, major condensed nuclei fixed at 4 hrs. and at 6 hrs., respectively, after pulse labelling; m, a macro-condensed nucleus fixed at 10 hrs. after pulse labelling; n, two micro-condensed nuclei fixed at 13 hrs. after pulse labelling. $\times 1350$.

extended chromatin. The first appearance of grains over condensed bodies in macro-condensed nuclei was noted in the cells fixed at 8 hrs. after pulse labelling. On these nuclei the grains were also observed over extended chromatin. Localized appearance of grains over condensed bodies in macro-condensed nuclei was noted for the first time in the cells fixed at 10 hrs. after pulse labelling (Fig. 1 m). Uneven labelling was found among condensed bodies in a nucleus.

Grains over micro-condensed nuclei were observed for the first time in the cells fixed at 9 hrs. after pulse labelling. The grains on the nuclei were found to have occurred restricted to extended chromatin. Grains over condensed bodies in micro-condensed nuclei appeared for the first time in the cells fixed at 13 hrs. after pulse labelling (Fig. 1 n). Localization of grains over condensed bodies was noted for the first time in the cells fixed at 15 hrs. after pulse labelling.

Observations showed that all the grains appeared over the condensed bodies were noted in the cells fixed at 2 hrs. or more hours after pulse labelling. The observations indicate that the condensed bodies had incorporated H^3 -thymidine 2 hrs. or more hours before the time of fixation. Therefore, it can be presumed that the DNA synthesis in condensed bodies occurred in the extended state of chromatin.

The number of extended nuclei labelled was the highest in the roots fixed immediately after pulse labelling. Decrease of the number of labelled extended nuclei was noted in the cells fixed at 3 hrs. after pulse labelling. Of 110 extended nuclei fixed at 3 hrs. after pulse labelling, 26 (24%) had grains over them. In the cells fixed at 7 hrs. after pulse labelling the lowest number of extended nuclei labelled was observed: of 95 extended nuclei only 4 (4%) had grains over them. The number of labelled extended nuclei was found to increase gradually in the cells fixed later than 13 hrs. after pulse labelling: 12 (9%) of 135 extended nuclei. At 14 hrs. after pulse labelling 16 (16%) of 100 extended nuclei had grains over them. At 20 hrs. after pulse labelling 34 (28%) of 120 extended nuclei had grains over them.

Decrease of the number of partly condensed nuclei labelled was found in the cells fixed at 7 hrs. after pulse labelling. Decrease of the number of major condensed nuclei labelled was found in the cells fixed at 9 hrs. after pulse labelling.

A schematic representation of the time of appearance of grains over nuclei was shown in Fig. 2.

4. *Autoradiography of mitotic chromosomes:* Chromosomes in root tip cells were counted to be $2n=30$. Twenty six of 30 chromosomes had centromere in subterminal position, and four of smaller chromosomes had centromere in median position. During prophase the 26 chromosomes which had subterminal centromere showed large heterochromatic segments in a proximal region of the long arm, while the short arm and the distal region of the long arm of the chromosomes were found to be euchromatic. The change from heterochromatic segments to euchromatic segments was steep without showing gradient of chromomere. The four smaller chromosomes with median centromere had no heterochromatic segment.

No grains were observed over mitotic chromosomes in the cells fixed immediately after pulse labelling. The first appearance of grains over chromosomes was noted in the prophase plates fixed at 6 hrs. after pulse labelling (Fig. 3 a). Four of the 17 prophase plates fixed at 6 hrs. after labelling treatment had grains over them. Chromosomes and their grains in a prophase plate were shown in Fig. 4 a. In the chromosomes most of the grains were observed over the short arm and over the distal region of the long arm. While few grains were found over the area adjacent

Fig. 2. Schematic representation of the first appearance of activated silver grains over the interphase nuclei and over the chromosomes of root tip cells of *Spiranthus sinensis* after 1/2 hr. pulse labelling with H^3 -thymidine. Ordinates show the time in hour after pulse labelling. A, micro-condensed nucleus; B, extended nucleus; C, partly condensed nucleus; D, major condensed nucleus; E, macro-condensed nucleus; F, prophase nucleus.

to centromere. Most of homologous chromosomes of a complement had grains over relative regions. Two of smaller euchromatic chromosomes had grains over relative regions, while the other two had no grains. Asynchronous labelling was found in some homologous chromosomes. The first labelled prophase plates must have incorporated with H^3 -thymidine during the final stage of DNA synthetic phase. Presumably the DNA synthesis in both short arms and the distal regions of long arms, both of them being euchromatic, appeared to have occurred at the final stage of DNA synthetic phase.

The first appearance of grains over heterochromatic regions of prophase chromosomes was noted in the cells fixed at 10 hrs. after pulse labelling (Fig. 3 b). Grains on the chromosomes were also observed over euchromatic regions. Localization of grains over heterochromatic regions was observed for the first time in the cells fixed at 11 hrs. after pulse labelling (Fig. 3 c). Most of the prophase plates which appeared later than 11 hrs. after pulse labelling had grains restricted to the heterochromatic regions. It was presumed that the DNA synthesis in heterochromatic regions occurred at the early stage of DNA synthetic phase.

Grains over heterochromatic regions were found to vary in the position of occurrence among chromosomes of a complement. In some chromosomes scattered grains were observed over heterochromatic region. In some others grains were observed on the

Fig. 3. Autoradiographs of root tip chromosomes of *Spiranthes sinensis* ($2n=30$). a, prophase chromosomes fixed at 6 hrs. after pulse labelling (Text-fig. 4 a); b, prophase chromosomes fixed at 10 hrs. after pulse labelling; c, prophase chromosomes fixed at 11 hrs. after pulse labelling (Text-fig. 4 b); d, metaphase chromosomes fixed at 8 hrs. after pulse labelling; e, metaphase chromosomes fixed at 13 hrs. after pulse labelling; f, g, anaphase chromosomes in two focuses fixed at 8 hrs. after pulse labelling. $\times 1350$.

area adjacent to centromere. In the others grains were found in both interstitial and distal positions of heterochromatic region. The chromosomes in a plate and their grains were shown in Fig. 4 b. Grains on the chromosomes were observed over the heterochromatic regions adjacent to centromere, and over both medial and distal positions of heterochromatic regions. Synchronous labelling was found in some chromosomes. Asynchronous labelling was found in some other chromosomes. No grains were observed over short arm and over the distal region of long arm. No grains were found over the four small chromosomes without heterochromatic segment. The observations indicated that the DNA synthesis occurred in multiple positions of heterochromatic region.

The first appearance of grains over metaphase chromosomes was noted in the cells fixed at 8 hrs. after pulse labelling. Grains on the metaphase chromosomes were observed over distal regions of chromosomes (Fig. 3 d). Metaphase chromosomes fixed later than 8 hrs. after pulse labelling had many grains over them. The difficulty in classifying the members of chromosomes made it impossible to localize the areas of labelling in any consistent manner, because the chromosomes at metaphase are clumped together to form blocks of chromosomes.

The first appearance of grains over anaphase chromosomes was noted in the cells fixed at 8 hrs. after pulse labelling. Grains on the anaphase chromosomes were observed over short arms reaching the poles (Fig. 3 f). Grains were also observed over distal regions of the long arm of daughter chromosomes (Fig. 3 e). Both the

Fig. 4. Drawings of the autoradiographs of prophase chromosomes in the root tip cells of *Spiranthus sinensis* ($2n=30$). Activated silver grains overlying chromosomes were drawn toward the side of the chromosomes. a, prophase chromosomes fixed at 6 hrs. after pulse labelling; b, prophase chromosomes fixed at 11 hrs. after pulse labelling. $\times 2400$.

short arm and the distal region of long arm showed euchromatic characteristics at prophase. The first labelled regions of anaphase chromosomes, therefore, must be the euchromatic regions of chromosomes. Grains restricted to the proximal heterochromatic regions of long arm were noted in the plates fixed at 14 hrs. after pulse labelling.

Discussion

DNA synthesis in heteropycnotic condensed bodies in interphase nuclei has been reported by Lima-de-Faria²⁾ in *Secale cereale*, by Pelc and La Cour¹²⁾ in *Fritillaria lanceolata*, by Kusanagi¹⁾ in *Rumex acetosa*, and in several animal species³⁻¹¹⁾. In all of these species DNA in condensed bodies was synthesized in the condensed state of chromatin during later stage of DNA synthetic phase (S phase). In contrast to these species the condensed bodies in the interphase nuclei of *Spiranthes sinensis* do not incorporate H³-thymidine, which indicates that the condensed bodies of this species do not synthesize DNA in the condensed state of chromatin.

It was found that the first condensed bodies to appear labelled were found in the interphase nuclei fixed at 2 hrs. after pulse labelling. This finding indicates that the DNA synthesis occurs before the condensation of chromatin, because there will be no remain of H³-thymidine in cells after removal of roots in water, as it was shown in root tip cells of *Tradescantia paludosa* by Wimber¹³⁾ and in those of *Vicia faba* by Evans and Scott¹⁴⁾. In contrast to the condensed bodies, extended chromatin in interphase nuclei appeared labelled in the cells fixed immediately after pulse labelling. Therefore, it can be presumed that DNA synthesis in condensed bodies must have occurred in the extended state of chromatin during early stage of S phase.

On the existence of polarity of DNA synthesis in chromosomes studies were made by several authors. Taylor¹⁵⁾ reported that DNA synthesis in the chromosomes of *Crepis capillaris* initiated early at end regions and progressed toward the proximal regions. Similar sequence of DNA synthesis was proposed in the chromosomes of *Secale cereale* by Lima-de-Faria²⁾ and in the chromosomes of *Vicia faba* by Woodard *et al.*¹⁶⁾ In contrast to these species. Wimber¹³⁾ reported in the chromosomes of *Tradescantia paludosa* that DNA synthesis occurred earlier in proximal regions and later in distal regions. German⁷⁾ and others^{11,17,18)} reported multiple occurrences of DNA synthetic loci in human autosomes in cultured leucocytes and in skin cells. A late replicating X chromosome in human cells had DNA synthetic center in interstitial regions of both arms. Similar multiple occurrences of DNA synthetic loci was proposed in the meiotic chromosomes of *Melanoplus differentialis* by Lima-de-Faria¹⁹⁾. In the chromosomes of *Spiranthes sinensis* the DNA synthesis occurred earlier in heterochromatic regions and later in euchromatic regions indicating a sequence of DNA synthesis from heterochromatin to euchromatin. The DNA synthesis in the chromosomes of this species, therefore, initiates earlier in the proximal regions of long arm and ends at the distal regions of both arms. In heterochromatic regions, however, DNA synthesis occurs simultaneously in proximal, interstitial and distal regions, which indicates that there is multicentric DNA synthesis.

The duration of mitotic cycle phases in root tip cells was measured by studying the relative changes in the proportions of labelled mitotic cells which were collected at various time intervals after H³-thymidine treatment^{14,20,21)}. In these reports estimation of average duration was measured by the time intervals between the beginning of H³-thymidine treatment and the time when the mitotic labelling index

reached maximum value or one half of its maximum value. In *Spiranthes sinensis* a comparatively small number of cells was labelled. It was also difficult to find the maximum value of each phase. In order to calculate the duration of mitotic cycle phases in this species a minimum duration of each phase was measured by the time interval between the beginning of H^3 -thymidine treatment and the appearance of the first labelled cells of each phase. Because there was a 1/2 hr. treatment period, the beginning of each phase will not be sharply determined because of the passage of cells out of the S phase during the time of labelling. The following estimation of the duration of each phase is therefore a rough one.

The first labelled prophase appeared in the cells fixed at 6 hrs. after pulse labelling. Therefore, the post-DNA-synthetic phase (G_2 phase)⁽²²⁾ can be estimated as about 6 hrs. in minimum duration. Each of the first labelled metaphase and anaphase appeared at 8 hrs. after pulse labelling giving the minimum duration of prophase to be about 2 hrs. The first labelled micro-condensed nuclei appeared at 9 hrs. after pulse labelling. The minimum duration of anaphase-telophase can be estimated as about 1 hr. The number of labelled extended nuclei increased for the second time in the cells fixed at 13 hrs. after pulse labelling. The minimum duration of the pre-DNA-synthetic phase (G_1 phase) is given by 13-9 to be about 4 hrs. It was observed that the number of labelled prophase increased for the second time in the cells fixed at 28 hrs. after pulse labelling. Thus, the minimum total duration of mitotic cycle is given by $28-6(G_2)$ to be about 22 hrs. The minimum duration of S phase is given by $22-13(G_2+M+G_1)$ to be about 9 hrs. The estimated minimum duration of mitotic cycle phases can be summarized:

G_1	4 hrs
S	9 "
G_2	6 "
M	3 "
1 cycle	22 "

The minimum duration of synthesis of DNA in euchromatin can be estimated by the time interval between the appearance of the first labelled euchromatic region in prophase chromosome and that of the first labelled heterochromatic region in prophase chromosomes. The time interval between the first appearance of labelled euchromatic region and the first appearance of restricted labelling of heterochromatic region is the minimum duration of the synthesis of DNA in euchromatin. This is given by 11-6 to be about 5 hrs. It is also calculated by 14-8 as about 6 hrs. from anaphase chromosomes. The calculation by prophase chromosomes is considered to be more accurate than that by anaphase chromosomes, because the appearance of grains over prophase chromosomes was sharper than that over anaphase chromosomes.

The minimum duration of the synthesis of DNA in heterochromatin can be given by following calculation. The time of DNA synthesis in both eu- and heterochromatin must be overlapped at the middle stage of S phase, because both eu- and heterochromatin in prophase chromosomes appeared labelled in the cells fixed at 10 hrs. to 11 hrs. after pulse labelling. Labelling of both eu- and heterochromatin was also noted in the metaphase chromosomes fixed at 11 hrs. to 13 hrs. and in the anaphase chromosomes fixed at 12 hrs. to 13 hrs. after pulse labelling. The time interval of labelling of both chromatins will be the overlapped time when both chromatins are replicating. This overlapped time can be estimated as about 2 hrs. The duration of S phase was about 9 hrs. Therefore, the minimum duration of DNA synthesis in

heterochromatin is given by $9-(5-2)$ to be about 6 hrs.

It was observed that DNA synthesis in the chromatin of condensed bodies in interphase nuclei occurred at early S phase. This observation correlated well with the time of DNA synthesis in the heterochromatin of chromosomes and added support to the idea that the earlier replicating condensed bodies gave rise to the heterochromatic regions of chromosomes. Similarly, the later replicating extended chromatin in major condensed nuclei might give rise to the euchromatic regions of chromosomes.

The interphase nuclei in meristematic cells of present species were classified into five categories: micro-condensed nuclei, extended nuclei, partly condensed nuclei, major condensed nuclei and macro-condensed nuclei. Pulse labelling experiments showed that both micro-condensed nuclei and macro-condensed nuclei appeared without incorporation of H^3 -thymidine in the cells fixed immediately after isotope treatment. The first appearance of labelling with H^3 -thymidine in micro-condensed nuclei and that in macro-condensed nuclei were noted in the cells fixed at 9 hrs. and 2 hrs. respectively, after pulse labelling. The time of the first labelling indicates that the micro-condensed nuclei are the nuclei in G_1 phase and that the macro-condensed nuclei are those in G_2 phase.

In three types of nuclei, extended nuclei, partly condensed nuclei and major condensed nuclei, a significant labelling occurred immediately after 1/2 hr. exposure of roots to H^3 -thymidine. Therefore, the three types of nuclei are considered to be the nuclei in S phase.

Characteristics of each of the three types of nuclei were as follows: The extended nuclei were those of early stage in S phase. In these nuclei all of chromatins were in the extended state and most of DNA in heterochromatin were doing replication. The minimum duration of the nuclei in mitotic cycle is given by the time interval between the time of DNA synthesis in heterochromatin and the overlapped time of DNA synthesis in heterochromatin and euchromatin. The extended nuclei can be calculated by $6-2$ to be about 4 hrs. in minimum duration.

The partly condensed nuclei were those of middle stage in S phase. In these nuclei a part of heterochromatin initiated condensation. Synthesis of DNA in these nuclei appeared to have occurred in both heterochromatin and euchromatin. Supposedly the DNA synthesis in heterochromatin will be completed in these nuclei. The minimum duration of these nuclei is given by the time interval between the first appearance of grains over the condensed bodies in major condensed nuclei and that over the condensed bodies in partly condensed nuclei. The partly condensed nuclei can be calculated by $4-2$ to be about 2 hrs. in minimum duration.

The major condensed nuclei were those of later stage of S phase. In these nuclei only synthesis of DNA in euchromatin appeared to have occurred. The DNA synthesis in euchromatin will be completed in these nuclei. Condensation of most heterochromatic regions will be completed in these nuclei. The minimum duration of these nuclei is given by $9-(4+2)$ to be about 3 hrs., where 9 is the minimum duration of S phase, 4 is that of extended nuclei and 2 is that of partly condensed nuclei.

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Summary

Spiranthes sinensis, Orchidaceae, had large heteropycnotic condensed bodies in interphase nuclei and had large heterochromatic segments in the proximal region of the long arm of somatic chromosomes. Present paper deals with the pattern of DNA synthesis in the condensed bodies, heterochromatin and euchromatin through an autoradiographic study with H^3 -thymidine.

1. Condensed bodies in interphase nuclei did not synthesize DNA. DNA in condensed bodies was found to replicate at the extended state of chromatin during early stage of DNA synthetic phase.

2. Condensation of chromatin in interphase nuclei started at middle stage of DNA synthetic phase.

3. DNA in euchromatin was found to replicate during the stages from middle to late DNA synthetic phase.

4. DNA synthesis in the chromosomes initiated in the proximal heterochromatic region of long arm and ended at euchromatic short arm and at euchromatic distal region of long arm. In the heterochromatic region the occurrence of DNA synthesis was found to be multicentric.

5. Mitotic cycle phases were as follows in minimum duration: pre-DNA-synthetic phase, 4 hrs.; DNA synthetic phase, 9 hrs.; post-DNA-synthetic phase, 6 hrs.; mitotic phase, 3 hrs.; minimum mitotic cycle, 22 hrs.

6. The interphase nuclei in the root tip meristematic cells of this species were classified into 5 categories by morphological and physiological characteristics: micro-condensed nuclei, pre-DNA-synthetic phase; extended nuclei, early DNA synthetic phase; partly condensed nuclei, middle DNA synthetic phase; major condensed nuclei, late DNA synthetic phase; macro-condensed nuclei, post-DNA-synthetic phase.

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摘 要

田中隆荘： ネジバナの染色質異常凝縮，異質染色質および真性染色質に関する
H³-チミジンオートラジオグラフィー

Spiranthes sinensis ($2n=30$), ネジバナは根端の分裂組織細胞核の中間期において多数の異常凝縮染色質を有し，染色体の長腕の動原体側に大きな異質染色質部を有する．根端を $2\mu\text{Ci/ml}$ H³-thymidine にて短時間処理し (1/2 hr.), オートラジオグラフィーを行なった結果次のことが判明した．中間期の核は主として染色質の形態によって小凝縮核，拡散状核，部分的凝縮核，真性凝縮核，巨大凝縮核の 5 型に大別される．DNA 複製は拡散状核，部分的凝縮核および真性凝縮核の拡散状の染色質のみにおいて起こり，凝縮状染色質には起こらない．凝縮状染色質の DNA 複製は DNA 複製期 (S 期) の初期の拡散状態のときに行なわれる．DNA 複製は染色体の長腕の動原体側の異質染色質部から始まり，長腕の末端部の真性染色質部および短腕の真性染色質部において終わる．異質染色質部には DNA 複製の始点が数カ所存在する．分裂環の各期の最短経過時間は大体において次のとおりである．G₁ 期 4 hrs., S 期 9 hrs., G₂ 期 6 hrs., M 期 3 hrs., 1 分裂環 22 hrs. その他，M 期の前期，中期，後期の経過時間，小凝縮核は G₁ 期，巨大凝縮核は G₂ 期であること，異質染色質および真性染色質における DNA 複製の経過時間がそれぞれ約 6 hrs., 5 hrs. であること，および中間期核の異常凝縮染色質と M 期の染色体の異質染色質との同一性などが論じられた．(広島大学理学部植物学教室)